Title: Structural MR imaging of gray matter in irritable bowel syndrome

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Abstract

Irritable bowel syndrome (IBS) is a functional gastrointestinal disorder. Cortical thinning of the anterior mid-cingulate cortex (aMCC) and insula has been previously reported in IBS (Davis et al., 2008). The aim of the present study was to examine cortical and subcortical structural gray matter integrity in IBS with particular attention to individual disease symptoms and personality characteristics such as pain catastrophizing. Eleven IBS patients and 16 age-matched healthy subjects (female, right-handed) underwent structural MRI. Voxel Based Morphometry and Cortical Thickness Analysis revealed that the IBS group had increased gray matter density in the hypothalamus, cortical thinning in the aMCC, strong (r = -66; p=0.015) negative correlation between dorsolateral prefrontal cortex and pain catastrophizing and anterior insula thickness was positively correlated to pain duration (r = 0.77, p=0.003) when controlling for age. These abnormalities may contribute to chronic pain in IBS.
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LIST OF ABBREVIATIONS

ACTH  adrenocorticotropin hormone
ACC  anterior cingulate cortex
aMCC  anterior mid-cingulate cortex
BOLD  blood oxygenation level dependent
BGA  brain-gut axis
BA  brodmann area
CBT  cognitive behavioural therapy
CRF  corticotrophin releasing factor
CNS  central nervous system
DC  dorsal column
DLPFC  dorso-lateral prefrontal cortex
ENS  enteric nervous system
fMRI  functional magnetic resonance imaging
GI  gastrointestinal
GLM  general linear model
IBD  inflammatory bowel disease
IBS  irritable bowel syndrome
IC  insular cortex
MPQ  McGill pain questionnaire
NTS  nucleus tractus solitarius
PAG  periaqueductal gray
PET  positron emission tomography
PFC         prefrontal cortex
rCBF        regional cortical blood flow
ROI         region of interest
S1          primary somatosensory cortex
S2          secondary somatosensory cortex
sMRI        structural magnetic resonance imaging
SPM         statistical parametric mapping
STT         spinothalamic tract
TIV         total intracranial volume
1. INTRODUCTION

Chronic pain is defined as pain that persists for longer than the usual temporal course of healing. The economic and societal burdens that a chronic pain condition creates are immense. Irritable Bowel Syndrome (IBS) is such a chronic pain disorder.

Pain is defined by the International Association for the Study of Pain (IASP) as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage” (Merskey and Watson, 1979). Pain has a sensory component as well as an affective component and is a complex multidimensional sensation. In addition, pain can be influenced by prior experience, preconceived beliefs, anticipation and the context in which the noxious stimulus occurs (Van Oudenhove et al., 2004). Acute pain typically originates from somatic or visceral tissue that contains nociceptors, receptors that are preferentially or exclusively sensitive to noxious stimuli. Repeated or sustained noxious stimulation can lead to two different scenarios. In one, the pain response is decreased, via habituation or fatigue. The other scenario can lead to serious consequences in that the pain response can actually be sustained or increased. In particular, prolonged or strong activity of primary afferent nociceptors or central nociceptors can lead to an increased neuronal responsiveness, otherwise known as sensitization. One potential outcome of sensitization is the chronification of pain. This thesis will explore the neuroanatomical underpinnings of this condition from an anatomical/structural point of view.
2. LITERATURE REVIEW

2.1 Irritable Bowel Syndrome

2.1.1. Clinical Aspects of IBS

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder which acts as a significant burden to our healthcare system and to society. The most common symptoms that characterize IBS are abdominal pain, discomfort and bloating, as well as altered bowel movements (Ringel et al., 2001). The condition is complex, as patients can present with different subtypes such as constipation dominant (IBS-C), diarrhea dominant (IBS-D), or even with mixed bouts of both constipation and diarrhea. Interestingly, most patients transition from one state to another, with the most common transition being between the mixed type IBS and IBS-C (Drossman et al., 2005).

A diagnosis of IBS is hampered by a lack of identifiable organic pathology, thus deeming it as a functional disorder. A functional disorder is one such disorder that disturbs function of a usually normal process, and that cannot be explained by any obvious structural or biochemical abnormality (Diamant, 1995). As such, attempts at diagnosis of IBS have historically been based on excluding other organic disease (Kruis et al., 1984). In recent years however, the Rome process classification system was developed to assist in diagnosing functional gastrointestinal disorders. The Rome process, currently in its third version, has provided insight into IBS and has allowed for more effective methods of diagnosis and treatment (Drossman, 2006).
IBS is highly prevalent in western societies, affecting 10-15% of the population. It is also prevalent outside western societies (Cremonini and Talley, 2005). In addition, IBS predominantly affects females, with a female to male ratio of up to 4:1 (Muller-Lissner et al., 2001). The prognoses of IBS shows considerable variation and heterogeneity among patients; with some patients presenting with relatively mild symptoms and responding well to treatments, while others experience severe symptoms that severely impact their social and occupational lives (Ringel et al., 2001). IBS can cause serious distress and frustration for patients seeking treatment and can significantly reduce a patient’s quality of life (Gralnek et al., 2000; Koloski et al., 2001). Indeed, it has been shown that the severity and intensity of IBS symptoms correlate with a lower quality of life (Coffin et al., 2004).

IBS patients frequently suffer from co-morbidities. The most commonly reported co-morbid conditions include fibromyalgia, chronic fatigue syndrome, temporomandibular joint disorder, and chronic pelvic pain (Whitehead et al., 2002). In addition, there can be co-morbidity with psychiatric disorders, the most common being major depression and generalized anxiety disorder (Whitehead et al., 2002). But, despite reports of high co-morbidity (50-90% of IBS patients have been found to suffer from at least one psychiatric disorder), it is not yet clear if the findings can confidently be applied to the condition in general.
2.1.2. Physiological and pathological mechanisms of IBS

**Visceral hypersensitivity**

Visceral hyperalgesia is a heightened perception of gastrointestinal sensation and is characterized as a significantly lowered pain threshold (allodynia) and increased pain evoked by visceral distension. Visceral hypersensitivity, specifically of the rectum, has been shown to be present in IBS and has even been considered to be a biological marker for diagnosis (Mertz et al., 1995). This proposition was based on Mertz’s finding of a lowered rectal distension threshold for aversive stimulation in 94 out of 100 tested IBS patients. Furthermore, another study tested 164 functional pain patients, 86 of them IBS sufferers, and found that IBS patients exhibited the lowest rectal distention-evoked thresholds (Bouin et al., 2002). Others have now consistently reported visceral hyperalgesia/allodynia in IBS. For example, Kwan et al., (2005) reported that compared to healthy controls, IBS patients have lower pain thresholds to rectal distension, pain and unpleasant sensations that persist longer after the stimulus is terminated (a ‘lingering’ of pain), and more intense affective responses evoked by prolonged stimuli (Kwan et al., 2005a).

One possible mechanism of visceral hyperalgesia is that there is sensitization of the primary afferent neurons that innervate the gastrointestinal tract. Peripheral inflammation, visceral nerve damage and alterations in receptors and ion channels have all been proposed as possible causes for peripheral visceral hyperalgesia (Anand et al., 2007). Sensitization of primary afferents can be expressed as an increase in their spontaneous activity, lower thresholds and/or enhanced stimulus-evoked responses (Knowles and Aziz, 2009) In addition, recruitment of ‘silent’ nociceptors to become
active is another postulated mechanism of peripheral sensitization (Bielefeldt and Gebhart, 2006). It may be that IBS patients have abnormal afferent impulses from the GI tract that may lead them to more readily perceive normal functions of gastrointestinal function such as motility and acid secretion (Musial et al., 2008). In order to elucidate the role of peripheral sensitization in IBS, one interesting study administered glycerol intraluminally to healthy volunteers, causing mucosal irritation of the rectum. This resulted in a lowering of their pain thresholds to rectal distention to the range usually found in IBS patients, suggesting that indeed peripheral sensitization plays a role in IBS (Bouin et al., 2001).

Another postulated mechanism for visceral hyperalgesia involves central sensitization, specifically of dorsal horn neurons in the spinal cord, possibly due to tonic, sustained activity in primary afferents. Increased input from the periphery can increase the excitability of central neurons at spinal and supraspinal sites (Bielefeldt and Gebhart, 2006). The theory of spinal central sensitization was tested in an animal model of IBS that used neonatal colonic inflammation induced in rats with daily applications of mustard oil enemas (Al Chaer et al., 2000). A control group was also administered neonatal daily saline enemas. In adulthood, after the initial irritation of the colon subsided, the rats had increased contractility of the abdominal muscles that indicated chronic visceral hypersensitivity. Electrophysiological recordings from visceral neurons in the lumbosacral spinal cord during rectal distention revealed increased responses compared to the control group (Al Chaer et al., 2000), pointing to the emergence of central sensitization.
In addition to central and peripheral sensitization, Knowles and Aziz (2009) propose that visceral hypersensitivity can also be caused by altered descending modulation processes and altered supraspinal mechanisms that could lead to the interpretation of non-noxious stimuli as noxious (both of these reasons will be explored in detail below). It is likely however, that both central and peripheral mechanisms are the underlying cause for visceral hyperalgesia and that multiple factors may contribute to these symptoms. This concept has been consolidated as an alteration of the ‘brain-gut axis’ (BGA), the bi-directional communication between the central and enteric nervous systems (Drossman, 2006). The BGA is an important system for healthy regulation of food intake, digestion and expulsion of bowel movements and its dysregulation (on any or multiple levels) may contribute to functional GI disorders, including IBS (Mayer et al., 2006).

**Psychological and social influences on IBS**

Psychological factors are thought to contribute to chronic pain disorders including IBS, especially because co-morbidities with psychological and psychiatric deficits are common (Drossman, 1999). Up to 60% of IBS patients report psychological and psychosocial problems including psychiatric disorders such as major depression, general life stressors, and physical and sexual abuse, with the most common disorders being mood, anxiety, and somatoform disorders (Van Oudenhove et al., 2004). However, it is not known whether the high occurrence of psychological disturbances is common to all IBS patients since the percentage may be due to the propensity of these patients to seek health care. It is now recognized that understanding the psychological and social aspects
of the patient is of great importance for effective treatment and improvement of symptoms (Levy et al., 2006). A study of risk factors for IBS reported that 86 of an initial sample of 2456 healthy individuals were diagnosed with IBS 15 months after the initial study enrollment, and that the presence of four of a variety of factors (for example, anxiety, high levels of illness behaviours, and sleep problems) predicted the emergence of IBS (Nicholl et al., 2008).

Psychosocial stressors, especially early life stressors, are common in IBS. These stressors usually comprise physical and/or sexual abuse (Drossman et al., 1999), and have been found in up to 40% of IBS patients. Using a large population based sample, Talley et al. (1994) reported a significant association between the symptoms of IBS and self-reported sexual, emotional and physical abuse. A more recent systematic review by Chitkara et al., (2008) identified many predictors that are associated with the development of IBS such as socioeconomic status, traumatic events during infancy or childhood (including parental deprivation, loss of parent), and social learning acquired from the environment. In sum, it is clear that IBS symptoms are at least partially influenced by psychosocial factors.

It has been suggested that patients suffering from IBS are more likely to have certain maladaptive personality traits. For example, Hazlett-Stevens and others (2003) reported that amongst a sample of undergraduate students, those who were identified as IBS sufferers were also more likely to demonstrate high levels of worry, neuroticism and anxiety. Predictably, visceral-specific anxiety was found to be the strongest association. The authors postulate that these personality traits could potentially manifest in a cycle of anxiousness regarding visceral activity, which in turn could lead to increased vigilance
towards bodily sensations. Indeed, IBS patients have been found to report high somatic focus (Verne et al., 2001), which may point to a hyper-vigilance to visceral symptoms. Another study evaluated 153 IBS patients and found that compared to controls, the patients had higher than normal levels of neuroticism, openness, and conscientiousness (Farnam et al., 2007). Interestingly, the authors also found differences in personality between the constipation- and diarrhea-dominant subtypes, suggesting that there may be differences in personality within IBS patients. Results using the NEO-PI and the Buss-Durkee Hostility Inventory found that functional gastrointestinal disease patients (including IBS) scored higher on measures of neuroticism and covert aggression (Tanum and Malt, 2001). Therefore, psychological factors may be associated with IBS. Although a causal link between IBS and psychosocial distress is not warranted at this time, there remains much to study to establish the nature of the relationship.

**The HPA and the neurobiological contribution of stress to IBS**

Stress is defined as either a real or perceived threat to the homeostasis of the organism and activates responses that are designed to ensure survival (Mayer et al., 2001). It has been suggested that stress is a key contributor to IBS symptoms, although its precise role is still unclear (Fukudo, 2007). For instance, one longitudinal study revealed that one of the major obstacles to improvement and management of symptoms of IBS was the perceived level of life stressors (Bennett et al., 1998). An organism’s response to stress is known to be generated by an integrated network of structures including the hypothalamus, amygdala, and periaqueductal gray (PAG) (Mayer et al., 2005). The hypothalamic-pituitary-adrenal axis (HPA axis) is an extensively studied stress pathway
that involves a variety of hormones and structures within the body. The HPA axis is activated when the brain perceives stress and results in release of corticotropin releasing factor (CRF) by the paraventricular nucleus of the hypothalamus. CRF stimulates the anterior pituitary gland to secrete adrenocorticotropin hormone (ACTH), and subsequently ACTH acts on the adrenal cortex to release cortisol, a molecule integral to the stress response (Tsigos and Chrousos, 2002).

Recent research has attempted to find biological substrates to link IBS symptoms and stress. For example, one study found that women suffering from IBS had increased levels of urinary catecholamines, norepinephrine, epinephrine and cortisol levels even when differences in demographics, lifestyle characteristics, and measures of anxiety and depression were considered (Heitkemper et al., 1996). In another study, increased salivary cortisol levels after rectal distention (but not normal daily activities) were found in patients with IBS but not healthy controls or subjects with non-IBS chronic constipation (Walter et al., 2006). The authors considered that because the IBS patients had experienced previous rectal examinations, they may have been experiencing stress from the memories of these previously painful examinations. However, the findings were believed to be IBS specific because the chronic constipation group was also subject to previous rectal exams, but did not exhibit marked increases in cortisol levels.

Animal models have also provided interesting findings pertaining to the stress response. For example, neonatal maternal separation in rodent models, analogous to early life stress in humans, was found to increase predispositions to develop many dysfunctions including visceral hyperalgesia and increased colonic motility (Coutinho et al., 2002; Soderholm et al., 2002). In another study, male rat pups were stressed by separating
them from their mothers daily for 3 hours. The stressed group displayed altered brain-gut axis responses and displayed an increased number of fecal boli, increased systemic immune response, and increased visceral sensation (O'Mahony et al., 2008). The authors suggested that these symptoms are essentially similar to the symptoms that humans with IBS possess. Other animal studies have investigated the role of CRF receptors, agonists and antagonists, and have concluded that there may be a strong link between this molecule and IBS-like symptoms (Tache and Bonaz, 2007). Although animal models do not fully replicate the symptoms experienced by IBS patients, they are valuable as they can be designed and manipulated in ways that human research understandably cannot (see Discussion).

2.1.3. IBS treatment

Treatment of IBS is a challenge because a biological substrate is unknown. The primary treatment most often involves treating the abdominal pain and altered bowel habits that the patients complain of (Hammerle and Surawicz, 2008). It has recently been suggested that treatment be individualized because different subtypes (constipation, diarrhea or both) may dominate in a given person. It is useful to note that a majority of IBS patients are treated in primary-care settings, and that lifestyle and diet changes can sometimes be sufficient to reduce symptoms, not requiring the expertise of a gastroenterologist or other specialist (Hammerle and Surawicz, 2008). Furthermore, patient education and a good physician-patient relationships can be effective in reducing patient visits and symptom related anxiety (Owens et al., 1995). Still, many patients do not benefit from these primary remedies. The treatment modalities most commonly studied can be divided into either pharmacological or non-pharmacological modalities.
**Pharmacological treatments**

Serotonin (5-hydroxytryptophan) is abundant in the GI tract and so it is thought to be an important neurotransmitter in the pathology of IBS. Research into IBS treatment has sought to alter serotonergic transmission in the GI tract. In fact, approximately 90% of the body’s serotonin stores are found within the enteric nervous system, and its release is triggered by luminal distention and other chemical signals. The specific serotonin receptor 5HT₃ has been found to modulate visceral pain and motility in the ENS, and also influences the emotional component of visceral sensation within the CNS (Gershon and Tack, 2007). It is not surprising then, that an 5HT₃ antagonist (Alosetron) can be effective in reducing abdominal pain and reducing stool frequency in IBS-D patients (Hammerle and Surawicz, 2008). Other 5HT₃ antagonists such as Cilansetro and Ramosetron have also shown promising results – suggesting that these kinds of drugs may be the most helpful to treat IBS-D (Hammerle and Surawicz, 2008). However, significant side effects have limited their approval for their use. Another serotonin receptor 5HT₄ has also been suggested to be involved in IBS symptoms mainly associated with constipation. For example, Tegaserod, a partial agonist, has been found to effectively reduce such symptoms as abdominal pain, constipation and bloating. Tegaserod’s main mechanism works to stimulate the intestinal secretion of water, as well as to decrease nociceptive responses to rectal distention (Prather et al., 2000).

When administering intravenous CRF to IBS patients, Fukudo and others (1998), observed an exaggeration of colonic motility and increased ACTH secretion. Accordingly, another promising avenue of treatment research has focused on is the CRF antagonist, alpha-helical CRF. This molecule has been shown to significantly reduce
abdominal pain and anxiety induced by electrical rectal stimulation in IBS patients (Sagami et al., 2004). Such trials strengthen the belief in a relationship between stress, IBS and the HPA axis.

Other pharmacological agents such as antidepressants, antispasmodics and opioid agents have also been suggested to help reduce IBS symptoms (Bradesi and Mayer, 2007). Nevertheless, the relatively few clinical trials, as well as the possibility of severe and unwanted side effects, have reduced their appeal. It is clear that new ideas and investigations will continue to emerge as scientists search for more effective and safe pharmacological substrates that will aid in the treatment of IBS.

**Non-pharmacological treatments**

Alternative treatment regimens, based more on the psychosocial aspect of IBS, have recently been used to mediate the symptoms. Cognitive behavioural therapy (CBT) is a psychological treatment method that is based on the idea that behaviour is shaped by consequences and that it can be socially learned (Toner, 2005). Thus, it is social factors that can bi-directionally affect cognition, physiology and motor behaviour. As a result, CBT attempts to address negative thoughts and behaviour and in fact strives to change them.

Studies of the effect of CBT on the improvement on IBS patients have been conflicting. CBT has been shown to significantly improve gastrointestinal and psychological symptoms related to IBS, however not necessarily better than routine clinical care or relaxation training (Boyce et al., 2003). Drossman and others (2003) showed that CBT was more effective in reducing the overall symptoms, but did not
significantly reduce patients’ pain scores. Although CBT seems promising, it is important to approach study results with caution, because CBT protocols are not standardized and each study may use a different approach under the umbrella term of “CBT”. For example, strategies can include various stress management methods, relaxation techniques, and other pain management routines that the choice of strategy may impact outcomes of the different studies (Toner, 2005). In addition, CBT can be conducted in individualized sessions, as well as in group settings. Some have criticized CBT studies due to small sample sizes, non-standardized classification of patients and uncontrolled study designs. In order to maximize the efficiency of CBT, it may be best to pair it with standard medical care. In one study, patients who were assigned regular treatment in addition to some form of CBT revealed better treatment outcomes than patients who were only assigned to the standard treatment (Heymann-Monnikes et al., 2000). Although the effects of CBT seem promising, more work needs to be done to fully understand their implications. Although they will not be reviewed here, it is noteworthy to mention that other alternative treatments for IBS exist, such as various forms of psychotherapy and hypnotherapy (for review, see Levy et al., 2006).

2.2. Anatomical substrates of visceral pain and sensation

Much of our understanding of basic mechanisms of pain derives from studies of somatic tissue. However, the scope of this review will focus on the specific topic of visceral pain. Therefore, this section outlines the anatomical pathways and structures that integrate and process sensation and pain arising from visceral structures.
2.2.1 Enteric Nervous System

The gastrointestinal tract in humans is a complex and unique structure because of its innervations by both the intrinsic enteric nervous system (ENS), as well as the extrinsic central nervous system (CNS). It is this dual innervation that has prompted the notion of the gut being under the control of a ‘big brain’ – meaning the brain, and a ‘little brain’, meaning the ENS. The ENS is a large network of neurons that are organized into two major plexuses of ganglia; the myentric plexus found between longitudinal and circular smooth muscle layers of the gut wall and submucosal plexus which is located between the mucosal layer of the intestine and circular smooth muscle layer (Kunze and Furness, 1999). The myentric plexus contains the cell bodies of a large number of intrinsic neurons including intrinsic primary afferents, some of which are responsive to stretch and some of which are sensitive to chemicals, excitatory and inhibitory motor neurons and ascending and descending interneurons that are responsible for mediating reflexes (Kunze and Furness, 1999). The submucosal plexus on the other hand, contains fewer types of neurons such as intrinsic primary afferent neurons and cholinergic motor neurons.

2.2.2 Central Nervous System Innervations

Visceral organs receive extrinsic innervation from either the vagal or spinal nerves. Spinal visceral afferents may be further divided into splanchnic or pelvic afferents. The axons that convey nociceptive information are mostly C fibers (unmyelinated) with some A-delta fibers as well ( thinly myelinated). Vagal afferents’ cell bodies reside in the vagal nodose ganglia. The bilateral vagus nerves innervate a very
large portion of the visceral organs, including most of the abdominal viscera, such as the stomach, small intestine, large intestine and liver (Ness and Gebhart, 1990). These fibers project to the nucleus tractus solitarius (NTS). Most of these second order neurons project to the parabrachial nucleus (PBN) which transmits information to viscerosensory cortical areas such as the insular cortex (Ness and Gebhart, 1990). There are also direct connections from the spinal cord to brain areas involved in regulation of arousal, emotions and behavioural responses, including the noradrenergic locus coeruleus, the amygdala, ACC and hypothalamus (Van Oudenhove et al, 2004). In the past, the vagus nerve was considered to have no role in visceral nociception, however new evidence suggests that it is important for contributing to chemonociception as well as to the affective and unpleasant qualities of visceral pain (Bielefeldt and Gebhart, 2006).

The innervation of the viscera by the spinal nerves are highly distributed, from cervical to sacral spinal segments. Spinal nerve cell bodies are found in the dorsal root ganglion and upon entrance into the dorsal horn of the spinal cord these afferents synapse in laminae I, II, V and X (Ness and Gebhart, 1990). Relevant to this review are the colonic and rectal afferents, that enter the spinal cord in the lower thoracic, lumbar and sacral segments (King and Szurszewski, 1989). It is currently believed that the spinal nerves are important for the transmission of visceral nociception. Such afferents are also subject to a significant convergence in the spinal cord with afferents from other viscerosomatic organs such as colon/rectum, bladder, uterus, cervix and vagina and with cutaneous afferents. Central somatovisceral convergence is believed to be the reason for the poor localization and high levels of referral that are experienced with visceral pain (Cervero and Laird, 1999; Foreman, 1999).
2.2.3. Ascending visceral pain pathways

In order to reach the brain, the transmission of nociceptive information ascends from the spinal cord via several anatomical tracts (Bielefeldt and Gebhart, 2006).

Traditional views implicate the spinothalamic tract (STT) as the main ascending pain pathway to the brain from the spinal cord. The STT terminates in several thalamic nuclei, including the ventral posterior lateral (VPL) and ventral posterior inferior (VPI) nuclei, considered to be components of the lateral pain system as neurons there project to the primary (S1) and secondary (S2) somatosensory cortices. This system is believed to be crucial for relaying sensory-discriminative information such as the intensity, duration and location of the pain stimulus (Brooks and Tracey, 2005). Conversely, there are STT terminations in medial thalamic nuclei such as the medial dorsal nucleus and neurons in these nuclei relay information to limbic and frontal cortical areas such as the anterior cingulate, orbitofrontal, and prefrontal cortex. This medial pain system is thought to be primarily concerned with affective, emotional and motivational aspects of the pain experience (Brooks and Tracey, 2005). In addition to the STT, nociceptive visceral input is transmitted from the spinal cord to the brain via the spinoisolitary, spinoparabrachial, spinoreticular and spinohypothalamic tracts terminating respectively in the nucleus tractus solitarius, parabrachial area, reticular formation and hypothalamus (Grundy et al., 2006; Burstein et al., 1987).

More recent additional evidence has shown another tract that is believed to convey visceral nociception: the dorsal column (DC) system. This system consists of large myelinated primary afferents that enter the spinal cord and ascend in dorsal columns to the ipsilateral DC nuclei which contain second order neurons that project to
the contralateral VPL nucleus of the thalamus. Al-Chaer et al. (1996) observed that neurons in the VPL thalamus continued to respond to colorectal distention in the rat after cordotomies that interrupted the STT. This was an important finding since it demonstrated that although the STT was important for transmitting somatic and cutaneous nociception, it was not necessarily unitarily responsible for the transmission of visceral nociception. Willis and others, (1999) confirmed this when they found that the visceral pain could be reduced by a DC lesion but not a STT lesion. Indeed, electrical stimulation of the DC in a patient with severe IBS produced widespread abdominal pain that was similar to her IBS symptoms, providing evidence that the DC has a role in visceral pain transmission (Malcolm et al., 2001). These findings implicate the DC system as a major tract for transmitting lower abdominal visceral pain.

2.2.4. Descending Modulation Pathways

Descending projections from supraspinal and brain stem areas can have modulatory effects on transmission of nociceptive information, and are implicated in antinociception and analgesia. Such descending projections mainly produce their effects in the spinal dorsal horn and spinal trigeminal nucleus (Basbaum and Fields, 1984). These effects can be inhibitory; however there may also be facilitatory processes that enhance the sensation of the pain perceived (For the most part, this thesis will concentrate on the inhibition process). Major sources of descending modulation arise from the PAG and the rostral ventromedial medulla (RVM), two highly interconnected structures. The PAG and RVM have dense concentrations of mu, delta, and kappa opioid receptors, and so it is not surprising that they are involved in antinociception mechanisms (Yaksh, 1997). Stimulation of these areas can have analgesic effects in humans and animals (Hosobuchi,
1986; Behbehani, 1995). For example, local anesthesia injected to the RVM of hindpaw-inflamed rats has shown to enhance nociceptive activity in the dorsal horn (Ren and Dubner, 1996). Furthermore, some experimental animal studies have replicated this stimulus evoked analgesic effect in visceral pain as well (Cervero et al., 1985; Giesler, Jr. and Liebeskind, 1976), suggesting this system is crucial for the healthy maintenance of visceral processes.

More recent data suggest that cortical and limbic areas such as the prefrontal cortex (PFC), anterior and mid cingulate cortex, (A/MCC), insular cortex (IC), amygdala and hypothalamus are also involved in the control of pain (Tracey and Mantyh, 2007). Anatomical tracing studies in animals have been especially useful for implicating cortical involvement of descending modulation. For example, An and others (1998) injected axonal tracers into the PFC of monkeys and visualized projections to the PAG that originated from medial prefrontal areas such as the anterior cingulate and dorsomedial areas. A recent seminal study by Hadjipavlou et al. (2006) used diffusion tensor imaging (DTI) to identify the white matter tracts implicated in descending modulation in humans. This study identified white matter tracts connecting the PAG and the DLPFC, amygdala, thalamus, hypothalamus, and RVM. These findings coincide with experimental animal data and provide further evidence for the anatomical circuitry for pain modulation.

Bingel and Tracey (2008) suggest that this inhibiting/facilitating mechanism may be functioning under two different contexts – either when the organism needs to ignore the pain by inhibiting it, or when it needs to increase attention to it for protection reasons, by facilitating it. Furthermore, it has been suggested that chronic pain may be a result of a malfunction of the modulation system that persists in keeping the pain facilitation “on”
for an abnormally long period of time (Mason, 1999). Finally, a reduction in the normal pain-induced inhibition may be a factor in chronic pain.

2.2.5. Brain Areas Implicated in Pain Perception

To understand the neuroimaging studies presented later in this literature review, this section will introduce some of the major brain areas implicated in the pain response. Although the focus will be on the roles these structures have on pain, it is important to recognize that these areas may also sub-serve other homeostatic functions in the body.

Prefrontal Cortex

The prefrontal cortex (PFC) is a highly complex area of the brain encompassing a variety of subregions which have been implicated in functions such as cognition, self-awareness and executive function (Rossi et al., 2007). Relevant to this thesis is the PFCs contribution to the control of pain. Animal studies have shown that medial PFC stimulation in rats reduces pain behaviour, promoting the notion of stimulus-driven analgesia originating from the prefrontal cortex (Hardy and Haigler, 1985). One subregion particularly relevant to pain is the dorsolateral prefrontal cortex (DLPFC). Utilizing repetitive transcranial magnetic stimulation (rTMS), Brighina and colleagues (2004) were able to relieve pain in patients suffering from chronic migraine, thus providing a human correlate to the aforementioned animal finding. Interestingly, another rTMS study reported that stimulating the right but not the left DLPFC increased pain tolerance to noxious stimuli, suggesting a laterality effect of pain control (Graff-Guerrero et al., 2005).
The modulatory effects of the PFC on pain have also been explored directly in patients with IBS. In a PET study, Lieberman et al. (2004) investigated a three week placebo trial in IBS patients to determine which brain areas were associated with a possible placebo response. Patients were scanned prior to the trial, and immediately after the trial had ended. The scanning session included both a resting period, as well as a rectal distention trial. Interestingly, activation in the right ventrolateral PFC was increased after the 3 week placebo trial. This activation was also positively correlated with the reported symptom improvement. Although not highlighted as a major finding, the DLPFC was found to be activated during the first scanning session, but after the placebo trial, this activation diminished. Due to the fact that the IBS symptoms improved for some of the patients, this lack of activation can possibly be attributed to a reduced need for pain modulation.

Indeed many neuroimaging studies have investigated various pain modulation mechanisms including attention and distraction (Bantick et al., 2002; Seminowicz et al., 2004), anticipation (Ploghaus et al., 1999), and placebo analgesia (Petrovic et al., 2002). These studies further support the notion of the DLPFC, working in concert with other brain regions, in the processing of pain modulation.

**Cingulate Cortex**

The cingulate cortex is an integral area of the brain that is involved in a variety of functions such as sensory, motor, cognitive and emotional processing (Bush et al., 2000). This diversity of function has led to several schemes for partitioning the cingulate cortex into sub-regions. Most recently, Vogt (2005) separated the cingulate into six regions consisting of the subgenual anterior cingulate cortex (sACC), pregenual ACC (pACC),
anterior mid-cingulate cortex (aMCC), posterior MCC (pMCC), dorsal posterior cingulate cortex (dPCC) and ventral PCC (vPCC).

The ACC and MCC regions have specifically been found to respond to painful stimulation. The meta-analysis of brain imaging studies conducted by Apkarian et al. (2005), indicated that parts of the ACC and MCC were active in a majority of pain studies. In general, the sACC and pACC are thought to be involved in the emotional aspects of pain, while the aMCC is believed to contribute to the cognitive salience of pain (Bush et al., 2000). In support of the role of the cingulate in pain perception, a seminal study by Hutchison et al. (1999) recorded from single cells in the human MCC and found neuronal activity evoked by noxious stimulation, while innocuous stimuli failed to elicit a neural response. Furthermore, in the same study, a neuron responded before the application of the stimuli, suggesting a “mirror neuron-type” response of anticipatory response to pain that has also been found in pain imaging studies (see Plo fhaus et al., 1999). Specific to visceral pain, electrophysiological evidence implicating the role of the cingulate in visceral nociception has come from animal studies. For example, Sikes et al. (2008) recorded from single neurons in several regions of the cingulate and found that during noxious rectal distention neurons in both the ACC and MCC were found to respond. A similar study by Gao et al. (2006) tested the response of ACC neurons in rats undergoing rectal distention. In this case however, control rats were tested against rats that were suffering from visceral hypersensitivity. The results of this study point to the fact that in visceral hypersensitive rats, ACC neurons fire at lower pressure thresholds and display enhanced response patterns compared to the ACC neurons in the control
group. According to the authors, these results suggest that the ACC can indeed be sensitized in a visceral hypersensitivity model (Gao et al., 2006).

Another suggested function of the ACC/MCC is the modulation and control of pain. This is not surprising, since this area has also been attributed to salience and attention to behavioural stimuli (Davis et al., 1997; Davis et al., 2000). In nerve injured rodents, electric stimulation of the ACC reduced the effect of repeated noxious tactile stimulation via the PAG system (LaBuda and Fuchs, 2005). Indeed, areas within the ACC and MCC were activated during placebo-analgesia and hypnosis conditions in human subjects (Bingel et al., 2006). This may be due to this area modulating pain processing through a sub-cortical system (such as the PAG), probably working with other frontal regions such as the DLPFC.

In sum, it is clear that the specific roles of the different regions of the cingulate cortex have not yet been completely consolidated. Nonetheless, the evidence to date implicates this region in pain response and modulation.

**Insular Cortex**

The human insular cortex (IC) is a lobe within the brain hidden by opercula of the frontal, parietal and temporal lobes and has been shown to be disproportionately large in the human compared to lower order primates (Ture et al., 1999). The middle cerebral artery passes across the surface of the IC and divides it into an anterior and posterior portion. Interesting, the anterior and ventral portion of the IC may be the most evolved parts as primate equivalents have not been found. Connections to and from the IC in primates and humans are abundant and include the prefrontal cortex, amygdala, cingulate cortex, and several thalamic nuclei (Augustine, 1996). In the past, the IC was thought to
function primarily for visceral sensation, visceral motor activity, and language functions. However, more recent evidence indicates that the IC is also critical for general somatosensory functions as well as integration of limbic activity (Augustine, 1996). Craig (2002) has proposed that the anterior IC, combined with homeostatic information integration from the posterior IC, forms a representation of the entire bodily state. Indeed, neuroimaging studies have implicated the IC in a variety of interoceptive processes such as time perception, self recognition, decision making, and error awareness (Craig, 2009).

The IC is also thought to be involved in pain processes because it is activated in many experimental pain studies, including visceral (Baciuc et al., 1999; Mertz et al., 2000), and thermal pain (Davis et al., 1998b; Coghill et al., 1999). However, the location within the IC that is most crucial is contentious. A case study of posterior IC occlusion reported significant impairment in pain perception (Greenspan and Winfield, 1992) and stimulation of the posterior IC has been shown to evoke pain (Ostrowsky et al., 2002). Conversely, the anterior IC has been implicated in relation to nociception in healthy volunteers (Davis et al., 1998a; Schweinhardt et al., 2006; Coghill et al., 1994), and even in emotional aspects of pain (Rainville et al., 1997; Singer et al., 2004). Interestingly, a recent study by Taylor and colleagues (2008) found that the anterior IC is functionally connected with the pACC/aMCC and suggested that this region is important for the emotional salience of the bodily state, including the perception of pain.

**Primary and secondary somatosensory cortices**

The primary somatosensory (S1) cortex comprises the post central gyrus and includes BAs 1, 2, 3a and 3b. Each area receives a different combination of thalamocortical projections which distinguishes them functionally (Jones and Friedman,
The S1 is known to have a somatotopic organization of the contralateral body, based on classic electrophysiological (recording and stimulation) and anatomical studies (Kaas et al., 1979; Nelson et al., 1980). The secondary somatosensory (S2) has been defined as Brodmann area 43, and is believed to have a boundary with the posterior IC (Brodmann and Garey, 1999). S2 receives input from S1 and projects to the IC and other areas such as BA 4, 5, 6, and 7 (Vogt and Pandya, 1978). Studies of the characteristics of the S2 response have reported that it is somatotopically organized, based on evidence from monkeys (Friedman et al., 1980), and humans (Ruben et al., 2001), and it can be activated by innocuous stimuli.

Despite the presence of nociceptors and input from the STT, the role of S1 in pain processing in humans is still being debated (Peyron et al., 2000). Most neuroimaging studies have reported that noxious stimuli can activate S1, but a few studies found either deactivation or no activation of S1 during noxious stimulation (Bushnell et al., 1999). Timmermann et al., (2001) found a strong correlation between S1 activity and pain intensity. However, classically S1 has been implicated in the sensory-discriminative aspect of at least somatic pain.

Our understanding of the role of S1 and S2 in the sensation of visceral pain is unclear due to inconsistencies in the literature. For example, Vandenbergh et al. (2005) has reported an activation of S1 and S2 during gastric fundus distention. Conversely, neither of these areas was activated during any kind of painful visceral stimulation in another study (Ladabaum et al., 2001). In sum, the role of S1 and S2 in the processing of visceral pain is still being debated, and thus further studies must explore the issue.
**Hypothalamus**

The hypothalamus resides at the base of the brain near the third ventricle and although small in size, is considered to be a major component of the diencephalon. The hypothalamus is divided into three longitudinal zones: the periventricular zone, the medial intermediate zone and the lateral zone. The most important nucleus within the hypothalamus is the paraventricular nucleus (PVN). The PVN contains neuroendocrine cells which can secrete a number of different molecules. This structure plays an integral role in food ingestion, energy balance, reproduction, immunity and emotional responses, including the response to pain (Cortelli and Pierangeli, 2007). The hypothalamus has extensive connections with other structures in the brain such as the amygdala, PFC and cingulate gyrus (Giesler, Jr. et al., 1994).

The hypothalamus contains neurons that respond to noxious stimuli transmitted to it via the STT and spino-hypothalamic tracts (Giesler, Jr. et al., 1994). These neurons are thought to be involved in the autonomic, affective and neuroendocrine changes that accompany pain (Giesler, Jr. et al., 1994). Neurons in the hypothalamus can respond to noxious stimulation by way of the HPA axis (discussed below), or through neuroendocrine pathways involving other hormones, such as vasopressin.
2.3 Brain imaging of pain

2.3.1. Functional imaging of acute pain

Brain imaging has been used since the early 1990s as a tool to locate supraspinal sites of pain-evoked responses. In the early 1990s, two seminal studies measured regional cerebral blood flow (rCBF) with positron emission tomography (PET) to identify noxious heat-evoked brain responses. One study (Talbot et al., 1991), reported rCBF increases in the contralateral S1, S2, and ACC whereas Jones and colleagues (1991a) observed rCBF increases in the contralateral thalamus, basal ganglia and ACC. Since then, many studies have used different techniques (PET, fMRI and EEG) with a variety of stimulation modalities to delineate pain-related brain activity (Apkarian et al., 2005). Attempting to summarize the large volume of studies in the literature today, Apkarian and colleagues (2005) conducted a meta-analysis review of the supraspinal brain mechanisms in response to pain. This paper reported the commonly identified brain areas showing pain-evoked activity. These areas were categorized into two systems: a lateral system and a medial system. The lateral system, important for the sensory and discriminative aspects of pain, includes the S1, S2, thalamus and some parts of the IC. The medial areas on the other hand include the ACC, the anterior parts of the IC and some parts of the PFC, and are mostly important for the affective and cognitive aspects of pain (Tracey, 2008). All of these areas are most consistently activated by painful stimuli, but their roles in the perception of pain are not completely understood. Although some have simplistically referred to them as the ‘pain matrix’ (Ingvar, 1999), it is not known whether they act as an integrated network. The increasing revelations of the complexities
of these areas have thus made this term become increasingly less common (Bingel and Tracey, 2008).

2.3.2. Functional imaging of chronic pain

Neuroimaging techniques have been extremely useful as they provide an opportunity to study human brain responses in patients suffering from chronic conditions and thus assess brain abnormalities in the diseased brain. There are some inconsistencies in the results of neuroimaging of chronic pain. Some studies have failed to find differences in the central pain response between chronic pain patients and healthy controls. For instance, in one study, both chronic back pain patients and controls revealed a similar activation response of thalamus, IC, MCC, PAG and cerebellum to acute noxious thermal stimulation (Derbyshire et al., 2002). In contrast, there have been studies that did indeed find differences in brain response between chronic pain patients and healthy controls. A meta-analysis of neuroimaging studies of chronic pain states reveals that the PFC is more frequently active in chronic pain states (when compared to acute pain), while other pain related areas such as S1, S2, thalamus and cingulate cortex are actually less active (Apkarian et al., 2005). The authors do note however, that due to the fact that such studies use the acute stimulations to create the painful effect, it is hard to translate the findings to chronic pain populations (Apkarian et al., 2005).

Some studies have focused on imaging the chemical distribution of neurotransmitters and receptors in the brains of chronic pain patients. These studies have shown reduced opioid receptor binding in DLPFC, ACC thalamus and IC (Jones et al., 1994), as well as reduced levels of N-acetyl aspartate and glucose in the DLPFC (Grachev et al., 2000) in chronic pain patients. It has been suggested that altered brain
chemistry may point to maladaptive plasticity in either function or structure that may be an underlying cause of chronic pain (Apkarian et al., 2005).

2.3.3. Functional imaging in IBS patients

Perhaps a more thorough approach to understanding the brain processes influenced by chronic pain is to attempt to mimic the perceived pain experienced by the specific condition. Thus, distention of the GI tract (mostly of the rectum) has been a popular tool for functional imaging studies of IBS.

Brain imaging studies have, for the most part, found that healthy volunteers undergoing painful visceral stimuli consistently activate major areas of pain processing within the brain (Rapps et al., 2008). These are essentially the same areas associated with other types of pain, namely S1, S2, cingulate regions, anterior IC, PFC and thalamus (Verne et al., 2003; Berman et al., 2000; Naliboff et al., 2000). Some studies however, have found slight differences between brain activations when comparing cutaneous versus visceral pain (Strigo et al., 2003) and anal versus rectal (non-painful) stimulation (Lotze et al., 2001).

The same areas have also been found to be activated in IBS patients undergoing rectal distention. However, major inconsistencies in activation appear when comparing the brain response of IBS patients to those of healthy controls. It appears as though the biggest discrepancy is in relation to the ACC and PFC and S1 (Rapps et al., 2008).

Silverman et al. (1997) were the first to conduct a PET study of painful rectal distention in IBS. Their main finding was that compared to controls, the IBS patients significantly activated the left DLPFC. Interestingly however, the patients failed to activate the ACC during the distension, an area that was found to be activated in the
healthy controls. This reduced activation, or in some cases, complete lack of activation of the MCC/ACC has been found in other studies as well (Wilder-Smith et al., 2004; Bonaz et al., 2002; Yuan et al., 2003). For example, Andresen et al. (2005) found reduced ACC/MCC activation in IBS patients during subliminal and liminal rectal distention when compared to healthy controls. Another study which used percept-related fMRI, a method that takes into account the temporal and perceptual attributes of a noxious stimulus, delivered rectal distention stimuli to IBS patients and healthy controls. This study found that patients had pain-related activity in the medial thalamus and hippocampus that healthy controls lacked, however the patients lacked urge and pain-related activity in the right anterior IC and right aMCC (Kwan et al., 2005b). One reason for the differences in findings in this study is that the reported activations are related to the time course of evoked pain, whereas other studies instead used the time course of the stimulus to search for activations and found that when compared to healthy controls, IBS patients actually display higher activations of the ACC/MCC during painful stimulation (Mertz et al., 2000; Verne et al., 2003).

Brain activity has also been compared between other gastrointestinal diseases such as ulcerative colitis (UC), and IBS patients (Bernstein et al., 2002; Mayer et al., 2005). By comparison with UC patients, the IBS patients failed to show increased activation in limbic regions such as the amygdala, hypothalamus, sACC and other medial parts of the PFC (Mayer et al., 2005). In addition, PAG and DLPFC failed to activate in the IBS patients, but were activated in the UC patients. The findings of this study support the idea that IBS activates a different response in the brain, and it is this response that may explain the symptoms associated with the syndrome.
Brain imaging studies examining the effects of pharmacological and/or behavioural treatment of IBS patients have provided evidence to support the concept of altered brain processing of affective and emotional aspects of pain. Berman and others (2002) found that Alosetron, a 5-HT\textsubscript{3} receptor antagonist (see IBS treatments above), reduces the emotional ratings associated with rectal distension and reduces activity in brain regions related to the emotional motor system; namely hypothalamus, amygdala PAG and sACC. Lackner et al. (2006) examined brain activations of IBS patients undergoing CBT sessions using PET and found that CBT sessions reduced baseline activity in the right sACC and the left medial temporal lobe. Additionally, the brain activation changes were accompanied with improvements in symptoms and a reduction in anxiety. The authors attributed the results to reduced vigilance towards visceral stimuli and/or reduced visceral-specific anxiety.

It may be that IBS patients have altered central processing of visceral and somatic stimuli – some sort of generalized hyperalgesic state that may explain their hyper-vigilance towards visceral stimulus. To elucidate this, brain responses to somatic (non-visceral) stimulation have also been studied in IBS patients. Verne et al. (2003) ran an fMRI study of rectal distention and hot water foot immersion to determine whether IBS have altered pain perception to both somatic and visceral stimuli and found that IBS patients displayed greater activation of the PFC, ACC, thalamus, S1 and IC for both types of stimulation. The visceral stimulation however only evoked greater activation within the thalamus and PFC. Intriguingly, although pain intensities were similar between the visceral and somatic stimuli, the IBS patients did report feeling more anxious about the visceral stimuli, as well as rating it as more unpleasant.
The lack of organic pathology in IBS has created a surge of interest in the functional properties of the CNS and the brain during visceral and autonomic stimulation. Still, functional imaging of IBS has proven to be a challenging endeavor for several reasons. One issue is that the IBS patients are heterogeneous with respect to their main symptomology (constipation, diarrhea, or mixed), and their severity. There are other sources of variability across studies due to different exclusion criteria and imaging tools (fMRI, PET) and parameters. In addition, many research studies have used varied methodologies and study paradigms to try and understand the issue. For example, different stimulation levels and methods have been used such as balloon distension stimulation at different levels of the gastrointestinal tract (rectum, sigmoid colon, gastric fundus, esophagus), electrical stimulation and acid infusion (Rapps et al., 2008). Furthermore, while some studies use standard stimulus-related analyses, others use more creative approaches such as a percept-related design (Kwan et al., 2005b). These differences in methodology may partially explain some of the inconsistencies between IBS imaging studies.

2.3.4. Structural Imaging of chronic pain states

Structural MRI (sMRI) is a relatively new application of MRI that can be used to study brain anatomy, including gray and white matter. Using sophisticated new techniques such as morphometry and cortical surface analyses (discussed in detail below), researchers are now able to determine whether specific conditions of chronic pain have an anatomical structural underpinning in the human brain.

One of the earliest sMRI studies in a chronic pain population examined gray matter in patients suffering from a common debilitating disorder, chronic back pain. In
this study Apkarian and colleagues (2004) reported reduced gray matter density in bilateral DLPFC and right thalamus. It was also reported that that the gray matter changes were strongly influenced by external factors such as pain severity and duration (Apkarian et al., 2004). Another sMRI study found that chronic tension type headache sufferers had reduced gray matter density in the pACC, bilateral IC, and orbitofrontal cortex, among others (Schmidt-Wilcke et al., 2005). More recently, chronic regional pain syndrome (CPRS), a condition characterized by spontaneous and exaggerated painful responses, abnormal blood flow and sweating in an affected region of the body, has been examined (Geha et al., 2008). When compared to controls, patients had reduced gray matter density in the right IC, ventromedial prefrontal cortex and nucleus accumbens.

Structural MRI studies have also focused on functional pain disorders such as fibromyalgia and IBS. For example, Kuchinad et al., (2007) revealed reduced gray matter density in the cingulate, IC, and medial PFC in addition to reporting an overall reduced gray matter volume in fibromyalgia, which the authors interpreted as equivalent to 9.5 times the rate of loss due to normal aging per year of illness. In IBS, only one study to date has examined possible structural abnormalities (Davis et al, 2008). In this study, 9 patients suffering from IBS were examined against 11 controls. The results revealed a reduction in gray matter density of the right anterior/medial thalamus and MCC. Additionally, cortical thinning in the right MCC and bilaterally anterior IC was found (Davis et al., 2008). The authors linked the findings to an alteration in descending analgesic processes (originating from the aMCC) as well as alterations in homeostatic responses.
Although most studies report gray matter decreases, increases in gray matter and cortical thickness have also been reported in some pain conditions. Chronic vulvar pain was examined by Schweinhardt et al. (2008) and was found to exhibit increases in gray matter density in brain areas thought to be involved in both pain modulation and stress responses such as the parahippocampus, hippocampus and basal ganglia. Furthermore, patients suffering from chronic migraine showed a thickening of the somatosensory cortex. This was especially true in the caudal portion of the somatosensory system, where the representation of the head and face is believed to reside (DaSilva et al., 2007).

It is important to keep in mind however, that although structural abnormalities exist, they do not allude to a causal relationship between chronic pain and gray matter differences. It could in fact be that the structural abnormalities found are in fact congenital and/or genetic and that they predispose the individual to develop such chronic pains. Future studies will further improve our understanding of this contentious issue.

2.4. Chronic Pain

2.4.1. Plasticity and Chronic Pain

What is it then that causes functional alterations in the brains of chronic pain patients? The answer may be related to the concept of neuroplasticity. Neuroplasticity is a term that is used to describe the changes that occur in the nervous system. In the past few decades it has become clear that the brain is a plastic organ that may change in both function and structure. This notion has been supported by studies that investigated functional reorganization in chronic pain conditions and found that these conditions may
also be a consequence of central plastic mechanisms (May, 2008). For example, patients suffering from chronic regional pain syndrome (type 1) exhibited a reduced representational field of the affected arm (Maihofner et al., 2003). Moreover, the extent of the finding was highly correlated with the perceived pain intensities and levels of hyperalgesia, meaning the more severe the pain, the smaller the representation field was.

One question that arises when considering the above findings is whether these structural changes can also be induced by acute pain. A well-controlled study explored the effects of repetitive painful stimulation on healthy controls to observe the possible brain changes such stimuli induce (Teutsch et al., 2008). The painful stimulation was applied over several days and an increase in gray matter in pain-related areas such as the cingulate and somatosensory cortices was found. The authors proceed to identify the disparity between the idea that continuous pain causes an increase in gray matter and the finding that most chronic pain populations that have been studied to date, the opposite direction has occurred. To explain this discrepancy, the authors propose that in a patient suffering from chronic pain, the healthy physiologic mechanisms of habituation have ceased to work well and as a consequence a decrease in gray matter is observed.

2.4.2. Does chronic pain arise due to a lack of control?

A possible contributor to the ‘chronification’ of pain may be the presence of a lack of control of either a dysfunction in the endogenous analgesic descending pathways, or some kind of attentional/affective alteration. More specifically, pain perception may be modulated by our own belief and attitude towards the painful response. As a result, many studies have focused on brain areas that are believed to contribute to the cognitive and attentional demands that are relevant to pain.
A study by deCharms et al. (2005) used real time fMRI to train subjects to modulate their perceived pain intensity by ‘controlling’ (both increasing and decreasing) the neural activity in the aMCC. Some, but not all, of the subjects were able to decrease the amount of pain intensity that they felt during the experimental session by reducing the activation of this area. Even the chronic pain patients that were tested using this technique reported decreased pain ratings which were inherently correlated with the degree of how well their aMCC was modulated. Although no cause-effect relations can be concluded from this study, it does raise the possibility that certain brain regions (in this case, specifically the aMCC) may modulate the perceived intensity of a painful stimulus. The exact mechanism underlying this effect is still unknown. Nevertheless, although costly, techniques such as this have enormous potential for providing a non-invasive and highly effective method for reducing pain in chronic states.

2.4.3. Pain Catastrophizing and chronic pain

Pain catastrophizing is a specific psychological variable that is associated with an exaggeration of negative beliefs and attitudes toward pain (Sullivan et al., 1995). It has also been shown to be associated with maladaptive coping systems, avoidance behaviour and hypervigilance to somatic sensations. Moreover, catastrophizing has been found to also be related to significant psychological distress, pain intensity and depression (Severeijns et al., 2001). The Pain Catastrophizing Scale (PCS) is a questionnaire used to measure this trait and has been extensively used in the context of chronic pain research. The PCS consists of thirteen items that assess three subscales known as helplessness, rumination and magnification. These subscales are thought to be the essential elements of catastrophizing (Sullivan et al., 1995). Pain catastrophizing has been found to be
associated with reports of more intense pain, as well as emotional distress in populations that suffer from chronic pain (Block and Brock, 2008). In addition, catastrophizing has also been found to predict poor health outcomes in post-operative pain (Pavlin et al., 2005), enhance the attentional demands on pain (Van Damme et al., 2004), and functionality impair the quality of life of migraine sufferers (Holroyd et al., 2007). A small number of studies have examined the impact of pain catastrophizing on IBS patients and found a link between high catastrophizing and poor health outcomes, although this was not the strongest predictor (Drossman, 1999). Lackner and colleagues (2004) found that IBS patients engage in high levels of catastrophic thinking and thus experience more intense pain, as well as activity limitations due to pain severity. Furthermore, catastrophic thinking was found to act as a mediator between depressive symptoms and pain.

Neuroimaging studies have revealed interesting relationships between pain catastrophizing and pain evoked responses in several key brain regions. For instance, in patients suffering from fibromyalgia there was a correlation between pain catastrophizing scores and activity in the dorsolateral and medial prefrontal cortices, as well as in the rACC (Gracely et al., 2004). In a study of healthy volunteers, Seminowicz and Davis (2006), reported strong positive correlations between pain catastrophizing scores and areas associated with emotional aspects of pain, such as the insula and rACC, during mildly painful stimulation. Furthermore, this study also found that the responses to moderately painful stimuli were negatively correlated to activity in the DLPFC, suggesting an alteration in brain areas related to top-down pain inhibition. The field of pain catastrophizing in regards to neuroimaging is growing as the psychological
characteristics begin to play a larger role in theories of pain processing. To date, there have been no neuroimaging studies published with regards to IBS and pain catastrophizing.

2.5. Structural MRI techniques

The first type of technique that was used to assess brain structures from MR images utilized manually drawn regions of interest (ROI) directly onto anatomical images. Although regarded as highly accurate and still used today to measure gray matter volumes, this approach has many limitations. First, human error in manually tracing the ROI can easily occur. Second, it is difficult to standardize such ROIs across subjects. Third, this approach is very time consuming. More recently, there have been great advances in MR imaging and software development with the introduction of new methods for assessing human brain anatomy. In this section, I will describe two methods that were used in this thesis.

2.5.1. Voxel Based Morphometry

Voxel Based Morphometry (VBM) is an automated whole-brain technique that is used to make voxel-wise comparisons of regional gray matter density – that is, the relative amount of gray matter between different images (Ashburner and Friston, 2000). In the past decade, VBM has gained immense popularity and has become one of the most commonly used methods for structural MRI (sMRI) analysis. In order to fully review the methodology of VBM, I will present the standard pipeline that is most often used today.
Although VBM may be used for structural analysis of both white and gray matter, this thesis will focus on the application to gray matter analysis.

**Segmentation:**

The first step of VBM consists of segmenting high resolution anatomical MR images into different tissue classes of gray matter, white matter and cerebrospinal fluid (CSF). Voxel MR signal intensities are used to differentiate between the different classes of tissue. Segmentation usually also utilizes a template in the form of a tissue probability map to help approximate the spatial distribution of the tissue classifications and this then increases the accuracy of the segmentation (Ashburner and Friston, 2007).

**Spatial Normalization:**

In order to make valid comparisons between different subjects in a sMRI study, it is critical to overlay the raw images to each other. Spatial normalization attempts to do just that, as the gray matter images are ‘warped’ into a standard stereotaxic common space. A template is usually used for the normalization. For example, in the Statistical Parametric Mapping program (SPM) it is the International Consortium for Brain Imaging or ICBM, template. The integrity of this step is crucial because it ensures that any density differences that are found are not attributable to actual disparity in the data. If mis-registration does occur, reported differences may in fact be artifacts due to poor spatial normalization (Bookstein, 2001).
**Optimal Modulation:**

The spatial normalization procedure changes and distorts the original volumes of brain regions, because the images are essentially stretched or compacted into the template. Consequently, this may undermine the results found. In order to adjust for these changes and to preserve the initial and true amounts of tissue within a specific region, “optimal modulation” is used. Optimal modulation uses the standard method of Jacobian adjustment but also multiplies the segmented images by their relative voxel volumes prior to and after the normalization (Ashburner and Friston, 2007). By doing so, the differences in volume are preserved so that sensitivity is increased and fewer false negatives are found. In addition, another method used to reduce possible confounding factors is to use total intracranial volume (TIV), a calculation of the sum of the gray matter, white matter and cerebrospinal fluid as a regressor in the statistical model. This method provides extra sensitivity to finding local differences (Ashburner and Friston, 2007).

**Smoothing:**

The smoothing step involves using an isotropic Gaussian kernel (usually between 8-12mm) to ‘smooth’ (blur) the data so that each voxel contains the mean average of gray matter from its neighbors (Ashburner and Friston, 2007). Smoothing is conducted in order to increase the sensitivity of finding differences. Also, using a smoothing kernel helps to at least partially alleviate the problem of inter-subject normalization. Lastly, smoothing is used in order to normally distribute the data so that it is more statistically valid.
Statistical Considerations:

After the preprocessing of the images, voxel-wise statistical tests can be performed. This is usually done using standard linear models such as the General Linear Model (GLM). The GLM can be utilized to perform a variety of different tests such as group comparisons, correlates with different variables and complex interactions. A statistical parametric map is then created and if differences are found, they show up as clusters (or blobs) and can be overlaid on an anatomical template of a human brain. It is also necessary to correct for multiple comparisons, since otherwise false-positive findings are more likely to appear. In SPM, random field theory is used most often (Ashburner and Friston, 2007).

2.5.2. Cortical Thickness Analysis

The human cerebral cortex is a highly folded sheet with a typical thickness of 1 to 4.5mm in different spatial regions (Fischl and Dale, 2000). The thickness of the cortex can be of great use to understanding normal development as well as the progression of diseases that may cause cortical neuron degeneration. Cortical thickness analysis (CTA) is a relatively new method that measures cortical thickness and that can be used to compare the differences between groups. There are several programs that can perform this analysis; however this section will focus on the method utilized by the program Freesurfer (http://surfer.nmr.mgh.harvard.edu), as it is most relevant to this thesis. Since the algorithms and methods of this procedure are highly complex and well beyond the scope of this thesis, I will provide here a brief, but complete summary of the process (For full review and detailed descriptions see: Fischl and Dale, 2000)
Intensity Normalization:

A high resolution T1-weighted anatomical image is used as the raw data in this procedure. The first step in CTA is intensity normalization of the voxels (Dale et al., 1999). This is required to reduce the intensity variations that are created by coil reception in-homogeneities. This is done by assuming that the highest voxel intensity belongs to the class of white matter. By doing so, white matter can be properly separated from gray matter and CSF (CSF having the lowest MR value), which will aid in the future segmentation. Next, the algorithm removes (“strips”) non-cerebral voxels, such as the skull from the intensity normalized image, and then normalizes the images to the Talaraich atlas (Dale et al., 1999).

Segmentation:

Following the normalization step is the actual segmentation of the cortex. Here, the gray-white matter borders are distinguished using a highly-integrated algorithm which is thought to provide better segmentation than the gray-scale method used by other segmentation procedures such as VBM. All white matter voxels are then used to construct a highly accurate estimation of the gray-white matter boundary. The cerebral hemispheres are then disconnected from each other and are also separated from subcortical structures. In order to do so properly, landmarks such as the corpus callosum and pons are used to produce a preliminary segmented brain. At this point, the gray-white matter border goes through a tessellation process, in that triangles are used to define the surface between the gray and white matter voxels. The advantage of this step is that the resulting tessellations accurately represent the topology of the surface. Finally, surface
deformation is employed outward toward the outer surface of the brain, stopping once the contrast between the gray matter and CSF or pial surface is reached. This method uses both intensity and continuity information from the entire MR volume to produce representations of cortical thickness. Once again, this method is highly useful to create an accurate topological surface with fine detail (Dale et al., 1999).

*Cortical thickness measurement:*

Cortical thickness is calculated as the closest distance from the gray/white boundary to the gray/CSF (or pial) boundary at each vertex on the surface. Thus, any point on the cortex can measured in millimeters, a scalar and quantifiable assessment.

*Transformation*

After each cortex has been successfully reconstructed, it is transformed into individual spheres. A sphere allows for the preservation of the topological structure of the surface without much distortion – thus creating a spherical surface that accurately represents the folding pattern of the cortex (Fischl et al., 1999). Once each individual brain volume is run through this process they are all aligned to a folding pattern of an average template so that they are accurately and correctly registered. Guassian kernel smoothing is then applied to further reduce registration imperfections. Facilitated by this spherical coordinate system, accurate structural properties of the cortex can be computed and analyzed in order to create statistical maps (Fischl et al., 1999).

The method outlined above for measuring cortical thickness has been validated by both histological analyses (Rosas et al., 2002) and also by manual measurements
(Kuperberg et al., 2003). As a result, CTA is a valid and reliable method for detecting small differences in cortical thickness between groups (Fischl and Dale, 2000).
3. STUDY RATIONALE AND HYPOTHESES

General Aim

The overall aim of this thesis is to determine if patients with irritable bowel syndrome (IBS) have structural abnormalities in cortical and subcortical gray matter.

The foundation for this thesis is built upon an earlier pilot sMRI study from our lab (Davis et al., 2008), as well as earlier functional imaging studies both in our lab and in others (see above). Thus, the goal is to expand on the previous findings to gain a deeper understanding of the brain plasticity in IBS. This thesis improves on the previous study in two ways. First, this thesis acquired data on a MRI with higher field strength (3 Tesla vs 1.5 Tesla). The increased field strength provides for a higher signal to noise ratio, leading to a clearer anatomical image, as well as better spatial resolution. This greatly improves the raw images used for sMRI analysis and allows for conducting more reliable studies. Second, this thesis examined the impact of additional individual variables that were not previously examined. The specific rationales and hypotheses are as follows:

Rationale for Hypothesis 1

There is clear evidence that at least some alterations in central processing of visceral afferent input influence the symptomology of IBS. Still, the results thus far have been inconsistent. Functional imaging studies have reported different results in terms of
activations, deactivation, or complete lack of the activations during rectal distention paradigms in IBS patients. More specifically, there have been inconsistencies in regards to how key pain processing areas operate in such a case. Furthermore, the recent rise in structural imaging of a variety of chronic pain disorders also provides evidence suggesting abnormal central anatomical substrates for a variety of chronic pain disorders such as chronic low back pain, fibromyalgia and migraine. Here too exist inconsistencies, but key pain and emotional processing areas have been shown to be affected in the majority of studies. Thus the rationale for this hypothesis is to find the anatomical substrate for the previous functional imaging studies on IBS (Specifically Kwan et al., 2005b), as well as determining how different IBS patients may be structurally different from other chronic pain disorder patients.

**Hypothesis 1:** Cortical thickness in key structures that are related to pain perception (S1/S2), affective and interoceptive processing (ACC/MCC, IC) and pain modulation (ACC/MCC, DLPFC) are abnormal in IBS patients compared to controls.

**Rationale for Hypothesis 2**

The relationship between stress and the symptoms of IBS has become fairly consolidated. The HPA axis is an important neuroendocrine pathway which controls the stress response and is likely to have major effects on normal gut regulation and psychological responses.
to pain. The integrity of the hypothalamus may thus have an impact on the symptoms experienced in IBS patients.

**Hypothesis 2:** Sites within the HPA axis are altered in IBS patients, compared to controls. Specifically, the hypothalamus is enlarged in size in IBS patients, due to the relationship with stress and the impact that it has on their symptoms.

**Rationale for Hypothesis 3**

Personality variables, such as pain catastrophizing and neuroticism, have been associated with painful disorders and their outcomes. Additionally, they have also been shown to affect brain responses to pain in functional neuroimaging studies. It is thus viable to determine if such personality variables can have an effect on the neuroanatomy of patients.

**Hypothesis 3:** Within IBS patients, pain catastrophizing will negatively correlate with cortical thickness in brain areas that are related to descending pain modulation, namely the DLPFC. This may indicate a structural abnormality that hinders the coping of pain in the catastrophizing patients. Furthermore, neuroticism will be correlated with cortical thickness in areas that are related to emotion and psychological well-being.
Rationale for Hypothesis 4

IBS patients are heterogeneous in terms of pain severity and duration. Such pain characteristics are important clinical features that may have important consequences for effective treatment regimes. It is thus important to study the effects such variables may have on gray matter changes because such results may shed some light regarding the question of cause versus effect, i.e – if there is a pre-existing vulnerability or the changes are in fact due to extraneous factors.

Hypothesis 4: Severity and duration of IBS related pain will correlate with cortical thickness in areas that are related to interoception and affective processing of painful stimuli (IC, MCC/ACC).
4. METHODS

4.1 Subjects

A healthy control sample was recruited by advertised postings throughout the University Health Network in Toronto, Canada. The healthy volunteers were screened via telephone for information such as date of birth, level of education and occupation. In addition, all controls were screened for exclusion factors, including any history of chronic pain, psychiatric illness, major surgery and medication, among others (see appendix for telephone screening form). Inclusion criteria required that the subjects be female, right handed, and between the ages of 18 and 55. Moreover, subjects needed to be fluent in English. Out of 30 healthy controls screened, 21 met inclusion and exclusion criteria and were scanned and 16 were used in the final analysis. Five of the subjects were not used due to a scanning acquisition problem brought upon by the screening goggles.

In parallel with the recruitment of the controls, IBS patients were recruited from the gastrointestinal unit of the Toronto Western Hospital, as well as from an affiliated clinic (The Brunswick Clinic), both of which are located in downtown Toronto. Inclusion criteria required that the patients also be female, right handed and between the ages of 18 and 55. The patients suffered from IBS in accordance with the Rome III criteria (for full details regarding the Rome Criteria see Drossman, 2006) and were additionally screened to rule out organic bowel diseases, other chronic pains and psychiatric conditions. Additional exclusion criteria required that the patients did not undergo any major surgery in the past 5 years, did not have a history of diabetes and did not possess any metal braces or implants in their body. During the course of patient
recruitment, 78 potential participants were screened, however only 12 fit the inclusion criteria and underwent the scanning protocol. The most common reasons for excluding a patient were due to not fitting in the age range, having other confounding medical conditions (psychiatric illness/diabetes/other chronic pains), and taking psychotropic medications. Due to additional information from one of the patients regarding a peripheral nerve injury only 11 patients were used in the final analysis.

4.2 Study Protocol

All subjects (both patients and healthy controls) were asked to attend a two hour and 15 minute session at the Toronto Western Hospital. The session consisted of collection of psychometric data and practicing a cognitive task (1 hour), 2) MRI (1 hour, 15 min). Prior to the experiment, all participants in the sessions were required to read and sign an informed consent form that was approved by the University Health Network Research Ethics board. Lastly, a monetary honorarium was provided for all participants in the study.

4.3 Questionnaires

Each participant was required to complete several questionnaires:

1) The Edinburgh Handedness Inventory - designed to classify the handedness of each person. The questions of the inventory focus on which hand is used for a variety of activities such as “writing”, “throwing”, “using a knife”, and so on (see http://www.brainmapping.org/shared/Edinburgh.php). The results of the questionnaire are
later scored and the degree of handedness is calculated based on a continuum ranging from -100 to 100. This test assured that the participants were all right handed.

2) **The Pain Catastrophizing Scale** - This questionnaire comprises 13 statements of thoughts and feelings that are concerning pain. The subject was asked to rate the degree to which s/he has these thoughts using a numerical scale of 0-4 (0 = not at all to 4 = all the time). Subjects were also instructed to consider their reactions to pain experiences in general, and not focus on one specific event or condition. A total score was calculated from the summed response to all questions. (Sullivan et al., 1995).

3) **NEO-FFI.** This test measures five personality facets, neuroticism, extraversion, openness to experience, agreeableness, and conscientiousness, and has been found to be universally reliable (Costa and McCrae, 1992; McCrae and Costa, 1997). It contains a list of 60 questions (12 per facet), and is self-administered. (NEO-FFI, Form S (Adult), Psychological Assessment Resources, Inc.).

4) **The McGill Pain Questionnaire (MPQ) (patients only)** – The MPQ presents 78 words that describe pain and sensation. For example, a sample of the words included are ‘pulsing’, ‘sore’, ‘stinging’ and ‘burning’, to name a few. Patients were instructed to choose any word that was applicable to their IBS pain. No limitations on the number of words chosen were imposed. The analysis included finding and reporting the most commonly chosen words by the majority of the IBS patients.
5) **Short Pain Assessment and Pain History (patients only)** - In addition to the questionnaires described above, the patient group was administered a short pain history (see appendix). The pain history was designed to get both a qualitative and quantitative perspective on the pain the patients’ experience. The survey asks questions such as “How long have you had IBS?” and “Describe how your pain feels”. In addition, questions regarding at what times of the day is the pain worse (morning, evening) and what triggers the pain were also included. The patients were also given the opportunity to add comments of their own and to freely speak about their experience with IBS. Quantitative information such as pain and unpleasantness ratings (scaled from 0-10, 0 = no pain/unpleasantness at all, 10 = most intense pain/unpleasantness imaginable) were also collected in order to find out what the severity of the pain is like in general and at the time of the session.

### 4.4 MRI Acquisition

Each participant was briefly interviewed by the MRI technician for safety reasons before entering the scanner. Each participant was then asked to lie supine inside the scanner and given a panic button to be held in their left hand. A vitamin E pill was taped to the right temple of each participant in order to easily distinguish between the right and left sides of the brain during data processing. Once the subjects were inside the scanner, they were given instructions through a microphone in the control room. The scanning protocol included a battery of different scanning sequences in a specified order which consisted of: 1) a low resolution anatomical scan, 2) functional resting state scan, 3) fMRI scans that were accompanied by a cognitive ‘stroop’ task, 4) a high-resolution
anatomical scan, and 5) 2 diffusion tensor imaging scans. Only the fourth scan was used for the analysis of this thesis and the other scans were used for other projects not discussed here.

During scanning, subjects were asked to keep their eyes closed for all of the scans except the fMRI session, as visual attention was needed for the cognitive task. The cognitive task was streamed through a computer in the control room to MR-compatible goggles that were fitted onto the subject prior to scanning. In order to reduce inhomogeneous confounds in the images, the goggles were removed before the last two scans (high resolution anatomical and DTI).

Anatomical MR image acquisition was obtained from a 3 Tesla MRI system with an 8 channel head-coil (GE medical systems, Milwaukee, WI, U.S.A). A T1 weighted 3D FSPGR sequence (TE=5ms, TR=12ms, flip angle = 45 degrees) was employed to generate 128 axial slices, each with a thickness of 1.5mm (256 x 256 matrix size, field of view = 24 x 24 cm). During the anatomical scanning, subjects were instructed to remain still and keep calm so as to not confound the images.

4.5 Statistical Analysis

Voxel-based morphometry was conducted in SPM5 using the VBM5 toolbox. Each subject's high resolution, T1-weighted anatomical scan was normalized to the International Consortium for Brain Mapping (ICBM) template (included in the software package) and was then segmented using the unified segmentation algorithm in SPM5. Segmented gray matter images underwent Jacobian modulation to adjust for the effects of spatial normalization. A smoothing kernel of 10 mm FWHM was used and an absolute
A threshold mask of 0.20 was applied to avoid partial volume effects and also restrict the results to gray matter only. Each subject's final gray matter image was then entered into an independent two sample t-test between patients and controls, with TIV entered as a covariate. In addition, a mask of the hypothalamus was used in order to test hypothesis 2 (Wake Forest University Pick Atlas http://fmri.wfubmc.edu/cms/software). The significance threshold was set at 0.05, False Discovery Rate corrected.

For the cortical thickness analysis, all scans were normalized to a Talairach template and were then segmented in order to identify the boundaries between the gray- and white matter. Inner and outer cortical surfaces were generated from these boundaries by Freesurfer and once created; the distance between them could be calculated. Each subject's surface map of thickness values was aligned to Freesurfer's default average surface map according to cortical folding pattern and data smoothed in the common surface space. Here, a 6 mm smoothing kernel was used. Significance was set at 0.05, corrected for multiple comparisons. For the group comparison, a two sample independent t-test was conducted with a mask encompassing the bilateral anterior insula, postcental gyri and cingulate region (figure 1). Using the same mask, a within-group correlation was conducted between cortical thickness and pain duration (peak vertex reported). In order to focus on two particular brain areas of interest, an a priori region of interest (ROI) analysis of the right DLPFC and left medial orbitofrontal cortex was conducted in Freesurfer by extracting mean values of cortical thickness which were then used in external correlation analyses in SPSS (SPSS Inc., Chicago).
Figure 1 - Mask for CTA
A mask consisting of S1 (post-central gyrus and sulcus), anterior IC, and cingulate region (consisting of the aMCC, pACC, and sgACC). ROIs for this mask were constructed using the built-in Freesurfer parcellation atlas.
5. RESULTS

5.1 Demographics and Pain Characteristics

All subjects who participated in the study (including both patients and controls) were confirmed to be right hand dominant by the Edinburgh handedness test. According to a two-tailed, independent sample t-test, no significant age differences were apparent between the two groups (IBS mean 30.2, standard deviation ± 8.45; Controls mean 31.5 ± 9.4).

The IBS patient group included 9 patients with mixed constipation-diarrhea type and 2 patients with constipation-dominant IBS. IBS duration varied from 2-20 years with an average duration of 9.1 ± 6.3 years. The patients reported a range of pain severity and unpleasantness from 4-10, average pain severity was 7 ± 1.8 (on a 10 point scale), and average unpleasantness rating of 7.3 ± 1.6 (on a 10 point scale, See Table 1). In the IBS group there were significant correlations between pain duration and neuroticism (r = 0.66, p = 0.02), as well as between pain rating and neuroticism (r = -0.70, p = 0.01). Additionally, a correlation of 0.57 was observed between the ratings of pain severity and pain unpleasantness, but surprisingly, this correlation did not reach significance, although there was a trend (p = 0.06). No significant correlations were found between pain duration and pain rating (r = -0.33, p = 0.31), or between catastrophizing and pain rating or pain duration (r = -0.07, p = 0.8 and r = 0.33, p = 0.32, respectively).

The MPQ analysis (see Table 2) showed that the most common word that was reported by patients with regards to their pain was ‘cramping’. This word was mentioned by 100% of the patients (11/11). Moreover, words such as ‘aching’, ‘sharp’, and ‘hurting’
were also high frequency words. The appearance of these words as the most common is understandable, since they all fit as good descriptors of abdominal pain.
Table 1 - Individual patient characteristics

Individual characteristics of all 11 IBS patients used in the final analysis. As can be seen, 9 of the 11 patients suffered from the mixed-type classification of IBS, while only 2 suffered from IBS-C. None reported being IBS-D. Additionally, this table shows the heterogeneity of the patients in terms of the duration, severity, unpleasantness and frequency.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Type</th>
<th>Duration (yr)</th>
<th>Pain Severity</th>
<th>Unpleasantness</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>42</td>
<td>mixed</td>
<td>10</td>
<td>7</td>
<td>7</td>
<td>2.3/day</td>
</tr>
<tr>
<td>2</td>
<td>24</td>
<td>constipation</td>
<td>12</td>
<td>10</td>
<td>10</td>
<td>2+/day</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>mixed</td>
<td>6</td>
<td>8.5</td>
<td>8.5</td>
<td>1.2/day</td>
</tr>
<tr>
<td>4</td>
<td>29</td>
<td>mixed</td>
<td>20</td>
<td>4</td>
<td>8</td>
<td>1/wk</td>
</tr>
<tr>
<td>5</td>
<td>28</td>
<td>mixed</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>1+/day</td>
</tr>
<tr>
<td>6</td>
<td>42</td>
<td>mixed</td>
<td>7</td>
<td>8.5</td>
<td>6</td>
<td>1/mth</td>
</tr>
<tr>
<td>7</td>
<td>20</td>
<td>constipation</td>
<td>5</td>
<td>8</td>
<td>8.5</td>
<td>1+/day</td>
</tr>
<tr>
<td>8</td>
<td>22</td>
<td>mixed</td>
<td>12</td>
<td>7</td>
<td>8</td>
<td>3/wk</td>
</tr>
<tr>
<td>9</td>
<td>44</td>
<td>mixed</td>
<td>2</td>
<td>8.5</td>
<td>8</td>
<td>2-3/wk</td>
</tr>
<tr>
<td>10</td>
<td>26</td>
<td>mixed</td>
<td>20</td>
<td>5.5</td>
<td>5.5</td>
<td>1/day</td>
</tr>
<tr>
<td>11</td>
<td>27</td>
<td>mixed</td>
<td>4</td>
<td>5</td>
<td>7</td>
<td>1+/day</td>
</tr>
</tbody>
</table>
Table 2 – Frequent MPQ words

Most frequent words that were chosen by the 11 IBS patients completing the McGill Pain Questionnaire. ‘Cramping’ was the only word that was consistently chosen by all (100%) of the patients. ‘Aching’, ‘Sharp’ and ‘Hurting’ were also chosen by a large portion of the patients (82%, 73% and 73%, respectively).

<table>
<thead>
<tr>
<th>Word</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cramping</td>
<td>11/11 (100%)</td>
</tr>
<tr>
<td>Aching</td>
<td>9/11 (82%)</td>
</tr>
<tr>
<td>Sharp</td>
<td>8/11 (73%)</td>
</tr>
<tr>
<td>Hurting</td>
<td>8/11 (73%)</td>
</tr>
<tr>
<td>Tender</td>
<td>6/11 (55%)</td>
</tr>
<tr>
<td>Stabbing</td>
<td>6/11 (55%)</td>
</tr>
<tr>
<td>Miserable</td>
<td>6/11 (55%)</td>
</tr>
<tr>
<td>Throbbing</td>
<td>6/11 (55%)</td>
</tr>
<tr>
<td>Annoying</td>
<td>6/11 (55%)</td>
</tr>
<tr>
<td>Intense</td>
<td>6/11 (55%)</td>
</tr>
</tbody>
</table>
5.2 Personality

Both the patient and healthy control groups included subjects scoring over the range of PCS and N scores. Although the mean PCS score of the IBS group (IBS 20.7 ±12.7 was higher than the control group (17.9 ± 9), this difference was not statistically significant (two-tailed, independent sample t-test, p = 0.54). In regards to Neuroticism, the two groups showed similar scores (IBS 19.6 ±6.3; Controls 18.6 ±6.0), and a two-tailed, independent sample t-tests revealed no significant statistical difference (p = 0.68).

5.3 Qualitative Patient Observations

Due to the psycho-sociological effect on IBS, it is also worthwhile to report some of the remarks made by IBS patients during the pre-scan pain assessment. This kind of qualitative assessment provides us with a glimpse of how this condition can impact on the lives of the patients. For example, patient 02 revealed that her pain “can make her feel like she wants to stay in bed” and that her IBS “grabs her attention and prevents her from daily, demanding tasks”. Patient 06 remarked that “at some points, it feels like my colon is going to explode”. Patient 11 said that the pain can range from “excruciating to simple stomach aches”. Conversely, some patients remarked that although the symptoms are still there, they have been controlled quite well, and do not bother them as much. For example, patient 08 said that she “learned how to cope and control” the symptoms. Ten out of the 11 patients reported that “stress” was a major trigger for their IBS symptoms, confirming the relationship between stress and GI symptoms. Some of the “stress” reported including such events as exams, exercise and arguments, however most patients
just referred to a general feeling of stress. Additionally, a majority of the patients reported that diet can be a factor as well, citing greasy, spicy and new foods as the top triggers. Moreover, in some cases, dairy, wheat and gluten was reported to be an additional factor in propagating the symptoms. The patient sample consisted of 9 mixed-type IBS sufferers, and only 2 constipation type, IBS-C, sufferers. None of the patients were classified as IBS-D.

An additional measure of the qualitative experience of pain in the sample of IBS patients are the results of the MPQ (see Table 2). Unsurprisingly, most of the words that were found to be chosen most frequently are in line with abdominal pain symptoms. For example, the word ‘cramping’ was chosen by all of the patients and is indeed a feeling that seems to be commonly reported in IBS patients.

5.4 Hypothesis 1 results

The first hypothesis tested whether there were cortical thickness abnormalities in structures that are related to pain perception (S1/S2), affective and interoceptive processing (ACC/MCC, IC) and pain modulation (ACC/MCC, DLPFC) in IBS patients compared to controls. CTA revealed cortical thinning in the anterior MCC (p = 0.03, corrected) compared to the control group (figure 2). Mean thickness for controls was 3.14 ± 0.40 mm, while the mean thickness for patients was 2.63 ± 0.42 mm. This constitutes a 16.2% reduction in cortical thickness in the IBS patients compared to controls. No statistically significant differences were found in S1, or the anterior IC region, counter to the hypothesis.
5.5 Hypothesis 2 results

The second hypothesis tested whether the hypothalamus was enlarged in size in IBS patients. For this analysis, we used VBM to test whether the hypothalamus ROI was increased or decreased in size in the IBS group compared to controls. No significant differences were found between the contrast of Controls > IBS. However, a significant finding occurred when the contrast was IBS > Controls (p < 0.05 FDR). Namely, increased gray matter density in the area of the hypothalamus was found. Figure 3 shows the location of this abnormality in the hypothalamus located at -2, 0, -11 (x, y, z).

5.6 Hypothesis 3 results

The third hypothesis tested whether pain catastrophizing in IBS patients negatively correlates with cortical thickness in brain areas that are related to descending pain modulation, namely the DLPFC and whether neuroticism correlates with cortical thickness in areas that are related to emotion and psychological well-being. The ROI analysis of the DLPFC resulted in a strong (r = -0.66; p=0.015) negative correlation between DLPFC and PCS, accounting for age (see figure 4). The control group showed an opposite trend (r = 0.41, p = 0.15, not significant). In addition, the ROI analysis of the medial orbitofrontal cortex revealed a negative correlation of r = -0.64, p=0.043 (figure 5) with neuroticism, accounting for age. The control group revealed a weak correlation of r = -0.22, p = 0.41. It is important to note however, that patient 10 may be driving this correlation as she had a high neuroticism score. If this patient is excluded from the
analysis, the correlation is not statistically significant ($r = 0.02$ $p = 0.94$). Thus, the confidence of this finding is weaker than the other ones.

5.7 Hypothesis 4 results

The fourth hypothesis tested whether severity and duration of IBS-related pain correlates with cortical thickness in brain areas that are related to interoception and affective processing of painful stimuli (IC, MCC/ACC). Extracted cortical thickness values from anterior IC showed that a correlation with pain duration was $r = 0.85$ ($p = 0.001$; figure 6). When age was accounted for however, the $r$ value dropped to 0.78 ($p=0.003$).
Cortical thinning in the aMCC of IBS patients

A two-sample t-test showed that IBS patients present with cortical thinning in the aMCC (Talairach coordinates at 12, 12, 33; red region), when compared to healthy controls ($p < 0.05$, corrected). Extracted cortical thickness values clearly show that the difference is approximately 0.5 mm in thickness (right side of figure).
Figure 3 - Increased gray matter density in hypothalamus of IBS patients
VBM results (under a ‘hypothalamus’ mask) showed increased gray matter density of the hypothalamus (TAL: -2, 0, -11) in IBS patients, when compared to healthy controls (p < 0.05, FDR).
Figure 4 - Correlation with DLPFC and PCS scores

Negative correlation between cortical thickness values from the right DLPFC and the PCS scores of both the patients (red) and controls (blue). A strong, $r = -0.64$ ($p = 0.03$), correlation was observed with the patients (shown in this graph). In addition, when age was entered as a covariate of no interest, the correlation strengthened to $r = -0.67$. The control group displayed a positive correlation of $r = 0.41$, but was not significant ($p = 0.15$). The left portion of the figure is the hand made ROI of the DLPFC, based on the built in Freesurfer atlas. Each symbol in the graph represents an individual patient or control.
Figure 5 - Correlation with medial orbitofrontal cortex and Neuroticism

Negative correlation between cortical thickness values from the left medial orbitofrontal region and neuroticism scores of both patients and controls. A negative correlation of $r = -0.58$ was observed between the neuroticism scores of the patients and cortical thickness, however this correlation was borderline significant ($p = 0.053$; shown in this graph). When age was taken into account however, a fairly strong, $r = -0.64$, $p = 0.043$, correlation was observed. The control group displayed a weak, non-significant, negative correlation of $r = -0.22$, $p = 0.41$. The left portion of the figure is an ROI of the medial orbitofrontal region. Each symbol in the graph represents an individual patient or control.
Figure 6 - Positive correlation with anterior IC and pain duration
Positive correlation between cortical thickness of the anterior IC and pain duration of IBS patients in years. Extracted values showed that the correlation was $r = 0.85 \ p = 0.003$ (as shown in the graph). Once accounting for age, the correlation became slightly weaker at $r = 0.78$. 
6. DISCUSSION

There are three main findings of this thesis: 1) Compared to healthy controls, IBS patients have increased gray matter density in the hypothalamus and cortical thinning in the aMCC. 2) IBS patients show a positive correlation between pain duration and anterior IC thickness. 3) IBS patients show a negative correlation between PCS and DLPFC thickness. As predicted by the apriori hypotheses, the brain regions found to be abnormal in the IBS patients are mostly implicated in relation to pain, stress and attention/emotion and are thus possible contenders for the development and maintenance of IBS.

6.1 Increased hypothalamic density in IBS

Is an altered hypothalamus related to the enhanced stress response in IBS?

The relationship between stress and colonic function is inherent to many of us. Stressful or uncomfortable situations can manifest as GI distress and have led to the coinage of such sayings as “butterflies in the stomach” (Knowles and Aziz, 2009). Indeed, psychological stressors can affect gastrointestinal symptoms in people who do not suffer from chronic symptoms (i.e. healthy volunteers). This has been experimentally shown in studies that have used stressful tasks to observe what effect they have on colonic motility (Mertz, 2002). For example, one study found that physical and psychological stress enhanced colonic motor activity. Interestingly, the psychological stress seemed to have a more adverse and prolonged effect (Rao et al., 1998). Jones et al. (2006) describes the hypothalamus as part of the limbic system – and as an integral part...
of the ‘visceral’ brain. Moreover, Mayer et al., (2001) proceed to categorize the hypothalamus as part of a greater ‘emotional nervous system’, a highly interconnected system with outputs to higher and lower CNS structures that are modulated by psychosocial and physical stressors. Abnormalities in this system can lead to physiological pathologies in the GI system, but also changes of vigilance, attention and emotion (Mayer et al., 2001). In particular, the hypothalamus is the initiator of the HPA axis, a neuro-endocrine pathway that is chiefly concerned with the stress response, releasing CRF and cortisol into the circulatory system. The HPA axis is one of the main pathways of communication between the brain and the gut (Jones et al., 2006), and so the hypothalamus is an integral structure for the healthy function of the gut. The enhancement of this structure found here in the IBS group suggests that the HPA axis is altered, and this could cause an imbalance in homeostasis that may result in the symptoms that characterize IBS. Other imaging studies in chronic pain patients have found abnormalities within the hypothalamus and surrounding structures. For example, May et al. (1999) reported an increase in gray matter density of the bilateral hypothalamus in patients suffering from idiopathic headache syndrome. Interestingly, this area was also functionally activated during an acute headache attack, in the same group of patients, supporting the notion that altered structure may lead to altered function (or the opposite relationship). Although headache syndromes are dissimilar to IBS, the two conditions share a largely misunderstood pathogenesis that may be associated with CNS dysfunction and the stress response. This alteration in the hypothalamus of IBS patients may impact a wide variety of bodily functions, including the emergence of pain and may
even be a tentative biological marker for IBS, and possibly also a target to consider for treatment.

The possible role of microglia

Although the understanding of pathological pain has mostly revolved around neuronal mechanisms, astrocytes and microglia are now thought to have a significant role to play in pain processing (Milligan and Watkins, 2009; Tsuda et al., 2005). Glia can be involved in the maintenance and creation of a variety of pathological pain conditions and can create hyper-excitability of nociceptive neurons and increase the concentration of other substances involved in the pain response, such as prostaglandins, nitric oxide, and others (Scholz and Woolf, 2007). In rodents, chronic stress has been shown to lead to microglial proliferation in areas surrounding the third ventricle, particularly the thalamus, hypothalamus, hippocampus and substantia nigra (Sugama et al., 2007). Although little is known of such mechanisms in the brain, a recent study by Zhou et al (2008) found that microglia activated in the thalamus influenced pain processing after a spinal cord injury. Recently, Bartley (2009) hypothesized that glial cell activation (and the cascades which are initiated thereafter) may be in part responsible for the hypersensitive state found in migraine. Thus, although highly speculative theory in nature, it could be that the increase of gray matter density in the hypothalamus of IBS patients may be due to a stress induced enhancement of microglia that in turn, can lead to the maintenance of the visceral pain experienced by these patients.
6.2 aMCC thinning in IBS

The cortical thickness analysis revealed that IBS patients had cortical thinning in the aMCC. This result concurs with the previous IBS sMRI study published by the Davis lab (Davis et al., 2008), and hence, supports the a priori hypothesis of this thesis. Moreover, although the current study used different analysis software than the previous study (i.e., CTA implemented in BrainVoyager versus CTA in Freesurfer) and derived from a second sample of IBS patients, roughly the same area was found to be reduced in IBS patients when compared to controls, increasing the confidence of the finding. Furthermore, cortical thinning in the cingulate cortex is consistent with functional imaging studies that reported an attenuated response to rectal distension in IBS patients compared to healthy controls (or complete lack thereof) (Kwan et al., 2005b; Silverman et al., 1997). Therefore, cortical thinning in the aMCC may represent a neuroanatomical substrate for at least part of the functional attenuation. However, the complex functions of the aMCC gives rise to several possible interpretation of this finding:

Interpretation 1 – Altered descending modulation of pain

Several supraspinal areas can directly, and indirectly, influence the nociceptive processing that occurs in the dorsal horn via a descending pain modulation system (Millan, 2002). The system is believed to include the PFC and ACC/MCC working together with subcortical structures such as the hypothalamus and PAG. When needed, this system modulates nociceptive information at the level of the dorsal horn, and thus controls the amount of pain we perceive (Wiech et al., 2008). The pain modulation system has also been associated with endogenous opioid mechanisms. For example,
studies of post-mortem brain tissue in humans and non-human primates have found high concentrations of opiate receptors in the PAG, medial nuclei of the thalamus, cingulate and PFC (Pfeiffer et al., 1982; Wamsley et al., 1982). In addition, PET imaging studies have reported opiate receptor binding in these brain areas (Jones et al., 1991b). The down regulation of opioid mechanisms in such areas may be especially evident in chronic pain patients. For example, a recent PET study of fibromyalgia patients found reduced binding potential of an opiate analogue in the MCC, indicating altered endogenous opiate activity (Harris et al., 2007). Cerebral decreases in opiate receptor binding were also found in patients with central post-stroke pain and central neuropathic pain (Willoch et al., 2004; Jones et al., 2004). Additional evidence implicating the aMCC as an integral part of the descending modulation system comes from the hypnosis/placebo analgesia literature, as this area is often activated during such states (Kupers et al., 2005). Therefore, cortical thinning of the aMCC in IBS patients might be due to a loss in neurons, or a loss of opiate receptors, leading to an impairment of the pain inhibition that the descending modulation system and endogenous pain processes exert. This may be due to the perpetuation of symptoms in various chronic pain conditions, and in this case, in IBS.

**Interpretation 2 – Alteration of attentional resources**

The modulation of pain need not be an isolated response, but may be influenced by cognitive and psychological factors as well. Attention is one such construct which allocates resources to stimuli that are relevant to the organism. By doing so, it can either amplify or extinguish unwanted stimuli (Corbetta and Shulman, 2002). Pain is inherently an attention-demanding perception that is biologically salient (Melzack, 1999). Pain has
been found to be modulated by attention; focusing on pain increases pain perceived, while focusing away from pain increases pain thresholds and tolerance (Levine et al., 1982; Bushnell et al., 1985). As discussed earlier, the aMCC region has been regarded as being part of the cognitive subdivision of the ACC (Bush et al., 2000). Several studies have associated the aMCC with distraction and attention (Downar et al., 2000; Davis et al., 2000; Davis et al., 1997; Coen et al., 2008). It is plausible that an abnormality in the aMCC may cause an inability to cognitively disengage from pain, leading the individual to focus more on the nociceptive activity, and eventually propagating the emergence of a chronic pain condition. This interpretation fits well with the relative success of the non-pharmacological treatments (such as CBT) for relieving the pain associated with IBS. To our knowledge, none of the patients in the current study were undergoing any CBT form of therapy, and it is thus possible that they cannot divert their attention away from their own bodily symptoms. The aMCC finding found here then may be the neuroanatomical explanation for this ‘reduced resource’ of modulation through attention.

6.3 Positive correlation between anterior IC thickness and IBS pain duration

As stated before, the anterior IC is implicated in a variety of different roles such as pain perception, emotional salience and visceral (and whole body) integration (Davis et al, 1999; Taylor et al., 2008; Craig et al., 2009). The result of a positive correlation between pain duration and the thickness of a portion of the anterior IC in IBS patients can be viewed in many ways. The most likely explanation is continual nociceptive inputs and persistent pain over many years leads to an enhancement of gray matter (whether it be
neurons, glial matter, etc.) that translates to a measurable change in cortical thickness. This theory is supported by the finding of increased gray matter following repetitive stimulation. For instance, a recent VBM study by Teutsch et al., (2008) found that repetitive painful stimulation administered over a number of days could cause significant changes in gray matter in the aMCC and somatosensory cortex (albeit not in the IC as was found in this thesis). The major caveat with the present finding is apparent when comparing it to the previous IBS sMRI study. The correlation between cortical thickening and pain duration in the anterior IC of IBS patients suggests that the patients who have had IBS for longer are actually approaching thickness measures that are similar to the healthy controls in the previous sMRI IBS study (Davis et al., 2008). This is indeed a puzzling notion, but can possibly be explained by the fact that perhaps the IBS patients who have been suffering from symptoms for the longest time have adapted to their symptoms and condition, and thus may have physiologically adjusted over time to the constant pain.

6.4 Negative correlation between PCS and DLPFC thickness in IBS

A main finding in this thesis was the negative correlation between DLPFC gray matter and the patient PCS scores. That is, high pain catastrophizing was associated with a thinner DLPFC. Catastrophizing is related to the coping mechanisms of pain, and is also associated with how well a patient will recover. It is plausible that patients with IBS, who are more catastrophic in their thoughts about pain, might have a more difficult time inhibiting their pain. Thus, this negative correlation may be a neuroanatomical substrate
of the poor pain control which manifests as the chronicity of pain. This is an interesting finding that links the catastrophizing variable with a neuroanatomical area. Moreover, the exact opposite trend was found in the healthy control group (positive correlation trend). Thus, it could be that in healthy controls, the positive correlation indicates a healthy system that is capable of controlling and inhibiting pain, while the converse occurs in IBS.

Consistent with this result, was the reported reduction in gray matter density in the DLPFC region in chronic low back pain patients (Apkarian et al., 2004). Furthermore, the finding in this thesis fits well with previous evidence from our lab. For example, Seminowicz and Davis (2006) found that during moderate pain stimulation, healthy controls showed a negative correlation between pain responses in the DLPFC and PCS scores. In contrast, this area did not show any response related to PCS during milder forms of pain. It is possible then, that in pain catastrophizers, the attenuated response to noxious stimulation in this area ‘allowed’ for the heightened sensation of pain and was due to a down-regulation of the top-down pain inhibitory influences. Indeed, the DLPFC is an area that is thought to be integral for the modulation of pain, however it does not work alone, but rather influences other cortical and subcortical areas to modulate pain (see section 2.4). For example, one fMRI study found that activity in the right DLPFC was positively correlated to activity in the midbrain, where the PAG resides (Wager et al., 2004). The DLPFC has also been associated with endogenous opioid mechanisms, much like the MCC/ACC, and thus contains the neurochemical ‘requirement’ to be part of a pain inhibition system.
In sum, the negative correlation between PCS and cortical thickness in the DLPFC in IBS suggests that the tendency to catastrophize about pain may predict the development of chronic pain, via a brain area that is related to heightened pain vigilance through a dysfunction with the recruitment of descending modulatory effects.

6.5 Negative correlation between neuroticism and medial orbitofrontal cortex

It has been suggested that the structural integrity of the orbitofrontal cortex is important for normal emotional appraisal (Rolls et al., 1994). Additional findings of atrophy/thinning of the orbitofrontal cortex in emotion impaired diseased states such as obsessive compulsive disorder (Pujol et al., 2004), schizophrenia (Kuperberg et al., 2003), and major depressive disorder (Lacerda et al., 2004), strengthen the relationship between this area and emotional capacity. Previous studies examining the relation of neuroanatomy to personality have found interesting correlations between gray matter and personality traits. For example, one group looking at both young adults (both female and male) found that neuroticism was found to correlate negatively with orbitofrontal cortex thickness (Wright et al., 2006). A similar negative correlation was found for both healthy controls and patients in this study; however it only reaches significance in the IBS population. As noted earlier, neuroticism seems to predispose people to display excessive sensitivity to negative cues, and cause excess worry. The negative correlation between neuroticism and cortical thickness of this area may thus point to emotional dysfunction brought upon by high neuroticism. It may be that the neuroticism experienced by IBS patients is highly driven by visceral specific anxiety and worry about their condition, and
thus creates a more robust relationship between cortical thickness and personality. It is important to note that this result is significantly driven by an outlier of one of the patients (namely, a vastly higher neuroticism score compared to the others), and thus any interpretation of the finding must be cautiously considered.

6.6 An integrated model of structural dysfunction

The results in this study likely fit with an overall integrated structural dysfunction. The brain is a highly interconnected organ, and consequently it is highly plausible that even anatomically distinct regions work together to process information and form perceptions. Structural alterations in some brain areas may lead to maladaptive behaviours, such as chronic pains. For example, the DLPFC and the aMCC are thought to be related, as the aMCC signals to the DLPFC when attentional signals are competing. It is then the DLPFC that allocates the resources to the appropriate signal (Botvinick et al., 2001; Kerns et al., 2004). It can very well be that in the current sample of patients, this network is structurally compromised, and thus a certain dominant attention signal, in this case being pain, is not efficiently dealt with. Interesting results from the placebo-analgesia literature may shed some light on the importance of these areas (as a network) on the modulation of pain. Craggs et al. (2007) found that when IBS patients were given a placebo treatment (during rectal distention), connectivity analysis revealed a network that included a functional connection between the DLPFC and the aMCC. This was interpreted as the activation of a cognitive-affective network that is responsible for moderating painful experiences and initiating the placebo-analgesic response (Craggs et al., 2007). A recent study specifically addressing visceral (esophageal) stimulation during
distraction found the aMCC and the right frontal cortex (probably including parts of the DLPFC) to be active during painful bouts (Coen et al., 2008). Other plausible functional networks can include the aMCC and the anterior IC, and the aMCC and the medial orbitofrontal cortex, although this has yet to be consolidated.

As discussed earlier, a recent DTI study by Hadjipavlou et al., (2006) found that there exists an anatomical connectivity between the PAG, hypothalamus and PFC (including the DLPFC). This anatomical evidence further suggests that perhaps a dysfunction in one area of the brain can lead to a structural alteration in another part of the brain. For example, it may be that a seemingly independent cortical thinning in the DLPFC or aMCC causes the hypothalamus to “over compensate” in order to re-achieve homeostasis.

6.7 What causes gray matter abnormalities detected by sMRI?

We do not currently know what causes the detected findings in sMRI studies. There are however, several possible factors that may contribute to changes in gray matter measurements. Possible factors include an increase or decrease in cell size, apoptosis of neural cells or neurogenesis, increased growth/death of glia and astrocytes, and alterations in dendritic spine and synaptic density (May, 2008).

The observation that environmental factors can affect the brain was proposed as early as 1966 when Rosenzweig found that rats subjected to environmentally complex settings were found to have heavier cerebral cortices and enlarged capillary diameters when compared to standard housing rats. Since then, other studies have attempted to replicate this result, albeit with more sophisticated methods. For example, using an
immunohistochemistry technique, Kempermann and others (1997) showed that mice living in an enriched environment, consisting of extra environmental stimuli and more food, had increased hippocampal astrocytes and neurons. In addition, this finding was accompanied by an increase in hippocampus volume in the enriched group (Kempermann et al., 1997). Consistent with this study, but more relevant to the topic of pain, Terada et al. (2008) studied how chronic pain can affect this enriched environment effect. Interestingly, mice with chronic pain due to litigated sciatic nerve which were housed in an enriched environment did not display the same kind of neural benefits (neuronal differentiation, migration) as the sham group that was also placed in the enriched environment. The authors suggest that chronic pain may stunt the effect of the enriched environment and negatively modulate neurogenesis, at least in the hippocampus.

To date, few studies have directly investigated the effect of chronic pain on neuronal morphology. One notable study investigated pyramidal neurons of the mPFC using brain slices from a rodent model of neuropathic pain. The results indicated that compared to controls, the neuropathic pain group had increased dendritic complexity and spine branch density in such neurons. This suggests that morphological changes are indeed associated with chronic pain, especially in areas that are believed to process painful responses. Still, these results do not necessarily mean that such morphological changes translate to increased gray matter, as the authors actually suggest that increased spine number may lead to increased calcium influx which can in turn even lead to glutamate toxicity and neuronal loss (Metz et al., 2009).
6.8 Gray matter changes in IBS: Cause or Effect?

An important question relating to the structural integrity of brain structures is whether they are a cause or a consequence of chronic pain. The debate is between one concept of the brain as ‘pre-wired’, either by genetic or congenital factors to cause some individuals to be more susceptible to chronic pain conditions or, a different concept that the brain can change due to external stimuli (such as repeated noxious stimuli). There is support for both of these concepts. In light of findings of structural differences due to personality traits which are seemingly supposed to be stable throughout the lifetime (Wright et al., 2006, Blankstein et al., 2009), it could be argued that functional chronic pains, such as IBS, may arise from a predisposition to hyper-vigilance of bodily symptoms (perhaps based on genetics, see Levy et al., 2006). The other, more peripheral, problems associated with IBS (and found by other studies, see section 2.1.2) may in fact be due to an initial brain abnormality (which has a significant role in the healthy development and proper regulation of the body). Thus, the speculation here is that the gray matter ‘changes’ we observe are not actually changes per se, but rather pre-existing anatomical abnormalities that cause, or at least lead to the emergence of the symptoms of IBS. The converse idea is that extraneous factors, such as pain and environmental forces are the true reasons for the changes observed in the gray matter, causing a form of maladaptive plasticity, much like the functional plasticity that is thought to cause the effects of phantom sensation and pain. This theory is supported by the correlation between the anterior IC and pain duration found in this study. The matter of fact is that we simply do not yet know the clear answer to this question. Only future research will allow us to fully comprehend the issue.
6.9 Negative Findings

The data obtained for this thesis did not support all of the proposed hypotheses. Namely, no anatomical changes were found in the somatosensory (S1 and S2) region, and no group differences were found in the IC. Several factors may have contributed to these results. Firstly, significant changes in these areas may have been too small to be detectable given the sample size of the patient group (see limitations). The second reason as to why this study did not find the exact same results as the previous sMRI study from our lab could be due to the heterogeneity of the patients in this sample group. As mentioned earlier, IBS exists in different forms (such as in different subtypes), and can have different causes. It could very well be that the results reported in this study are unique to at least, this sample, and it would thus be difficult to try to compare them to a different study with a varied sample of IBS sufferers. This disparity in results is interesting, as it does not sway away from the general trend of inconsistencies between functional imaging studies of IBS. It could be that IBS patients do have CNS abnormalities, however they cannot be generalized into one common ‘cerebral signature’, but rather a constellation of differing maladies that occur in different sub-sets of patients, but of which all lead to symptoms of functional GI disorders, such as IBS.

6.10 Limitations

The foremost limitation of this study is the relatively small sample size of patients collected during recruitment. Although many patients were screened, most did not fit the criteria, as efforts were made to include only subjects that met our inclusion and
exclusion criteria, while others did not agree to enter the study due to various reasons such as aversion to the scanner, scheduling conflict, or general disinterest. Nonetheless, the number of patients studied is in line with the average number of participants in many human imaging studies.

Interestingly, the majority (9 out of 11) of our patients suffered from the ‘mixed-type’ classification of IBS, meaning that their symptoms rotated between bouts of constipation and diarrhea. It is still unclear as to whether differences in subtypes of IBS result in differences in psychometrics or brain function. This is further hindered by the fact that most studies comparing IBS subtypes focus on the differences between the IBS-C and the IBS-D subtypes, and have largely ignored the mixed type classification. This is surprising, since in some cases, the prevalence of patients suffering from the alternating symptoms can be up to 46% of an IBS sample (Wilson et al., 2004). There is no doubt that IBS is a heterogeneous illness that can be caused by a variety of different reasons. Hence, a major caveat of this, and any other small size IBS study, is that it may not be possible to generalize the results to the whole clinical population. Another limitation to generalizing the results to the entire IBS population is that patients suffering from additional co-morbidities and/or undertaking medications were excluded from participating in the study. Nevertheless, the patients recruited from this study indicate a genuine clinical picture, and thus the results are meaningful for the current sample.

The existence of a clinically diagnosed psychiatric illness such as major depression disorder was ruled out in both IBS patients and controls through our exclusion criteria questionnaire which was conducted prior to the testing session. Still, a detailed psychological profile was not conducted. It is therefore impossible to know whether the
subjects in the present study had sub-clinical ailments (such as depression), or other confounding factors that would have affected the results. As stated earlier, IBS patients have been found to score high on anxiety and depression scales (Whitehead et al. 1990; Munakata et al. 1997; Verne et al. 2001). Structural abnormalities have been found to exist in a myriad of psychological and psychiatric disorders, as well as even in extreme personality variants (Wright et al., 2006; Blankstein et al., 2009). Thus, any sub-clinical ailment IBS patients of the present study may have had an adverse confounding impact on the findings.

A final important caveat of this study refers to its ‘incompleteness’ in terms of identifying what exactly causes IBS. It is important to consider that the findings of this thesis are ‘limited’ as that they only refer to the brain. As mentioned earlier, the BGA is a complex multi-level construct which includes the spinal cord, as well as the peripheral nervous system and the ENS. A dysfunction can be present at more than one level at a given time. Thus, the findings here are only a piece of the overall puzzle that is the complexity of IBS.

6.11 Future Directions

Since we do not fully understand the mechanisms underlying the changes observed in this sMRI study, additional studies are needed to validate the findings and to elucidate the exact mechanisms that cause said changes. For example, a controlled animal model would be a major step forward towards this goal. Innovative and creative models of IBS have already been used in some rodent studies, focusing mostly on rectal distention paradigms (Al Chaer et al., 2000). However, none of those have directly
investigated brain structures. Applying sMRI techniques to animals, while also using post mortem histochemistry analyses, may help determine the mechanisms that bring forth these structural changes. Furthermore, careful manipulations of study designs could possibly lead to potential treatment regimens. In humans, future sMRI studies should be conducted longitudinally to assess pre/post treatment the effects of different treatment options (pharmacological, CBT). An example of this study method was recently published by De Lange et al (2008) which found that after a long session of CBT, chronic fatigue syndrome patients exhibited a significant increase in PFC volume. Such studies allow us to determine whether these structural changes are malleable, and could also aid in the development of different treatment options. It would also be interesting to collect additional personality variables such as trait and state anxiety measures or a depression measure. Using such measures in the analysis may deepen our understanding of how the brain operates in chronic pain. IBS patients may benefit enormously from these and other study designs.

7. CONCLUSION

This thesis has supported the role of the supraspinal CNS sites in contributing to IBS symptoms. Specifically, the results implicated regions related to descending modulation, attention, and stress as being crucial in the pathogenesis of IBS. Nonetheless, the myriad of previous experimental studies cannot be ignored. These studies point towards other possible avenues of dysfunction, mainly peripheral and spinal mechanisms. The most complete explanation for the emergence of IBS is probably rooted in a
combination of dysfunction at several levels of the brain-gut axis. Still, psycho-social factors, such as psychological well-being and stress are undoubtedly extremely important for the development of the condition. It is these multi-level complexities that cause this condition to be so difficult to treat and understand. IBS is a challenging condition both for the patients suffering from it and for the clinicians treating it. There are many possible domains of dysfunction, and for that reason, any information we acquire will hopefully lead us to fully unravel the mechanisms of the condition.
8. REFERENCE LIST


Bookstein FL (2001) "Voxel-based morphometry" should not be used with imperfectly registered images. Neuroimage 14:1454-1462.


9. APPENDICES

Appendix 1 – Telephone screening form with exclusion criteria

Screener: JC / UB

Date of screen: _______________

Subject Code: _______________

Subject Name: _______________

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**IBS STUDY SCREENING QUESTIONNAIRE**

=============PERSONAL INFORMATION==============

Sex  M / F  Handedness  R / L  D.O.B  y_____/m_____/d_____

Phone # __________________________  Address: __________________________

Email: _______________  ____________________________________________

Occupation ______________________  Work Hours _______________________

Highest Level of Education ______________________

English as 1st language? __________

If not, at what age did you learn English? _____________________________

=============INCLUSION CRITERIA===============

Positive diagnosis for IBS by a GI specialist  Y   N

Name ___________________________  Date (how long?) _______________

**IBS Symptoms:** Constipation dominant / Diarrhea dominant / Mixed type

Comments: ______________________________________________________

=============EXCLUSION CRITERIA===============

History of bowel disease (other than IBS)  Y   N   __________

History of chronic pain (other than IBS)  Y   N   __________

Recent injuries or pain (other than IBS) ___________________________________

In the last 6 months? ____________________________________________

More than 3 months in the past year? __________________________________

History of fibromyalgia / TMD  Y   N   __________

History of diabetes  Y   N   __________
History of psychiatric illness prior to IBS diagnosis   Y   N   
Major surgery in the past 5 years   Y   N   
Other chronic illnesses   Y   N   
Medications (current/recent)   Y   N   

History of claustrophobia   Y   N   
Metal implants, braces, etc…   Y   N   
Pregnant   Y   N   

========================================ADDITIONAL INFORMATION========================================
Date of last menstruation               Oral contraceptives   Y   N
Have you recently seen a doctor / psychologist regarding a health problem? If yes, please specify type of problem, date and outcome:

Other health related information:
Do you have glasses/contacts? If so, what is your prescription?

Reminders for scanning:
Payment: $75
Must be analgesic-free for 24 hours prior to Scanning
Wear contact lenses if possible; if not, know your prescription.
Avoid wearing a bra with a metal underwire or metal parts on the straps, clothing with metal and metal hairclips.
Avoid drinking too much water or coffee before the scan.
Appendix 2 – Short IBS pain assessment

IBS PAIN ASSESSMENT

Date: ......../......./.......  
DD  MM  YY

Patient Name: ......................................................
Patient No.: ......................................................
Date of Birth: ......../......./.......  
DD  MM  YY

What is your highest level of Education:  
ES  HS  UNI  PS

Phase of the menstrual cycle  
1  2  3  4

What day did your last period start: ......../......./.......  
DD  MM  YY

Oral contraceptive  Y  N

1. How long have you had IBS? _________________________
2. Describe how your pain feels? _________________________
3. McGill Pain Questionnaire
4. Frequency: Constant-..............
   Occasional- ...................... per week
   ...................... per day

Pain History:
Worse in a.m.?  Y  N

Worse in p.m.?  Y  N

Responsive to analgesics?  Y  N

Current medication/treatment  Y  N  ___________________________
Other Pain Conditions?  

- Y  
- N  
Arthritis, fibromyalgia, IBS

Are there any triggers that make your pain better or worse (diet, stress, etc…)?

________________________________________________________________________

________________________________________________________________________

Are there daily fluctuations with your pain?

________________________________________________________________________

________________________________________________________________________

Sleep affected?  

- Y  
- N  
Prevention, disturbance, wake early a.m.

Do you have pain in regions in other parts of your body?

- Y  
- N  
Specify

Current Pain Rating: 

NPS 0 (no pain)-10 (most intense pain imaginable)

Intensity- ________

NPS (0 not unpleasant)-10 (most unpleasant pain imaginable)

Unpleasantness- ________

History of symptoms: Since onset, pain has improved, stayed the same, worsened?

- Has the nature of pain changed since onset?