The Modulation of Cerebral Pain Responses Using Desipramine in the Treatment of Irritable Bowel Syndrome (IBS)

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7.5.4: Research Plan
A. Statement of Hypotheses and Specific Aims

Irritable bowel syndrome (IBS) is a common abdominal pain disorder diagnosed by symptom-based criteria (Rome III). Since there are no objective measures available to establish a formal diagnosis, IBS likely encompasses a biologically heterogeneous group of patients with distinct pathophysiology. The majority of studies examining the mechanistic basis of IBS have focused on bowel-specific factors. Recent neuroimaging studies using functional magnetic resonance imaging (fMRI) have demonstrated abnormal brain activation patterns with noxious visceral stimulation in some, but not all, IBS patients. Regions comprising the homeostatic afferent processing network (HAPN), important to cognitive and affective interpretation of pain signals, demonstrate striking visceral pain response abnormalities in many IBS subjects. It is possible that HAPN pain responses have pathophysiologic relevance in a subset of IBS patients. We thus seek to characterize a putative IBS subgroup—those with abnormal HAPN visceral pain activations (IBS-HAPN).

A clearer understanding the role of the HAPN in IBS pathophysiology would be of substantial clinical importance on two accounts. First, centrally-acting antidepressant therapies, shown to influence the HAPN responses to visceral pain, are effective in only one-half of IBS subjects. The identification of objective features which predict IBS response to antidepressant therapy (i.e., HAPN pain responses patterns) would potentially allow for the development of more rational IBS treatment approaches. Second, abnormal HAPN pain activations may have broader implications in IBS pain experiences. One-third of IBS patients have comorbid somatization, a condition exemplified by multiple unexplained pain syndromes. It is possible that in IBS patients with somatization, the abnormal HAPN activations observed with visceral stimulation may also occur with non-visceral (somatic) noxious stimuli.

The overarching hypothesis of this proposal is that aberrant brain responses to visceral pain signals within the homeostatic afferent processing network (HAPN) represent the primary mechanism responsible for bowel pain experiences in a subset of IBS patients (IBS-HAPN). Our preliminary data has identified a pattern of brain activation differences within the HAPN following visceral stimulation in IBS subjects compared to healthy controls. Using fMRI, I will further characterize the HAPN pain responses of IBS-S subjects to additional non-visceral stimuli and following antidepressant therapy. I predict that this protocol will reveal a pattern of increased HAPN pain activations to both visceral and somatic stimuli—activations that are refractory to antidepressant pharmacotherapy. Ultimately these findings will demonstrate the mechanistic importance of the HAPN to pain experiences in the IBS-S subgroup.

These goals will be realized through the pursuit of the following Specific Aims:

Specific Aim 1. To compare HAPN activations following noxious somatic stimulation in IBS subjects based on the presence of somatization.
Hypothesis 1: As a result of a generalized abnormal pain response within the HAPN, IBS-S subjects will demonstrate greater HAPN activations with noxious somatic stimuli, compared to IBS subjects with no somatization and healthy controls.

Specific Aim 2. To compare the effects of antidepressant therapy HAPN activations following noxious visceral stimulation in IBS subjects based on the presence of somatization.
Hypothesis 2: Consistent with clinical refractoriness to antidepressant treatment, IBS-S subjects will demonstrate a persistent pattern of abnormal HAPN visceral activations following desipramine therapy, whereas significant improvements in HAPN visceral activations will be observed in IBS subjects with no somatization following antidepressant therapy.

B. Background and Significance.

1. Irritable bowel syndrome (IBS) is biologically heterogeneous, symptom-based diagnosis.

Irritable bowel syndrome (IBS) is recognized as a cluster of functional bowel symptoms, predominantly abdominal pain, in association with a change in bowel habits. 10-20% of US adults have IBS, and the diagnosis accounts for 20-50% of gastroenterology referrals.[4] IBS results in significant functional impairments, imposing a major impact on affected patients' assessments of their overall global health.[5] Without identifiable pathophysiologic findings upon which a diagnosis can be established, IBS remains a diagnosis based on clinical symptoms (Rome III criteria).[4] Over the past four decades, studies have examined multiple processes as potentially relevant to IBS pathophysiology, including gut inflammation, bowel motility, and visceral hypersensitivity. While many of these putative abnormalities have been found to be more prevalent in IBS compared to control populations, each measure likewise been insufficient in explaining IBS in all patients.

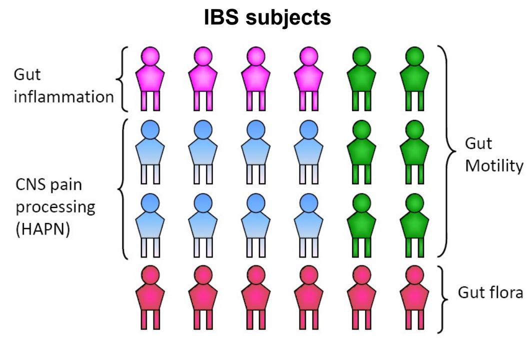


Figure 1. The biological heterogeneity of irritable bowel syndrome (IBS). Several unique mechanisms have been proposed as relevant to IBS pathophysiology. However, no single mechanism has been sufficient to explain IBS symptoms in all patients. Given that IBS is a symptom-based diagnosis, it is likely the result of a multiple of underlying mechanisms, emphasizing the importance of identifying pathophysiologically relevant IBS subgroups.

When studied as a syndrome—a collection of nonspecific symptoms—IBS almost certainly represents a very heterogeneous condition with a multitude of underlying pathophysiologic mechanisms (Figure 1).[4] It is likely that many of the previously proposed mechanisms underlying IBS are relevant in a subset of IBS patients. To date, however, the majority of clinical and physiologic studies of IBS have examined IBS subjects as a single group. The notion that important IBS subgroups likely exist has been largely disregarded in previous IBS investigations, and efforts to identify clinically- or physiologically-relevant subsets of IBS patients have been few. As a consequence, the biological basis for IBS remains largely unknown. *Establishing mechanistically-relevant IBS subgroups will be paramount to furthering our understanding IBS pathogenesis.*

2. Somatization is an important clinical feature in IBS, and portends greater IBS morbidity.

Somatization is a condition defined by the expression of pain syndromes across multiple organ systems (bowel, musculoskeletal, etc.) in the absence of any identifiable physical abnormality to explain the reported pain. It is likely that the multiple pain symptoms associated with somatization are the result of a common abnormal CNS interpretation of peripheral pain signals, rather than a simultaneous sensory malfunction of several organ systems.[6-9] We and others have found that 25-42% of IBS subjects attending gastroenterology referral clinics have identifiable somatization with standard instruments (PHS-15) and medical history.[10, 11] Clinically, the presence of somatization in IBS patients (IBS-S) bears prognostic importance: compared to IBS patients without somatization (IBS-No S), IBS-S subjects report significantly greater numbers of: a) GI and non-GI symptoms, b) missed work days, and c) urgent care visits and physician consultations.[12-17] Recently we demonstrated somatization in IBS predicts both more intense abdominal pain experiences, and poor bowel symptom responses to centrally-active pharmacotherapies (e.g., tricyclic antidepressants). We found that IBS-S subjects were 4.7 times more likely to report unsatisfactory treatment responses using antidepressant therapy, resulting in premature medication discontinuation (Figure 2).[1, 16-18]

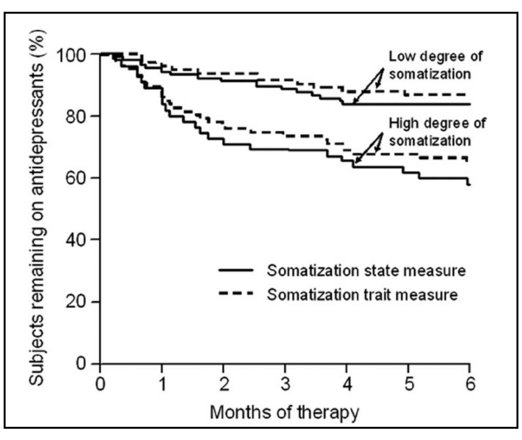


Figure 2. The importance of somatization to antidepressant treatment discontinuation in IBS subjects. IBS subjects with greater somatization using two different somatization measures (somatization state and trait) were significantly more likely to experience premature antidepressant treatment discontinuation due to inefficacy and side-effect experiences. From Sayuk G, et al.[1]

Thus, it is known that somatization is common in IBS, and IBS-S patients experience more severe clinical courses which are less responsive to centrally-active IBS treatments.

3. **Abnormal pain processing within the homeostatic afferent processing network (HAPN) is relevant to IBS pathophysiology in the IBS-S subset of patients.**

Visceral pain is, by definition, the symptomatic hallmark of IBS. Visceral pain in the absence of an identifiable cause (as in the case of IBS) theoretically could originate as a consequence of pain processing abnormalities at two distinct levels: 1) abnormal perception of chemo-mechanical stimuli by the enteric nervous system within the wall of the gut,[19] or 2) abnormal central nervous system (CNS) interpretation or modulation of the afferent sensory signals derived from the gut. The use of functional neuroimaging techniques (position emission tomography [PET] and functional magnetic resonance imaging [fMRI]) have resulted in recent advances in our understanding of the brain regions responsible for visceral and somatic pain processing. Over the past decade, several brain regions important to the affective and cognitive interpretation of visceral pain have been identified in animal models and healthy controls. [2, 20-26] These areas, including subregions of the anterior cingulate cortex (ACC), the insula, thalamus, orbitofrontal, and prefrontal cortices, collectively have been described by Craig and others as comprising the "homeostatic afferent processing network" (HAPN) (Figure 3).[22, 27, 28] An early study conducted by Mertz *et al.* found activations of several HAPN regions, including the ACC, prefrontal cortex, insula, and thalamus in both IBS subjects and controls, supporting the physiologic relevance of the HAPN to visceral sensation (Figure 4). In comparing HAPN activations with noxious visceral stimulation in IBS versus healthy controls, greater HAPN activations, particularly within the perigenual ACC, were appreciated in the IBS population. Several studies have replicated these findings, though the magnitudes of these IBS-healthy control differences in HAPN activation are less consistent across studies.[26] In part, these observations may reflect significant protocol differences, including the use of different imaging modalities (PET and fMRI), study designs (noxious stimulation type, intensity, and duration) and data analysis methodologies. However, another likely explanation is the mechanistic heterogeneity that exists within IBS patients when examined as a single population. It is likely that only a subset of IBS patients experience significant HAPN overactivation with noxious visceral stimulation. Thus, when HAPN pain responses are examined in all IBS subjects, the inclusion of IBS subgroups in which HAPN overactivation is not mechanistically-relevant may bias findings toward the null.

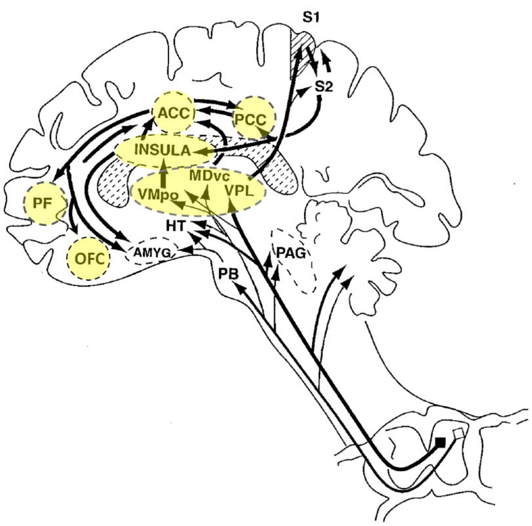
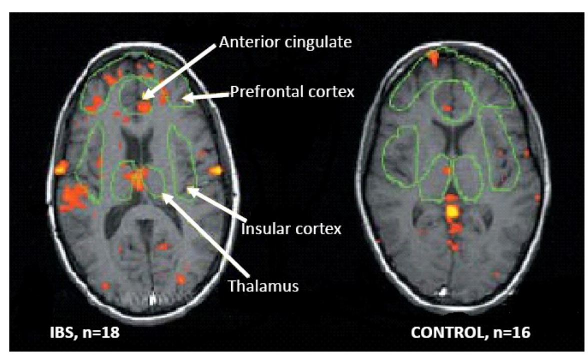


Figure 3. The Homeostatic Afferent Processing Network (HAPN). Regions comprising the homeostatic afferent processing network (HAPN) are responsible for the affective and cognitive modulation of pain signals, and are highlighted in yellow. PF, prefrontal cortex; OFC, orbitofrontal cortex; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; VMpo, MDvc and VPL, thalamic nuclei (ventromedial portion of the posterior nuclear complex, ventrocaudal portion of the medial dorsal nucleus, and ventroposterior lateral nucleus, respectively) Adapted from Price, DD. *Science* 2000.[3]



bowel subjects (IBS) and controls following rectal balloon distention. IBS subjects demonstrate greater activity within the homeostatic afferent processing network (HAPN) in response to visceral distention compared to controls. (from Mertz, *et al.*[2])

The HAPN is not only important to the emotional and cognitive responses to visceral pain, but likely is also relevant to somatic pain experiences in somatization syndromes.[27] A study by Chang *et. al.* found that in IBS subjects, a greater HAPN response within ACC was seen in response to somatic stimulation, but only in those with comorbid fibromyalgia (a somatization syndrome).[22] This finding supports my hypothesis that IBS subjects with somatization may experience activations of the HAPN with a broader spectrum of noxious stimuli, including painful somatic stimuli.

The recognition of specific a IBS population in which CNS pain processing abnormalities have mechanistic relevance would represent an important step in advancing our understanding of IBS pathophysiology. Recently, I completed a preliminary study to examine the influence of somatization on cerebral activations within the HAPN in an IBS population based on the degree of somatization present (see Preliminary Studies, Section C). *This work successfully demonstrated significant differences in HAPN activations in IBS-S compared to IBS-no S subjects, strongly suggesting that abdominal pain experiences in IBS-S subjects are a consequence of abnormal HAPN responses to afferent intestinal sensory input.*

4. Antidepressant therapy and homeostatic afferent processing network (HAPN) pain responses in IBS.

Clinical experience and controlled studies have shown that centrally-active therapies, such as the tricyclic antidepressants (TCAs), are of symptomatic benefit in the management of IBS.[18, 29] In a recent randomized, double-blinded study by Drossman *et al.*, desipramine therapy was shown to result in superior global IBS symptom responses compared to placebo. The *post hoc* analyses from that study found that somatization scores predicted treatment non-response using desipramine.[18] It is believed that TCAs (e.g., desipramine) modify CNS responses to noxious visceral stimulation within the HAPN and related regions in IBS.[31, 32] The effect of antidepressant pharmacotherapy on similar brain regions have been demonstrated in the treatment of mood disorders. For example, decreases in activations within ventral regions of the prefrontal cortex have been previously associated with response to selective serotonin reuptake inhibitors (SSRIs) when used in mood disorders. [30, 31,32] The influence of another TCA, amitriptyline, on cerebral pain responses has been examined in IBS in a single study. This investigation demonstrated that in an IBS population, compared to placebo amitriptyline therapy was associated with reduced visceral pain-related activations within the HAPN, specifically within the perigenual anterior cingulate cortex. [33] Based on previous these observations, and our earlier cited work suggesting that IBS symptom response to TCA therapies is less robust in IBS-S subjects, I hypothesize that somatization in IBS results in less robust responses due to a refractoriness to antidepressant medications within the brain's homeostatic afferent processing network.

5 . Study significance.

IBS is a common gastrointestinal diagnosis, imparting significant morbidity and effects on quality of life, and in turn substantial costs to society. Despite recognizing IBS for decades, the likely heterogeneity inherent in the condition has continued to hamper our understanding of the mechanistic basis of this condition. IBS patients with somatization (IBS-S) have more severe symptoms and more refractory treatment courses. It is anticipated that IBS-S subjects possess a generalized central pain processing defect within the HAPN as the primary mechanism underlying their pain symptom experiences. Consistent with the diffuse pain symptoms experienced in IBS subjects with somatization, it is anticipated that this abnormal HAPN activation can be evoked by both visceral and somatic stimulation. IBS-No S subjects, by comparison, may have a primary visceral hypersensitivity which originates at the intestinal level. In IBS-No S subjects, this would be expected to lead to greater afferent signaling and activation of the HAPN in response to noxious visceral stimulation, but not somatic stimuli (Figure 5). Additionally, observations of poorer treatment responses with centrally-acting antidepressants in IBS-S subjects raise the possibility that this abnormal HAPN pain response in IBS-S subjects accounts for the refractoriness to centrally-active IBS therapies. These objective responses of the HAPN to noxious stimulation and pharmacologic intervention will allow for a more objective definition of IBS-S as an important, physiologically-relevant IBS subgroup. As such, this study has the potential to highlight somatization as an important feature to measure in future IBS studies, and moreover to provide mechanistic insights into a specific IBS subgroup. The broad scientific and clinical implication of this work lies in its emphasis of IBS as a heterogeneous condition, deriving from a multitude of unique pathophysiologic pathways, necessitating the detailed characterization of relevant IBS subgroups in order to move forward in our understanding of IBS pathogenesis and treatment.

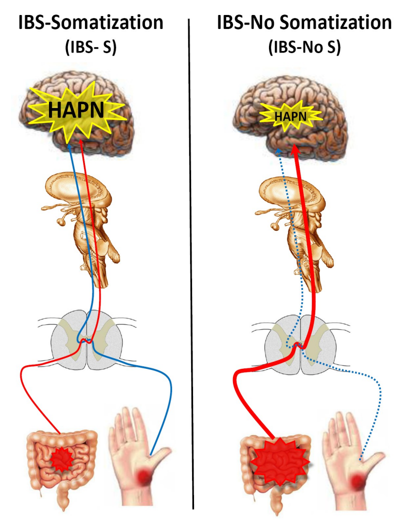


Figure 5. Proposed brain-gut interactions of IBS based on the presence of somatization. It is hypothesized that IBS subjects with somatization, IBS-S (left panel) experience abnormal activations of the brain's homeostatic afferent processing network (HAPN) in response to visceral- and somatic afferent inputs. Thus, greater HAPN activations would be expected with both visceral and somatic stimuli in IBS-S. In contrast, IBS subjects without somatization, IBS-No S (right panel) likely have abnormal afferent signaling from the bowel as their primary neurosensory abnormality. IBS-No S subjects thus would be expected experience greater HAPN activations with visceral stimulation, but not with somatic stimulation. These hypotheses will be assessed in the Aims of this proposal.

C. Preliminary study: The Importance of Somatization to Central Pain Responses in IBS

Rationale

fMRI studies demonstrate consistent differences in CNS homeostatic afferent processing network (HAPN) activations in IBS patients compared to healthy controls. However, within IBS populations substantial variation in HAPN responses to visceral pain is observed.[20, 34] Somatization is common in IBS, and results in worse clinical outcomes when present. This study tested the hypothesis that within an IBS population, the presence of somatization is associated with measurable differences in HAPN regional activations as measured by fMRI. We thus sought to demonstrate somatization as a clinical feature responsible for a portion of observed heterogeneity in CNS pain responses in IBS patients.

Specific Aim

To determine whether IBS subjects with somatization (IBS-S) demonstrate different patterns of central homeostatic afferent (HAPN) activations following noxious visceral stimuli compared to IBS-No S subjects and healthy controls. *It was hypothesized that IBS-S patients would exhibit significantly greater HAPN activations with noxious visceral stimulation compared to the IBS-No S, and healthy control groups.*

Methods

Female IBS subjects meeting inclusion and exclusion criteria (Rome III criteria)[35] and somatization (IBS-S) as defined in the Methods section (3.B.1, below) were eligible for study participation; female IBS patients with no somatization (IBS-No S) and healthy controls served as comparator groups. Baseline depression measures, including the Beck Depression Inventory (BDI), the Hamilton Depression Rating Scale (HAM-D) and anxiety scores (Beck Anxiety Inventory, BAI) were assessed as potential confounders. Subjects also reported on global bowel symptom burden and IBS symptom severity using visual analog ratings. The study design is illustrated in figure 6.

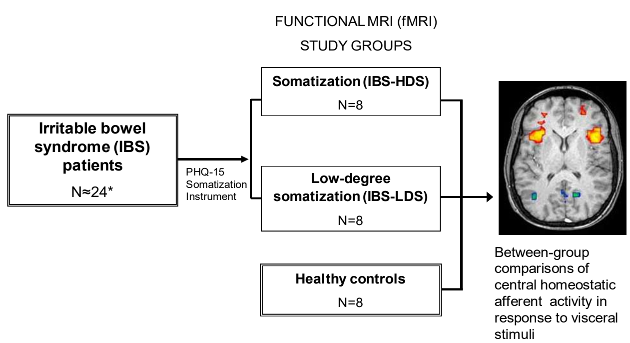


Figure 6. Study design, preliminary data. Functional MRI (fMRI) in IBS subjects with somatization (IBS-S) compared to those without somatization (IBS-No S) and healthy controls. Subjects with intermediate degrees of somatization were excluded from participation.

Intervention

Functional MRI studies were performed while applying simultaneous visceral stimulation (rectal balloon distension) at low- (25-mmHg) and higher-pressure (50-mmHg) levels in random order following established protocols.[2] Subjective ratings for distention discomfort were assessed for each distention using a 10-point Likert scale at the end of each distention sequence.

Data interpretation

Cerebral activations included any statistically significant changes in fMRI activity (voxels) in *a priori* regions of interest (ROIs) within the HAPN following visceral stimulation compared to rest. These ROIs included the thalamus, bilateral prefrontal, orbitofrontal, anterior cingulate, and insular cortices. Between-group differences in cerebral activations to noxious visceral stimuli were evaluated with 2-way ANOVA analyses. Preliminary analyses were conducted using data collapsed across the two visceral stimuli ("visceral stimulus") versus "rest periods". Corrections were made for multiple comparisons in regions of interest.

Findings

Clinical features and subjective pain ratings: 24 right-handed female subjects (8 in each study group) underwent fMRI during the rectal balloon distention protocol from April 2007 to June 2008. A summary of the clinical features and subjective pain ratings in response to the balloon distentions are reported in Table xx. Subject ages were not significantly different across study groups. However, there were significant differences in IBS symptom ratings between the IBS-S and IBS-No S groups, with greater symptom severity, frequency, and global symptom burdens reported by the IBS-S group. Depression and anxiety scores on the Beck Inventories also were significantly higher in the IBS-S group compared to IBS-No S and healthy controls. Subjective experiences with the low-pressure (25 mm-Hg) rectal balloon distentions trended toward significantly greater discomfort reporting in the IBS-S group (p=0.3), but no differences were appreciated across groups with the higher pressure (50-mm Hg) distentions. (Table 1).

Table 1. Clinical features and subjective distention pain ratings in preliminary study.

	IBS-S subjects (N=8)	IBS-No S subjects (N=8)	Healthy Controls (n=8)	P value*
Age	47.2 ±8.6	45.0 ±9.7	36.5 ±9.2	0.07
PHQ-15 total	18.1 ±3.8[a,e]	5.6 ±2.6	3.7 ±1.6	0.001
Non-GI Somatization Diagnoses	2.9 ±1.9[a,d]	0.2 ±0.4	0.5 ±0.5	0.001
Beck Depression Inventory Score	19.1 ±10.9[a,d]	3.5 ±3.3	3.6 ±3.8	0.001
Beck Anxiety Inventory Score	19.9 ±6.1[a,e]	3.1 ±2.9	1.5 ±5.4	0.001
IBS Symptom Severity Rating	6.9 ±1.9[c]	4.2± 2.8	--	0.04
IBS Symptom Frequency	9.1 ±4.8[b]	2.4 ±2.1	--	0.006
IBS Global Burden Rating	7.5 ±1.9[b]	3.2 ±3.0	--	0.005
Subjective pain rating, 50-mm Hg	5.1 ±2.0	4.5 ±2.2	4.6 ±2.2	0.8
Subjective pain rating, 25-mm Hg	3.0 ±1.4	2.8 ±1.4	2.0 ±0.9	0.3

* Reported p value is reflective of an ANOVA where all three study groups compared; t-tests performed to assess between-group statistical significances; a = p <0.001 between IBS-S and IBS-No S groups; b = p <0.01 between IBS-S and IBS-No S groups; c = p <0.05 between IBS-S and IBS-No S groups; d = p <0.001 between IBS-S and Healthy controls; b = p <0.01 between IBS-S and IBS-No S groups.

Regional activations within the HAPN by study group

Consistent with findings previously reported in the literature, during noxious visceral stimulation a significant difference in a HAPN brain region activations was seen in comparing IBS subjects and healthy controls (Table 2 and Figure 7). Between-comparison of HAPN activations in the IBS-S and IBS-No S groups revealed significantly greater activation of the right prefrontal cortex, insula, and thalamus in the IBS-S subjects. The

IBS-S subjects also were found to have greater activation of the bilateral anterior cingulate and orbitofrontal cortices compared to their IBS-No S counterparts.

Table 2: Regional differences in cerebral activations within the homeostatic afferent processing network (HAPN) in response to visceral stimulation in IBS-S subjects compared to controls, and IBS-S compared to IBS-No S subjects.

Region	Hemisphere	Coordinates (x,y,z)*	All IBS vs. Controls		IBS-S vs. IBS-No S	
			Z	p	Z	p
Anterior cingulate cortex	Left	-8, 38, 16	2.91	0.004	2.67	7.3×10^{-3}
	Right	9, 34, 11	2.19	0.02	4.15	7.6×10^{-4}
Insula	Left	-39, 2, 7	5.20	9.7×10^{-8}	1.09	0.27
	Right	32, 23, 10	5.47	2.8×10^{-8}	2.33	0.02
Orbitofrontal cortex	Left	-25, 13,-10	4.27	1.9×10^{-5}	3.13	0.001
	Right	34,22,-3	4.82	1.4×10^{-6}	4.84	6.5×10^{-7}
Prefrontal cortex	Left	-41, 40, 11	4.87	5.4×10^{-7}	1.37	0.16
	Right	41, 50, 5	5.78	3.5×10^{-8}	4.17	2.4×10^{-5}
Thalamus	Left	-19, -22, 11	5.58	1.2×10^{-8}	1.51	0.12
	Right	12, -8, 11	4.02	5.8×10^{-5}	2.09	0.003

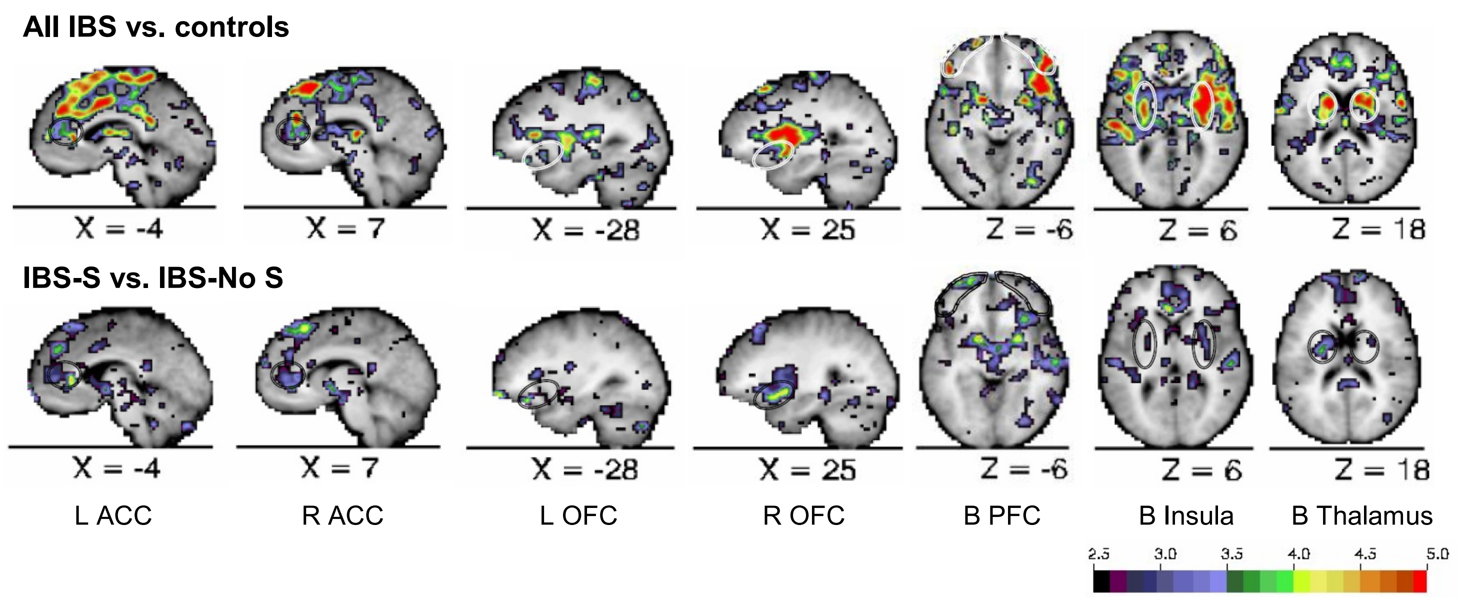


Figure 7. Brain activations following noxious visceral stimulation. Colored pixels indicate brain regions with significant activations (Z score) in response to rectal balloon distention. The regions comprising the homeostatic afferent processing network (HAPN) are circled and labeled. HAPN regions were activated to significantly greater degrees in the IBS subjects compared to healthy controls (top row). Comparing IBS-S and IBS-No S subjects (bottom row), many of the same HAPN regions demonstrated greater activation in the IBS-S group. L=left; R=right; B=bilateral; ACC=anterior cingulate cortex; OFC=orbitofrontal cortex; PFC=prefrontal cortex.

Significance of findings
In this study, IBS subjects clearly differed in their HAPN brain responses to visceral stimulation compared to healthy controls. A similar pattern of HAPN activation in IBS has been observed in several other studies, suggesting that dysfunctional affective and cognitive processing of visceral pain signals within the HAPN may be of mechanistic relevance to IBS symptom experiences. [34, 36] Hypothesizing that these HAPN pain responses are of particular importance to IBS subjects with comorbid somatization, a similar comparison of HAPN visceral activations was performed between IBS-S and IBS-No S subjects. In this small study sample, significantly greater activations of many of these same HAPN regions were observed in the IBS-S subjects compared to their IBS-No S counterparts. This finding supports my hypothesis that abnormal HAPN processing of afferent visceral signals may be of greater pathophysiologic relevance to the subset of IBS patients that have comorbid somatization.

An important HAPN region that demonstrated greater activation in the IBS-S subgroup was the perigenual portion of the anterior cingulate cortex (ACC). The ACC is the region most consistently activated by visceral stimulation, and thus is regarded by some as "the visceral pain center".[25] Electrical stimulation of this region results in a potent fear response, suggesting the key role of the ACC in emotional responses to aversive stimuli.[37] This region additionally has prominent descending connections to the dorsal vagal complex and periaqueductal gray, and as such is believed to play a central role in the endogenous modulation of pain and autonomic tone. The ACC influence on autonomic function could explain the alterations in bowel patterns often accompanying IBS pain experiences. Similarly, the right insular cortex (IC) demonstrated significantly greater activation in the IBS-S subgroup. Studies have shown that lesions within the IC result in modulation of the affective pain response, but not visceral sensory responses.[38] This region, along with the ACC, also has been shown to activate in response to viewed fearful faces (negative emotional condition). Together, these findings suggest that abnormal right IC and ACC affective responses to noxious visceral stimuli may be important to IBS-S symptom experiences.[40]

Greater activations of the bilateral orbitofrontal cortices (OFC), and right prefrontal cortex (PFC) were seen in IBS-S subjects. These regions play a central role in the higher-order processing and integration of the cognitive and affective responses to visceral sensory signals, resulting experience-related behaviors and thoughts. The dorsolateral subdivision of the PFC is involved in the cognitive processing (attention, memory, and executive functions) as related to visceral sensory signaling.[41] The PFC has direct reciprocal connections with brain areas known to be involved in mood and emotional processing, including the ACC.[42-44] Via inputs from the regions involved in emotional regulation, and different sensory afferents (visceral, gustatory, visual), the orbitofrontal cortex, in conjunction with the IC, is important to the integration of emotions and visceral sensation.[41] The preferential right sided frontal activation pattern seen in IBS-S subjects has been described previously. Right sided laterality has been reported in both humans and animals to be associated with higher cortisol levels and autonomic responses, and greater negative affective experiences (fear, disgust).[45] A similar pattern of right frontal activation previously was reported in one IBS study. The composition of this IBS group, in terms of comorbid somatization, was detailed by the investigators.

Thalamic activations were significantly greater bilaterally in IBS subjects compared to controls. As a key center in the initial processing and subsequent relay of visceral sensory information to multiple cerebral cortical regions for higher-order processing, these thalamic activations could be deduced as reflecting greater afferent signaling in response to noxious visceral stimulation. The thalamic nuclei are also the recipients of substantial descending signals from other HAPN regions, however, thus complicating this interpretation of activations in this region. IBS-S subjects did also have significantly greater activations than IBS-No S subjects, though the magnitude of difference between groups was small by comparison to the other HAPN regions.

Finally, these observed differences in HAPN activations between the two IBS subgroups must be considered in the context of the greater levels of bowel and mood symptom reporting in IBS-S subjects. These factors potentially could serve as confounders in attributing the HAPN activation differences to the presence of somatization alone, and additional analyses will thus need to be performed on this preliminary data to establish the independent importance of somatization to HAPN visceral activations in the IBS-S subgroup.

D. Research Design and Methods

1. Overview

It is likely that irritable bowel syndrome (IBS) is the result of multiple disease mechanisms which lead to the expression of a common set of symptoms. The goal of this proposal is to identify and characterize a more homogeneous subset of IBS patients—specifically, IBS patients with comorbid somatization (IBS-S). I will define the IBS-S subgroup by their CNS responses within the homeostatic afferent processing network (HAPN), regions important to the higher level (cognitive and affective) responses to pain. *The overarching hypothesis of these investigations is that aberrant central pain responses within the HAPN result in somatization pain symptoms, and as such may have pathophyiologic relevance to the bowel pain experiences of the IBS-S subgroup.* Building upon my initial observations that IBS-S subjects demonstrate greater HAPN activations to visceral stimuli, Specific Aim 1 will examine whether these responses generalize to non-visceral (somatic) stimuli. Given treatment refractoriness to antidepressants in IBS-S subjects, and the known influence of these medications on the HAPN, Specific Aim 2 will assess the effect antidepressant therapies on IBS-S central pain activation patterns. Ultimately, this work will provide greater insight into the neuropathophysiologic basis of IBS-S, allowing for the objectively definition of IBS-S as an important irritable bowel subgroup.

Specific Aim 1. To compare HAPN activations following noxious somatic stimulation in IBS subjects based on the presence of somatization.

Rationale*:* It has been demonstrated that CNS responses to noxious visceral stimulation differ within the HAPN from healthy controls subjects in some, but not all, IBS subjects. My preliminary data supports the hypothesis that IBS subjects with comorbid somatization (IBS-S) demonstrate a pattern of greater CNS activation within the homeostatic processing afferent network (HAPN) following visceral stimulation compared to those with no somatization (IBS-No S). Specifically, the regions responsible for higher-level cognitive (bilateral orbitofrontal, right prefrontal cortex) and affective (right insula, bilateral anterior cingulate cortex) pain processing had greater activations in IBS-S subjects. Given that IBS-S subjects experience multiple somatic pain symptoms, in this Aim I will determine whether the previous difference in HAPN pain responses in the two IBS populations (IBS-S and IBS-No S) is specific to visceral stimuli, or is a more generalized pain response which can also be elicited by somatic stimulation.

Design and Methods*:* HAPN responses to both visceral and somatic stimuli will be evaluated in 40 female IBS patients stratified by the presence of somatization (20 IBS-S and 20 age-matched IBS-No S subjects) and 20 healthy controls. Measures of current IBS symptoms and concurrent mood symptoms will be assessed as potential confounders using self-report instruments and structured psychiatric interviews (see “Assessment measures”, below).

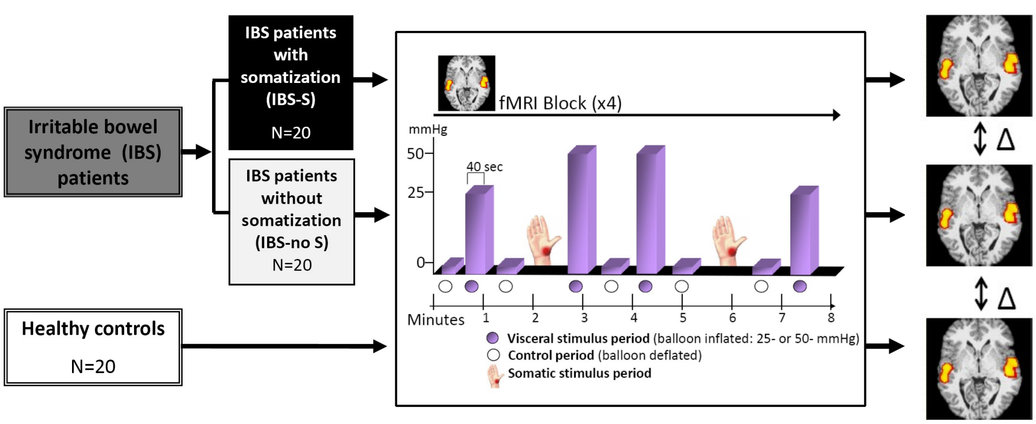


Figure 8. Overview of study design for Specific Aim 1. Female IBS subjects with somatization (IBS-S) and without somatization (IBS-No S) and healthy controls will undergo a series of four functional magnetic resonance (fMRI) blocks, each assessing brain activations in response to randomized visceral and somatic stimuli. Between-group (IBS-S vs IBS-No S) differences in brain activations within the homeostatic afferent processing network (HAPN) in response to these stimuli will then be evaluated.

All three subject groups will undergo a baseline functional MRI study of 4 functional imaging blocks obtained during a protocol of randomized visceral (25- and 50-mm Hg rectal balloon distentions) and somatic stimuli (1.4 kg-cm^2 nailbed pressure) (Figure 8, above). The primary endpoint of interest is differences in CNS activations of *a priori* regions of interest comprising the HAPN following somatic stimulation.

Analysis/interpretation*: As a result of a more generalized abnormal pain response within the HAPN, it is hypothesized that IBS-S subjects will demonstrate greater activations of the HAPN with noxious somatic stimuli, compared to IBS no-S subjects and healthy controls.* This hypothesis thus predicts a "IBS Group x Somatic stimulus" effect on two-way ANOVA analysis of activations within *a priori* HAPN regions. Specifically, it is hypothesized that the significantly increased HAPN activation pattern observed in IBS-S subjects compared to IBS-No S subjects that was observed following noxious visceral stimulation (preliminary data) will be present following noxious somatic stimulation in the IBS-S subjects, but not in the IBS-No S or healthy control groups (Figure 10, below). If this HAPN activation response to somatic stimuli is indeed observed in IBS-S subjects, it would support my hypothesis that in IBS-S patients, the presence of somatization reflects a less discriminate HAPN response to noxious stimuli, which includes not only visceral, but also somatic stimuli. This global overactivation of the HAPN in IBS-S subjects thus could have mechanistic relevance to the multiple pain experiences in affected individuals.

Alternately, if HAPN activation differences in IBS-S subjects are found only to be specific to visceral stimulation, the possibility that the HAPN is relevant to visceral, but not somatic, pain experiences in IBS-S subjects would be considered. This would imply that several distinct, system-specific sensory defects (i.e., gut, musculoskeletal, etc.) may be present in somatization, rather than the global pain processing abnormality within the HAPN as is hypothesized to be relevant to the multiple pain experiences of IBS-S subjects.

If greater HAPN activations are detected in both IBS-S and IBS-No S subgroups, this would be interpreted as possibly indicating that somatic afferent sensory signals are more potent activators of the HAPN than are visceral afferent signals, resulting in HAPN activation patterns with noxious somatic stimulation which are indistinguishable in the two IBS groups. This conclusion might be valid particularly if the difference in visceral pain responses seen in the preliminary data are again observed in this IBS cohort. It would thus still be conceivable that differences in HAPN somatic responses could be detected in future studies which implement either less intense somatic stimuli (i.e., lower level thumb pressures) or alternative somatic stimuli (thermal stimuli).

Pitfalls/Limitations*:* Critical to the study is an accurate determination of study group assignment (IBS-S or IBS-No S) and the limitation of potential confounders. Thus, substantial effort will go into the determination of somatization status, including the use of validated self-report instruments (PHQ-15), medical chart review and history by the investigator, and a formal structured interview (C-DIS). Other potentially important confounders will also be considered. To address differences in brain activations due to handedness, gender, and age, subjects will be age-matched within 5 years, and only will be right-handed females will be studied. Subjects with major depression, anxiety, or current antidepressant use will also be excluded. Current depression, anxiety, and duration and severity of bowel symptoms will also be assessed using both validated self-report and standardized interviews. Additional ANOVA models will be run to confirm that HAPN activation differences attributed to somatization are not accounted for by other IBS disease factors (duration, symptom severity) or psychological features are also relevant to observed differences in brain activation. Subject movement during the fMRI study is also a consideration given the noxious nature of the study stimulus. Preliminary data acquired using a smiliar protocol did not suggest this to be a major issue, either in terms of subjective reports of discomfort or on assessment of movement during quality-control assessments. No subjects withdrew from the preliminary study due to protocol intolerance. However, if movement is introduced as a problem in this investigation, it is anticipated that it will be increasingly present in the latter blocks of the protocol (i.e., fMRI blocks 3 and 4). The proposed block design of the proposed fMRI study allows for the exclusion of study blocks demonstrating excessive subject movement. Study recruitment may present a potential pitfall, though in the past I have been successful in meeting recruitment goals through my outpatient practice given my clinical emphasis on IBS and related functional GI disorders. If recruitment does prove to be an issue, recruitment efforts will be expanded to include the Washington University Volunteers for Health database and the use of recruitment advertisements on the WU Medical Center campus.

Specific Aim 2. To compare the effects of antidepressant therapy HAPN activations following noxious visceral stimulation in IBS subjects based on the presence of somatization.

Rationale: Case-control and randomized clinical trial data have established that somatization predicts symptom responses to IBS therapy with the tricyclic antidepressant desipramine. Specifically, IBS-S subjects are more refractory to antidepressant therapies, while IBS-No S subjects have better overall responses to the same treatment. A recent study has demonstrated improvements in HAPN visceral activations (anterior cingulate cortex) following desipramine therapy in an IBS cohort. Given these observations, the goal of this Aim is to assess whether differences in visceral HAPN activation patterns following despiramine treatment can be detected in IBS-S and IBS-No S subjects.

Design and Methods: The proposed study implements open-label desipramine therapy in 40 IBS (20 IBS-S and 20 age-matched IBS-No S) subjects. Participants from Aim 1 will be invited to continue on to open-label therapy in Aim 2. IBS-S and IBS-No S subjects will receive a 4-week treatment course with the tricyclic antidepressant desipramine (DES), as detailed in section 2.D., below. Subjects will undergo a fMRI protocol with noxious visceral and somatic stimuli as in Aim 1 at baseline, and then immediately following the 4-week desipramine therapy. Subjects will undergo baseline, and post-therapy assessment of psychiatric comorbidity (depression/anxiety) and IBS symptom severity (as described in 3.A. and 3.B., below). The primary endpoint is differences in visceral stimulation-induced brain activations of the HAPN on fMRI, ascertained between-groups following desipramine therapy. The proposed study design is outlined in Figure 9.

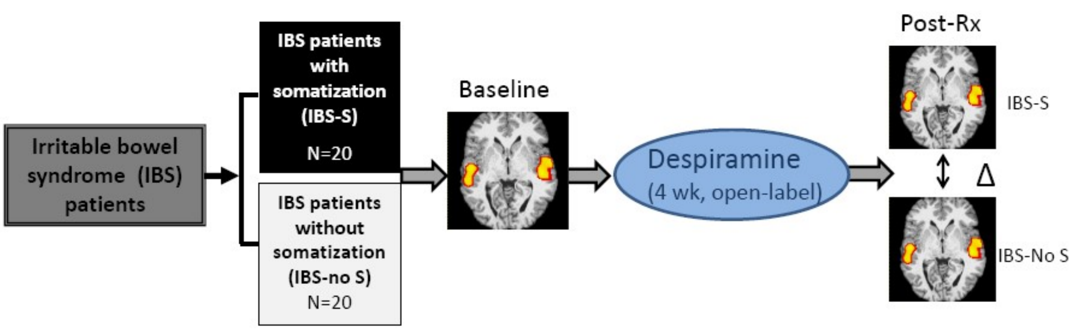
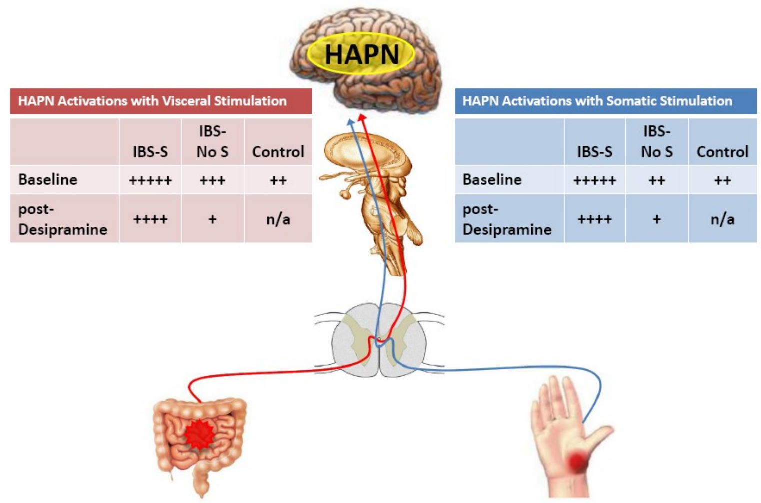


Figure 9. Overview of study design for Specific Aim 2. IBS patients with somatization (IBS-S) and without somatization (IBS-No S) will be identified. A baseline fMRI protocol (as in Aim 1) will be obtained at baseline, followed by a 4-week, open-label treatment with desipramine. A second fMRI protocol will be performed post-desipramine therapy at week 4. The primary endpoint of interest is post-treatment differences in visceral HAPN activations between IBS-S and IBS-No S subjects.

Analysis/interpretation: *Consistent with the clinical refractoriness to antidepressant therapy observed in IBS-S subjects, it is hypothesized that IBS-S subjects will exhibit a persistent pattern of abnormal HAPN activations in response to visceral stimulation following desipramine therapy.* By comparison, it is hypothesized that the IBS-No S group will exhibit decreases in HAPN activations in the post-treatment fMRI protocol (Figure 10, below). Region of interest two-way ANOVA models will be developed in comparing Δbaseline-DES activations, with IBS Group (IBS-S and IBS-No S) as a between-subject factor. If the stated hypothesis is supported by the fMRI findings, it would suggest that the persistent HAPN activations in response to visceral afferent signals may result in the less robust clinical responses to desipramine therapy in the IBS-S. This finding would be an important addition to the existing literature, providing both insight into the central mechanisms involved in IBS antidepressant response, and also into the specific relevance of HAPN pain responses to the IBS-S subgroup.

Conversely, should HAPN activations be found to improve in both IBS groups following desipramine therapy, the possibility that HAPN visceral activations are indeed partially refractory to desipramine in IBS-S, but the detected between-group differences (IBS-S vs. IBS-No S) in post-desipramine changes in HAPN activation were smaller than expected, and thus not statistically significant. If this were the case, the ANOVA analyses would be likely demonstrate statistical trends between the IBS groups, and enrollment of additional subjects would be considered if feasible following power calculations. This additional step might lead to findings which allow for rejection of the null hypothesis.

Pitfalls/Limitations: Pitfalls and limitations as described in Aim 1 are also applicable to this Aim, and will be addressed as outlined previously. Also a consideration is the use of repeated fMRI measures within the same individual, and the influence that previous experiences will have on subsequent fMRI studies (e.g., anticipation). Theoretically this effect should be distributed across treatments and IBS study groups equally, such that the anticipated effect, if any, bias towards the null. Existing literature discussing this phenomenon suggests that the proposed regionwise analyses based in *a priori* hypotheses are more immune to this phenomenon. This study is proposed without a placebo control arm. This design was decided upon after discussion with my mentors in order to facilitate subject recruitment and protocol adherence. While an obvious limitation of the study design, given previous data that demonstrates the effects of tricyclic medications over placebo within the HAPN, foregoing a placebo-controlled arm was felt to be a reasonable approach.



	IBS-S	IBS-No S	Control
Baseline	+++++	+++	++
post-Desipramine	++++	+	n/a

	IBS-S	IBS-No S	Control
Baseline	+++++	++	++
post-Desipramine	++++	+	n/a

Figure 10. Summary of the anticipated findings in Specific Aims. It is hypothesized that IBS-S subjects will demonstrate greater HAPN activations with noxious somatic stimuli, compared to IBS subjects with no somatization (IBS-S) and healthy controls (Specific Aim 1). It is also anticipated that IBS-S subjects will demonstrate a persistent pattern of abnormal HAPN visceral activations following desipramine therapy, whereas significant improvements in HAPN visceral activations will be observed in IBS subjects with no somatization following antidepressant therapy (Specific Aim 2). n/a=not assessed--control subjects do not participate in treatment arm of Specific Aim 2.

The observed changes HAPN activation seen following desipramine therapy in this Aim likely represent the direct effects of desipramine on these brain regions. This conclusion can be deduced from previous pharmaco-radiologic and animal model studies demonstating the actions of tricyclic antidepressants in these regions. The possibility remains, however, the desipramine is influencing the CNS proximal to the HAPN (i.e, at the level of the viscera, spinal cord, or brainstem), in turn decreasing HAPN activations via a reduction in visceral afferent signals. This possibility can be reconciled in future analyses which examine the data generated by this Aim, utilizing sophisticated functional connectivity models to establish the temporal relationships of these regional activations.

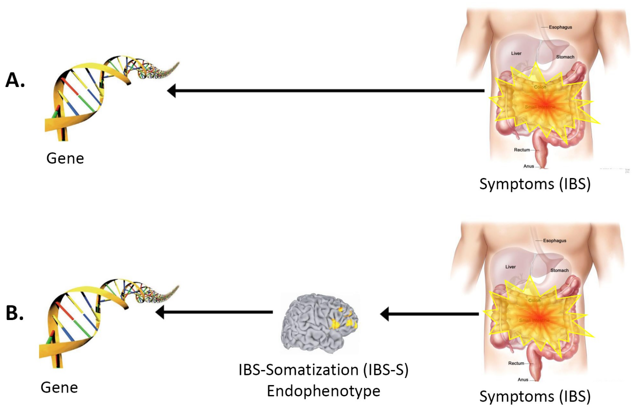


Figure 11. Strategies for the future study of genetics in irritable bowel syndrome. Gene-to-disorder approaches (A) have relied on self-report of symptoms in identifying study subjects with IBS, and assume direct linear relationships between genes and IBS. (B) By interposing objectives measures (e.g., more specific symptom subsets, differences in fMRI findings), an intermediate IBS "endophenotype" may be identified, potentially bridging the gap between single gene effects and the clinical phenotype.

Future directions. Recent epidemiologic studies have demonstrated greater prevalence of functional genetic polymorphisms of monoaminergic neurotransmission, including the serotonin transport protein (SERT), in IBS compared to healthy controls. Neurophysiologic studies in animal models and mood disorders have identified serotonin as important to nerve signaling within the HAPN. In future studies, I will attempt to link established serotonergic genetic polymorphisms (such as SERT) to the presence of CNS activation patterns in IBS subjects. Studies to-date evaluating the genetic basis of IBS have evaluated IBS subjects as a single group. The presumed heterogeneity in IBS pathophysiology has driven the need for large samples of several hundred individuals to ascertain even weak associations in previous IBS genetic studies (Figure 11A). These observations emphasize the need for the rational dissection of IBS into more discrete, homogeneous "endophenotypes"—intermediate phenotypes that help establish the causal link between genes and overt symptom expression.[46] The assumption of the endophenotype is that it represents a more homogeneous population, and thus one which is more likely to be impacted by a particular system defect, and in turn a more specific set of genes (Figure 11B).[47-49] Identification of mechanistically relevant IBS endophenotypes has been endorsed as a way forward in understanding the genetic basis of IBS and related somatization disorders.[50-53] The IBS-somatization (IBS-S) endophenotype is an example of such an IBS subset. IBS-S subjects can be identified on the basis of patient history and questionnaires, but also have a distinct clinical phenotype (e.g., more severe symptoms, multiple pain syndromes, treatment refractoriness) and are also associated with an objective measure (brain HAPN responses to noxious stimulation). Applying these objective filters over subjective symptom reporting alone, described functional genetic polymorphisms have the potential to yield much more robust impact on the syndrome.

Functional neuroimaging techniques, in which substantial data sets are acquired from a single subjects from hundreds of repeated measures of brain function, is a powerful tool when coupled with genetics. This is the concept of *"imaging genomics",* in which signal detection power allows for the study of tens, rather than hundreds of subjects in making significant gene-brain activation associations. This technique has been applied successfully in other similar conditions, including a recent National Institute of Mental Health study of 14 depressed subjects found that those carrying the SERT promoter s allele were found to have hyper-reactive responses of their amygdala to stressful environmental stimuli.[55-58] Genetic factors, particularly relating to candidate neurotransmitter systems, present an appealing area for future pursuit of a biological basis for irritable bowel in the IBS-S endophenotype. In preparing for the application of these techniques in future investigations, I have enlisted Dr. Alison Goate as a consultant and advisor, and will integrate formal and practical genetics training opportunities into my career development plan.

2. Source of subjects/calculation of estimated subject pool.

A. Irritable bowel syndrome and somatization. Based on published reports, it is conservatively estimated that approximately 15% of the US population is affected by irritable bowel syndrome.[59, 60] It has been observed by our group and others that somatization is present in 25-42% of IBS patients.[10, 11] Similarly, we have previously identified that within our gastroenterology referral clinic population that approximately 30% of IBS patients have no somatization features (IBS-No S).[61]

B. Subject pool. Patients undergoing evaluation for, or presently under medical management for, a diagnosis of IBS at the Washington University Gastroenterology clinic by from the period of Jan 2009 until the completion of the study enrollment period, if interested, will be assessed for participation eligibility. Given my clinical focus on IBS and related functional GI disorders, it is estimated that approximately two-thirds of the patients seen in my outpatient office meet criteria for IBS. I see an estimated 400 new patients a year in the office, of whom around 260 would be expected to have IBS. Primary recruitment efforts for the study population thus will be based in this setting. This strategy was successful in recruiting subjects to generate the primary data for this proposal.

C. Screening, eligibility, and patient enrollment. IBS subjects with- and without somatization features will be recruited. To be eligible, subjects will have to be between 18 and 90 years of age (inclusive) and qualify for a diagnosis of irritable bowel syndrome according to the criteria set forth in the Rome III criteria.[4] Comorbid somatization in these IBS subjects will be determined using the Patient Health Questionnaire-15 (PHQ-15).[62] Only subjects with high somatization (PHQ-15 ≥ 10), or low somatization (PHQ-15 ≤5), excluding GI symptoms, will be considered for enrollment. Medical history and chart review will be used corroborate the PHQ-15 score, requiring that IBS-S subject ≥2 additional somatization symdromes, while IBS-No S subjects have a history of ≤ 1 non-GI somatization syndrome.[1, 61] Persons are eligible to participate without regard to race or ethnicity. Given sex differences in cerebral responses to noxious stimuli and the greater prevalence of IBS in women, only female participants will be sought in this study.[63, 64] In view of brain hemispheric differences between left- and right-hand dominant individuals, all participants must be right-handed.

Persons are excluded from participation for having various psychiatric, medical, and other characteristics.

Psychiatric/cognitive exclusions include any of the following: active suicidal or homicidal ideation or a history of attempted suicide, current excessive alcohol use or other substance abuse disorders, active major depression, anxiety disorder, bipolar depression or any psychotic disorder, unwillingness to be randomized or provide informed consent, inability to communicate with staff or significant cognitive impairment.

Medical and other exclusions include any of the following: renal or hepatic disease or impairment, diabetes, cardiovascular disease, cardiac arrythmia, cerebrovascular disease, or breastfeeding, pregnant, or imminent intention of pregnancy, history of seizures or primary neurological disorder, head trauma, brain damage, hyper- or hypothyroidism, history or abdominal surgery (other than cholecystectomy/appendectomy), or known structural GI disorder (Crohn's disease, etc.), contraindication to MRI (metallic implant, pacemaker), or rectal balloon distention (e.g., proctitis/colitis).

Exclusions related to medications: 1. Analgesics (narcotics, NSAIDs; acetaminophen OK), 2. Muscle relaxants 3. Psychoactive agents (antidepressants, antipsychotics) 4. Other medications (phenytoin; amphetamines, prescription weight-loss drugs, or bezodiazepines); 5. Thyroid medication; 6. Anticholinergic medications or other IBS medications (hyoscyamine, dicyclomine), 7. Cytochrome p450 substrates, 8. Participation in any clinical trial using any other drug.

D. Desipramine therapy (Aim 2). Aim 2 utilizes desipramine, a standard-of-care option in the clinical management of IBS.[65, 66] Desipramine is a tricyclic antidepressant that has considerable clinical experience, and controlled-trial evidence supporting its use in the management of IBS symptoms.[67-69] *Dosing of desipramine is as follows:* Starting dose: Desipramine 25 mg/day administered in the evening. Dosing may be increased dependent upon side-effects and clinical response to a maximum of 100 mg/day. Absent significant side-effects, all patients are increased at the one week visit to 50 mg/day at bedtime if they have not achieved a report of "Adequate relief" (Assessment measures, B.3., below). Thereafter, up to week 4, the daily desipramine dose may be increased weekly by 25 mg up to the 100 mg/d maximum. All doses will be dispensed by the Barnes-Jewish Hospital (BJH) study pharmacy, and supervised by the investigator.

3. Assessment measures

Comprehensive assessment of psychiatric comorbidities (e.g., depression, anxiety) are critical for testing study hypotheses. A semi-structured psychiatric diagnostic interview and DSM-IV criteria will be used to make the diagnosis of any co-morbid psychiatric conditions.

(1) The Research Diagnostic Questions for Adult Functional GI Disorders in order to satisfy the presence of FGID(s) based on established diagnostic criteria (Rome III).[1]
(2) The Gastrointestinal Symptom Questionnaire (GISQ) which contains the following questionnaires:

a) GI symptom assessment (GISQ Part A):
   1. two 100-mm visual analog scales assessing recent bowel symptom burden and severity, and
   2. a quantification of bowel symptom frequency
b) Somatization state and trait measures (GISQ Part B):
   1. the PHQ-15 somatization form,[12] a validated self-report of recent somatic symptoms (somatization state measure), and
   2. a somatization diagnosis checklist (somatization trait measure).
c) Mood disorder measures (GISQ Part C):
   1. the Beck Depression Inventory
   2. the Beck Anxiety Inventory
d) Health-related quality of life measures (GISQ Part D & E):
   1. the Short-Form General Health Survey-36 (SF-36),[13] an instrument applied previously in the assessment of health influence on global well-being in FGID and other somatic disorders.
   2. a visual analog scale assessment of global symptom response to therapy.
e) FGID treatment measures (GISQ Part D):
   1. a visual analog scale assessments of GI symptom response to therapy.
   2. a self-report of subject compliance with medical therapy.
   3. a validated questionnaire measuring beliefs about medications.

(3) The Gastrointestinal Symptoms Questionnaire Supplement (GISQ-S) which contains the following questionnaires:

a) State-Trait Anxiety Inventory for Adults (STAI) (Part A). The State-Trait Anxiety Inventory Form Y (STAI) is an instrument for measuring anxiety in adults. The STAI differentiates between the temporary condition of "state anxiety" and "trait anxiety." The qualities evaluated by the STAIS-Anxiety scale are feelings of apprehension, tension, nervousness, and worry.
b) Pain Catastrophizing Scale (PCS) (Part B) is widely used to assess cognitive and affective responses to pain and to evaluate pain management program outcomes.
c) Somatic Symptoms Inventory (SSI) (Part C): The SSI is a self-report questionnaire composed of 26 bodily complaints drawn from the hypochondriasis scale of the Minnesota Multiphasic Personality Inventory and the Hopkins Symptom Checklist somatization scale.
d) SOMS-7 (Part D) is a 7-item instrument for the evaluation of treatment effects in somatoform disorders. It covers all somatic symptoms mentioned as occurring in somatization disorder, according to DSM-IV and ICD-10.
e) Gastrointestinal Symptom Rating Scale for IBS (GSRS-IBS) (Part E): The Gastrointestinal Symptom Rating Scale is a disease-specific instrument that includes 15 items combined into five-symptom clusters addressing different gastrointestinal symptoms. The five-symptom clusters depict reflux, abdominal pain, indigestion, diarrhea and constipation. The GSRS has a seven-graded Likert type scale where 1 represents absence of bothersome symptoms and 7 very bothersome symptoms. The GSRS is well documented to be reliable and valid and norm values for a general population are available.

f) Life-Stress Questionnaire. (4) Additional questionnaire measures

a) SES Ladder (SES-L)
b) Brief Pain Inventory (BPI)
c) Hospital Anxiety and Depression Scales (HADS)
d) Coping Strategies Questionnaire-Catastrophizing scale (CSQ-C)
e) IPIP Neuroticism and Extroversion scales (IPIP-NEO)
f) Early Trauma Inventory-Self Report (ETISR)

g) Positive and Negative Affect Scales (PANAS-SF)

(5) Diet Assessment

a) Harvard School of Public Health Food Frequency Questionnaire (FFQ): https://regepi.bwh.harvard.edu/health/FFQ/files/2007%20BOOKLET%20FFQ.pdf

C. *Functional neuroimaging testing.*

1. *Rectal balloon placement/distention.* A rectal latex balloon with an external diameter of 5 cm and a length of 9 cm (Mui Scientific, Canada) will be placed with its distal end 4 cm from the anal verge. A 20 min rest period will elapse to allow the patient to become acclimated to the presence of the rectal balloon before initiation of the protocol. The rectal balloon catheter will be attached to a one end of silastic tubing, and the remaining end of the tubing attached to the rectal balloon will be connected to a barostat, a device designed to safely deliver controlled inflation of a rectal balloon at pre-determined pressures following a programmed protocol.

2. *Dolorimetry methodology*. A pre-MRI baseline session will be performed to determine a tolerance for pressure pain sensitivity thresholds. A discrete pressure stimulus of 40 seconds will be applied to the left thumbnail using a 1-cm^2 hard rubber probe attached to a hydraulic piston as described previously.[27, 81-83] Dolorimetry using standard thumb pressure has been shown to accurately and reproducibly reflect an individual's overall pressure-pain sensitivity.[83] A pressure of 1.4 kg-cm^2 will be applied during the stimulus period, as this is the threshold previously established as "moderate" on a pain scale used in a fibromyalgia population.[27]

3. *Structural MRI scan.* All scanning will be performed on a 3.0T Siemens Allegra MR scanner at the WU Center for Clinical Imaging Studies (CCIR). Acquisition of a scout scan with three orthogonal slices, followed by a coarse 3D sagittal T1-weighted MP-RAGE (magnetization prepared rapidly acquired gradient echo)[84] will be used in slice registration. The coarse MP-RAGE will be used to automatically compute fMRI slice tilts and offsets that optimize whole brain. High-resolution structural images will be acquired using a 3D sagittal T1-weighted MP-RAGE acquisition optimized for contrast-to-noise and resolution.[85] High-resolution multislice oblique axial spin density/T2-weighted fast spin echo (FSE) structural images will be acquired using slice tilts and positions computed by slice registration. The acquisition of T2-weighted data with slice pre-registration provides very similar slice positions in serial studies. The T2-weighted FSE data will be used in the fMRI atlas registration procedure.

4. Functional MRI (fMRI) scanning. Functional images will be collected in runs using asymmetric spin-echo echo-planar sequence (same as blood oxygen level-dependent, "BOLD", contrast (T2*)). Each functional run will consist of sets of 39 contiguous 3.2 mm thick axial images acquired parallel to the anterior-posterior commissure plane, allowing complete brain coverage at high signal-to-noise ratio.[86] MRI data will be reconstructed into images, then normalized across runs by scaling whole-brain intensity signal to a fixed value and, removing the linear slope on a voxel-by-voxel basis to counteract effects of drift.[87] MR data will be aligned to correct for head motion using a six-parameter rigid body rotation and translation correction which mutually registers all frames in all runs for each subject.[88] Between-subjects analyses will be conducted after transforming data to a common atlas space and then blurring images with an optimal Gaussian filter[89, 90] To transform MR data into standard atlas, T1-weighted MP-RAGE and T2-weigted FSE anatomic images will be obtained (see above). This overall strategy has been described previously.[91] A sequence of linear (affine) transformations will be computed involving a combination of intra- and cross-modal registration procedures (e.g., T2- or T2*-weighted EPI to FSE to MP-RAGE to an atlas-representative MP-RAGE target). The transforms are combined by matrix multiplication such that reslicing of data in conformity with the atlas then will involve only one interpolation. The cross-modality registration algorithm used for this procedure is related to methods described previously.[92] The intra-modality registration algorithm is equivalent to minimization of difference image variance.[93] This is identical to the method used for MR-based co-registration of PET data[88] This method provides a straightforward means of conducting quantitative group comparisons while preserving the enhanced temporal resolution of fMRI. This analysis technique has been validated against single-subject analyses conducted with the N-back test.[94]

6. *Rectal balloon distention/dolorimetry protocol.* All balloon distensions and dolorimeter activations will be performed with the participant in the supine position with eyes closed to minimize extraneous visual stimuli (potentially confounding fMRI data). Participants will be informed that a series of stimuli are about to be delivered prior to a series of balloon distensions/thumb pressure deliveries, but will be given no insight into the degree of the impending distension or pressure. The balloon distension protocol will consist of a series of rectal balloon distensions and intermixed thumb pressure deliveries performed using a block design as reported previously.[95] Each "fMRI block" will constitute a total of 6 (six) 40-second "Control periods" alternated with 6 (six) 40-second "Stimulus periods". A continuous fMRI scan will be obtained for each of the fMRI blocks (Figure 11). Each 480-second fMRI block will be followed by a resting period of two (2) minutes between each functional scan for recovery of baseline MRI signal between scans. The individual fMRI blocks will randomly alternate two (2) rectal balloon distensions of 25 mm Hg (sensation of stool or mild pressure),[2] two (2) distensions of 50 mm Hg (sensation of moderate pressure or discomfort),[2] and two (2) thumb pressure applications of 1.4 kg/m2 ("Moderate pain"), with 40 second periods of rest interposed between these stimuli. Following fMRI rectal distention/dolorimeter protocol sequence, the participant will be removed from the MRI scanner using routine protocol.

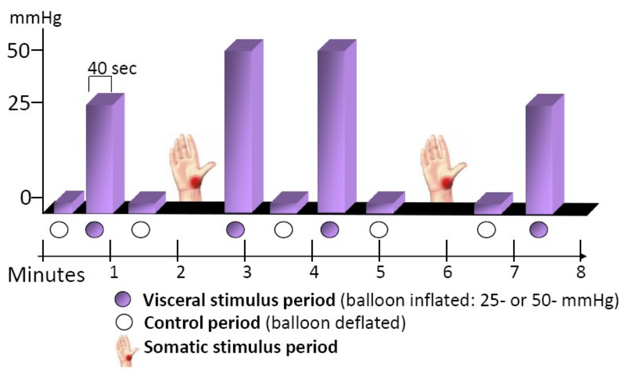


Figure 12. Rectal balloon distention/dolorimetry protocol.

Obtaining samples for genetic analysis.

We will obtain 5 ml of saliva using an established collection technique.[32, 33] All DNA samples will be collected by the principal investigator. Samples will be de-identified and delivered to the lab of Dr. Rodney Newberry for single nucleotide polymorphism (SNP) genotyping. Dr. Newberry is a molecular geneticist with more than 20 years' experience studying the genetic basis of neuropsychiatric disease. His laboratory will employ a Pyrosequencer or Sequenom mass spectrometer for SNP genotyping. Using these technologies, to date Dr. Newberry's lab has genotyped several hundred SNPs in several thousand study subjects.

Stool microbiome assay

Recent work has confirmed that, independent of bowel pattern, IBS patients possess different patterns of intestinal flora than control subjects[107] with reduced microbial diversity[108] and via detailed microbiotal examination using high-throughput pyrosequencing, the existence of IBS subgroups, with one IBS subgroup resembling controls, and the other with portportional differences in *Bacteroidetes* and *Firmicutes*.[109] Further, the sensitivity to colonic distention was successfully transferred to germfree rats inoculated with the fecal microbiota from IBS patients.[18]

Sample collection (stool). Patients will be provided a sterile stool sample collection container and sterile tongue depressors for sample acquisition. Stool samples will be collected as soon as possible, placed within sterile CryoVial tubes, snap-frozen in pre-cooled methyl-butane in dry ice, and stored at -80°C until use.

Data Analysis and Sample Size Calculations

The proposed experimental include visceral and somatic stimuli which will be studied in a block design, and the obtained functional MRI activations will analyzed using the general linear model, combined with appropriate corrections for multiple comparisons.[90, 93, 96, 97] These methods are implemented within a set of propietary display and analysis tools developed by Washington University investigators within the Mallinckrodt Institute of Radiology (MIR). The general linear model will be applied to produce statistical and magnitude maps. All analyses will be done assuming a standard Boynton hemodynamic response curve; this assumption has been valid in previous experiments conducted by Dr. Sheline which have examined similar brain regions of interest (ROIs) in this protocol. The statistical maps are then used to define activated regions within subjects. Random effects models for group comparisons will be performed in several ways. The increases or decreases in strength of response

(Delta S/S) will the metric that is used. To statistically evaluate this, ROI analysis will be conducted in anatomically constrained areas along with voxel-by-voxel analysis. Both ROI-directed and voxel-by-voxel repeated measures ANOVAs (using parameters described below) are done across subjects using model estimates as inputs. Interaction effects and other activations resulting from specific comparisons of a subset of the events will be reported at an uncorrected threshold of $p < 0.001$ for descriptive purposes. For the insular cortex, prefrontal cortex, orbitofrontal cortex, anterior cingulate cortex, and thalamic nuclei, predetermined ROIs will be used and will include any activations within these regions. A small volume correction[98] will be applied, in keeping with the *a priori* hypotheses regarding these regions. Predetermined ROIs from the HAPN in Talairach space will be drawn using the Talairach atlas coordinate system[99] and the software ANALYZE™ will then be applied to all the participants. Regions which would not be expected to differ between the study populations will also be selected for analysis, and will provide internal activation standards to provide for the specificity of the findings (i.e., control regions). All selected ROIs will be used as a mask to constrain the regions in which test for differences are conducted. These regions will be used as a mask in an overlay on functional datasets to test the hypotheses. Only areas with significant F-values on the ANOVA and falling within these regions will be used in statistical analyses. There will be 3 event types included in the model, resulting from the combination of all possible stimulus and response conditions: Visceral-Low distention (25-mm Hg), Visceral-High distention (50-mm Hg), and the Somatic stimulus. Voxel-by-voxel analyses will be conducted primarily to ensure that unpredicted differences a priori ROIs which are significant are not overlooked.

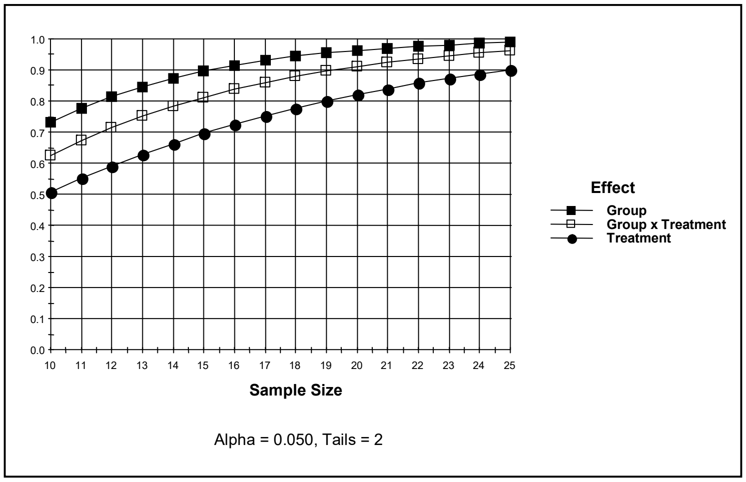


Figure 13: Study power as a function of sample size and effect.

B. Sample size calculations. The power calculations performed to assess the adequacy of the proposed sample size are based on the preliminary data, and are driven by the hypotheses proposed in Aim 2. These calculations assumed accrual of all subjects prior to the 10-week interval, a 10% attrition rate in each arm (2 subjects per group) over the course of the trial, observed group mean differences in blood flow within the anterior cingulate cortex from the IBS subjects studied in the Preliminary Studies section, and the previously reported post-desipramine reductions in ACC activations (effect size = 0.33).[33] With the proposed sample size (n=20 per group), the anticipated 2-way ANOVA design examining the fMRI data with one between-subjects factor (Group) would be powered at 0.95 for detecting the anticipated difference using a conservative treatment effect size of 0.33, while assessment of Group x Treatment effect would be powered at 0.82 (figure 12). This sample size would allow the detection of a desipramine effect size of 0.25 at $p<0.01$, and also permit the interpretation of predicted non-significant interactions as consistent with the stated hypotheses. These power calculations were conducted using SamplePower v2.0 (SPSS, Inc).

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