CHARACTERISTICS OF SOMATIC PAIN SENSITIZATION IN IRRITABLE BOWEL SYNDROME

By

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This document is dedicated to my family for their unending support.
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I thank my mentor, Andre P. Mauderli, for patiently guiding me through this journey.
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CHARACTERISTICS OF SOMATIC PAIN SENSITIZATION IN IRRITABLE BOWEL SYNDROME

By

Anthony Carl Rodrigues

August 2005

Irritable Bowel Syndrome is a common gastrointestinal disease which is often associated with extra-intestinal abdominal pain. Abnormalities in visceral perception have long been reported, but conflicting results have clouded similar findings on somatic pain perception. Recent results with IBS patients, as well as results from a relevant animal model, reveal an increase in pain sensitivity throughout the body, including in areas segmentally distant from the gut. The results of the present study suggest that the spatially diffuse somatic hyperalgesia is independent of the presence/absence/intensity of current visceral pain in IBS subjects and is not diminished by topical rectal lidocaine administration.
CHAPTER 1
GENERAL INTRODUCTION AND BACKGROUND

The clinical and socioeconomic importance of pain cannot be overestimated because it is the primary motivator for the utilization of the health care system by accounting for over 70 million physician visits annually (Turk 2002). Besides the large number of acute pain problems, an estimated 50 million people in the United States suffer from chronic pain (Joranson 1994). Medications used to treat the pain are the second most frequently prescribed class of drugs, and over the counter analgesics are the most popular non-prescription medicines (Isaacson 2002). The currently available pain medications typically are effective against acute pain that is caused by peripheral tissue injury, while many chronic pain conditions remain difficult to treat. In chronic pain the perceived intensity often has no clear relationship with the severity of tissue pathology in the region where the pain is perceived. In fact, it is possible that pain is present in an anatomical area where obvious pathology cannot be found. The development of pharmacotherapeutic agents of predictable efficacy against chronic pain has been stifled by the fact that the underlying mechanisms are largely unknown. It is becoming increasingly clear that models of pain where the causative input signal, the transmission of the signal to the central processor, and the processing / perception stage are organized in a linear hierarchical manner are inadequate for studying chronic pain. It is more likely that chronic pain can be explained by a network concept where functions contributing to the interpretation of a signal are widely distributed throughout the CNS, autonomic-, and immune system and are interconnected through a multitude of neural and endocrine
channels. In this model not only peripheral nociceptive inputs but any event that changes the operational steady state of the network has the potential to result in pain. A first step toward a better understanding of chronic pain mechanisms is to identify factors that change the way pain signals are processed and the time constants of these changes. Any chronic pain condition where pain does not heavily depend on nociceptive inputs from the periphery, e.g., Irritable Bowel Syndrome (IBS) and Myofascial Pain Syndrome (MPS), may serve as a model for the investigation of factors that may lead to a persistent and widespread increase in pain sensitivity.

IBS patients typically present with unpleasant sensations in the bowel, such as spasms and bloating and recurrent episodes of abdominal pain. MPS, on the other hand, is a regional pain syndrome where the pain is predominantly localized in muscles of the upper body (masticatory muscles, shoulder girdle, neck region) or the lumbar region. MPS may be characterized by the presence of focal points of pain hypersensitivity (trigger points) in muscles and/or connective tissues. Both IBS and MPS are frequent comorbidities in patients with fibromyalgia syndrome (Whorwell et al., 1986; Veale et al., 1991; Whitehead et al., 2002). MPS, IBS as well as FMS are all known to be associated with hyperalgesia and/or allodynia that extends to symptom-free parts of the body. This raises the question whether the difference between regional and generalized chronic pain disorders is more of a quantitative rather than qualitative (mechanistic) nature and a transition is possible from a regional to a generalized chronic pain condition.

IBS was chosen as the model in this series of studies to investigate whether and how a regional pain disorder can render a patient pain-prone anywhere in the body and potentially set the stage for the development of a generalized pain problem such as
fibromyalgia syndrome (FMS). IBS was originally described as a functional gastrointestinal disorder thought to be limited to the gut. Early research therefore focused on visceral pain phenomena by probing pain sensitivity in the rectum. Many found that rectal sensitivity was higher in the IBS population (Mayer and Gebhart, 1994; Mertz et al., 1995; Naliboff et al., 1997). Infection or inflammation injury (McKendrick and Read, 1994; Gwee et al., 1996), stress (Locke, III et al., 2004) and abnormal central pain processing (Gebhart 2000) were proposed as possible causative factors. Later it was discovered that often hypersensitivity was not limited to the gut: many patients with IBS had pain complaints in body regions distant from the gut, e.g., the head and neck (Whorwell et al., 1986). In addition, several recent studies have demonstrated that IBS patients are hyperalgesic to nociceptive stimuli (hot and cold) applied to cutaneous areas of the hands and feet (Verne et al., 2001; Bouin et al., 2001; Verne et al., 2003a). Therefore, it was concluded that a central mechanism must lead to hyperalgesia in remote asymptomatic regions and possibly set the stage for a spread of the symptoms (Mayer and Raybould, 1990; Mayer and Gebhart 1994). In the present series of studies two alternate hypothetical mechanisms were considered: (1) A feedback-based mechanism where a nociceptive focus, localized in the symptomatic area, is responsible for inducing and maintaining a sensitized state which, through propriospinal or other convergent projections, expands into asymptomatic regions (“vicious pain cycle” hypothesis); (2) a concept where generalized hypersensitivity precedes the onset of the regional chronic pain disease and renders the patient prone to developing a chronic pain problem upon even a minor local insult that would otherwise only lead to a transient problem. One can
speculate that a generalized hyperalgesic state could be the result of plasticity induced by an insult early in life, and/or by genetic factors.

The vicious cycle hypothesis finds support in neurophysiologic studies that demonstrated that prolonged nociceptive activity can cause second order neurons to become more sensitive to incoming signals from primary afferents (Price et al., 1977; Woolf 1996). Some of the mechanisms involved in feedback-based sensitization rely on NMDA receptor activation in the spinal dorsal horn and are known to affect not only the area of the nociceptive focus but also its surround. The spread of sensitization in part may be mediated by diffusion of sensitizing molecules such as nitric oxide. The sensitization may manifest itself in distant healthy tissues when the nociceptive afferents from these tissues project to second order neurons that are sensitized by convergent inputs from elsewhere. Feedback-based sensitization was demonstrated in reflex sympathetic dystrophy (RSD) patients who complained of mechano-allodynia in areas surrounding the initial site of pain (Gracely et al., 1992). Blocking the nociceptive input with either a cuff block or local anesthetic caused the central processing of the secondary areas to revert to normal, abolishing the symptoms for the duration of the block (Gracely et al., 1992). In addition, a case study of an MPS patient with elevated sensitivity not only in the face but also on the arm suggested that the sensitized region can extend many segments beyond the level of the local (facial) pain generator (Fillingim et al., 1998). The case study found that sensitization resolved upon therapeutic intervention with an oral NMDA receptor antagonist pointing to a hyperalgesic state maintained by nociceptive input.
Whether or not a vicious cycle is the most likely mechanism leading to central pain sensitization in IBS patients depends in part on answers to the following questions: (1) Is the level of pain hypersensitivity inversely related to the segmental distance from the visceral pain focus (gradient effect)? (2) Can experimentally-induced sensitization help to model and explain the long term sensitization that is typical for IBS patients? (3) Are the mechanisms that induce or maintain these sensitized states related to the magnitude of clinical pain? (4) Can local anesthesia that is directed at the putative nociceptive focus initiate reversal of the widespread somatic pain sensitization? A question related to the alternate hypothesis was the following: can a visceral insult early in life induce a lifelong sensitized state that extends to somatic tissues?

The study described in chapter 2 tested the vicious pain cycle hypothesis by determining whether sensitization of IBS patients follows a spatial gradient by being progressively less pronounced with increasing segmental distance from the presumed nociceptive focus in the gut. Pain sensitivity to brief thermal stimuli was sampled in widely spaced test locations across the body, i.e., on the calf where direct viscero-somatic convergence with the symptomatic gut can be expected and on two remote areas (forearm and cheek) with differing distances from the pain generator. According to the hypothesis, it was expected that hyperalgesia of IBS patients would be large on the calf and small on the cheek. If a putative local pain generator is the basis of the phenomenon, one would also expect that the degree of sensitization would correlate with the amount of clinical pain. However, complicating this correlation is the fact that low levels of nociceptor activity may not always be perceived as painful, yet be sufficient to maintain a feedback-based cycle of sensitization. Furthermore, it cannot be ruled out that the time constant of
the vicious cycle based sensitization is so slow that a constant flow of nociceptive activity is not necessary to maintain the sensitized state. Spontaneous pain therefore may have to be sampled not only during each test session but over a longer period of time. This was accomplished by using an automated telephone system that allowed the subjects to communicate information regarding their pain and other symptoms on a regular basis over many days.

The research described in chapter 3 attempted to model the putative feedback-based sensitization of chronic pain patients with experiments that used prolonged thermal stimuli. In IBS patients and normal controls an experimentally-induced sensitization was superimposed over disease-induced long-term sensitization (when present). The goal was to study how the disease affects the induction and maintenance of the experimental sensitization. The vicious cycle hypothesis would gain support if it were possible with prolonged experimental stimulation to induce long-lasting sensitization in healthy subjects.

The study described in chapter 4 investigated whether it is possible to interrupt the vicious cycle of pain with topical local anesthetic, thereby allowing the sensitized state to return to normal. The subjects rated the intensity of their clinical (disease related) pain and of experimentally induced pain before and after their presumed nociceptive focus had been targeted with a topical local anesthetic. The study included controls for potential systemic and placebo effects of the drug.

In these experiments, the following findings would support the vicious pain cycle hypothesis: (1) reduction of spontaneous visceral pain and experimentally measured somatic pain sensitivity beyond the expected duration of local anesthesia; (2) reduction of
experimentally measured somatic pain sensitivity following rectal lidocaine administration is most pronounced in patients with abdominal pain and in segmental proximity of this pain (i.e., the lumbo-sacral region). The “vicious pain cycle” hypothesis would have to be questioned if (1) the level of somatic hypersensitivity does not correlate with clinical pain; (2) baseline somatic hypersensitivity does not exhibit a spatial gradient; (3) rectal lidocaine does not reduce sensitivity to experimental pain in the IBS group; (4) anesthetic effect on somatic thermal pain sensitivity does not diminish in a gradient fashion with increasing segmental distance from the area of abdominal pain.

The study described in chapter 5 was conducted in a rodent model and addressed the alternate hypothesis that assumes that widespread sensitization \textit{predates} the onset of the chronic pain disease and makes the individual pain-prone and thus a likely candidate for future pain problems. The study investigated whether a visceral chemical insult early in life can mark the onset of lifelong pain hypersensitivity by inducing plastic changes in neural or endocrine components of the pain processing system. An operant escape assay was used to compare the responses of neonatally injured and naïve rodents to thermal nociceptive paw stimuli.
CHAPTER 2
HYPERSENSITIVITY TO CUTANEOUS THERMAL NOCICEPTIVE STIMULI IN IRRITABLE BOWEL SYNDROME

Introduction

Irritable bowel syndrome (IBS) is an intestinal ailment that may affect up to 20% of the U.S. population (Verne and Cerda, 1997). It is one of the most frequent gastrointestinal disorders seen by physician in the US, prompting up to 50% of referrals to gastroenterologists and as many as 3.5 million clinical appointments annually (Sandler 1990). In spite of its common nature, the pathophysiological mechanisms of IBS are not well understood. It is now established that most patients with IBS demonstrate hypersensitivity in response to distension of the gut lumen (Mayer and Gebhart 1994; Mertz et al., 1995; Naliboff et al., 1997). This hypersensitivity may account for typical IBS symptoms of urgency, bloating, and abdominal pain. Several causative mechanisms have been proposed for visceral hypersensitivity including inflammation injury (McKendrick and Read 1994; Gwee et al., 1996)), stress (Locke, III et al., 2004), and abnormal pain sensitization (Gebhart 2000). This study focuses on the contribution of abnormal pain sensitization due to positive nociceptive feedback (vicious pain cycle) that affects somatic tissues through viscero-somatic convergence. One could argue that the effectiveness of a feedback-based sensitization mechanism would decrease with increasing segmental distance from the nociceptive focus, and a recent report provides support for this argument (Verne et al., 2001). The sensitization of IBS patients was more pronounced on the feet (segmentally close to visceral pain focus) than the hands.
when a hot water immersion stimulus was used. Shortcomings of the cited report are the relatively poor spatial and temporal definition of the immersion stimulus and the fact that a third segmentally more distant site was not tested to confirm the putative sensitization gradient. This study revisits the issue with improved methodology. The specific objectives are to probe cutaneous thermal pain sensitivity along the segmental axis, including in dermatomes that are remote from the visceral pain focus. The hypotheses to be tested are (1) that IBS subjects have elevated somatic pain sensitivity and (2) that sensitization in IBS patients follows a gradient from lower to higher spinal segments, i.e., from the symptomatic region of the body toward the (asymptomatic) face. In other words, the study attempts to determine whether sensitization is most pronounced in symptomatic segments or alternatively, whether it is generalized without a segmental gradient. Pain sensitivity was probed with cutaneous thermal stimulation to the lower and upper extremities and the face. The stimuli were administered with a contact thermode in order to assure that size of the stimulated area and stimulus duration were clearly defined and identical in all locations.

Methods

Subject Recruitment

The recruitment and study procedures were approved by the University of Florida Institutional Review Board and the Veterans SCI Committee. Written informed consent was obtained from all participants. The criteria for members of the control group required absence of (1) significant spontaneous pain anywhere in the body, (2) ongoing pharmacotherapy with narcotics or antidepressants, (3) disease that might significantly affect pain perception or unduly increase risks (e.g., neurological disorders, serious psychiatric disorders, diabetes, hypertension, serious cardiovascular disorders, and
chronic pain diseases such as fibromyalgia syndrome). The criteria for the disease group required a diagnosis of ongoing IBS based upon the Rome II criteria (Thompson et al., 1999), supplemented by additional criteria: absence of other diseases (including other chronic pain diseases), risk factors, and ongoing drug treatments, as described for the control group. Patients diagnosed with Fibromyalgia Syndrome were excluded from the study. Initial screening consisted of blood pressure measurement, completion of a health questionnaire and --for IBS patients-- a physical exam, administered by an experienced gastroenterologist. Considering that all IBS patients (n=9, all diarrhea-predominant) were females of childbearing age, only female individuals of the same age bracket were recruited for the control group (n=12). Subject recruitment did not control for menstrual phase and for whether or not contraceptives were taken. There is no reason to believe that menstrual phase was not randomly distributed within groups and systematically different between groups. Synchronization of menstrual phases is most likely among females that live together, e.g. among college roommates (McClintock 1971).

Participants of our study were recruited from diverse social settings and had no close contact with each other. Test sessions were scheduled independently of whether or not IBS patients had an acute exacerbation of symptoms on the day of testing. Some but not all of the patients reported spontaneous pain or discomfort during some of the sessions.

Daily Protocol

Participants began each daily session of the three-day study by mapping and rating their spontaneous clinical pain. Subsequently, pain sensitivity was probed with thermal contact stimuli at three locations on the right side of the body: on the lateral aspect of the calf (same spinal segment as visceral symptoms, i.e., S1), the volar forearm (dermatomes C6, T1) and the cheek (V2, V3). Stimulus locations were reproducibly defined relative to
anatomical landmarks. Skin temperature at the stimulation site was measured with an Exergen Dermatemp infrared temperature scanner model DT-1001 (Exergen Corp., Watertown, MA, USA) before and immediately after each stimulus series.

**Pain Measurement**

Spontaneous clinical pain and experimental pain (induced by thermode) were measured with an electronic version of a visual analog scale (Price DD and Harkins SW, 1987). The electronic visual analog scale (eVAS) consisted of a low-friction sliding potentiometer of 100 mm travel. The left endpoint of the scale was identified as “no pain,” while the right endpoint was labeled as “intolerably intense pain.” There were no divisions between these two anchors. The position of the slider was electronically converted into a pain rating between 0 and 100%. The slider automatically returned to the left (“no pain”) position after each rating. The eVAS was mounted into the surface of a small inclined desk positioned to facilitate precise operation with minimal fatigue. The custom-built testing system integrated all inputs (temperature process value, eVAS signal) and outputs (stimulus temperature control, stimulus timing) and allowed automated execution of test protocols with preprogrammed parameters, including limits for temperature and pain intensity.

**Mapping and Rating of Clinical Pain**

At the beginning of all experimental sessions, subjects were asked to shade the locations of spontaneous pain on an anatomical diagram and to rank these sites according to pain intensity. Subsequently, the intensity of disease-related pain of the upper (head, neck, shoulder, upper back, arms, hands) and lower (low back, bowel, legs, feet) parts of the body were rated on the eVAS. The subjects were then asked to rate the unpleasantness of the single most intense clinical pain. All these ratings were required to
be below 5% (on the 0-100% eVAS scale) for subjects to be admitted to the control group.

**Thermal Stimuli**

Thermal stimuli were administered with a flat copper contact thermode of 23x23mm in size. The thermode was electronically held at the desired temperature by a Peltier thermoelectric device. It was brought into light skin contact of reproducible force by solenoid activation. A thermistor in the center of the thermode, very close to the surface, sampled the temperature during each skin contact to record potential temperature deviations resulting from the skin's heat sink effect. The stimulator assembly was mounted on an adjustable arm (comparable to those that support dental x-ray machines) for convenient and stable positioning for any desired stimulation site.

The relationship between stimulus temperature and pain intensity was assessed by rating series of stimuli across a pain intensity range from threshold to at least 45%. The thermode was preheated to the desired temperature before it contacted the skin. Contact duration was short (3 seconds), and the intervals between each stimulus were relatively long (30 seconds), to minimize sensitization. The temperature was set to 42.5°C (i.e., below pain threshold of healthy individuals) for the first stimulus, and increased in 0.7°C increments from one pulse to the next until a pain rating of 45% was reached (ascending temperature series). At that point, a descending temperature series of equal length was initiated. The series ended with the starting temperature of the ascending series (i.e., 42.5°C). The pain intensity was rated retrospectively within 5 seconds of the end of the stimulus, at which time the eVAS slider automatically returned to the left endpoint. It could be argued that randomized stimulus intensities should have been used to minimize
expectation-based response-bias. We chose to use the ascending and descending intensity series instead in order (1) to avoid uncomfortably high pain intensities and anxiety in subjects with unexpectedly high pain sensitivity and (2) to avoid unpredictable distortion of the stimulus response function due to effects of stimulus history on pain sensitivity (Lamotte and Campbell, 1978; Grill and Coghill, 2002).

Data Analysis

Analysis was performed with SPSS computer software. A two-parameter regression function ($pain\ intensity = exp \ (-A-B*temperature)$) was used to estimate the stimulus-intensity response relationship of each subject. Subsequent comparisons used an ANOVA model and were limited to three representative points on the stimulus-intensity response curve, i.e., 10% (near threshold), 20%, and 40% pain intensity. Pain intensities up to 40% were used because the intensity of disease related pain of IBS patients tends to be within this range. We argue that a test paradigm is most likely to engage pathophysiologically relevant mechanisms (and thus to produce clinically relevant data) when it represents disease-typical pain characteristics and takes into account the possibility that some modulatory mechanisms might be active only within a certain signal intensity range. Therefore, comparisons of pain sensitivity were made not only near pain threshold but also at higher intensities.

The two-parameter regression function was also fitted to the pooled data of each group (all three test sessions combined) in order to generate stimulus-intensity response curves representative of the entire groups (Figure 2-1).

Results

Nine diarrhea-predominant female IBS patients (age range 21-53, mean age: 36.3 years) and 12 healthy female controls (age range 19-45, mean age: 27 years) successfully
completed the study. There was no statistically significant age difference between the groups (p=0.345). The two groups differed in their clinical pain ratings but not in their pretest blood pressure or heart rate. None of the control subjects had any spontaneous pain anywhere in the body. For the IBS group the mean spontaneous pain ratings for the upper part of the body was 9.5% (standard deviation=5%), 20% for the lower part of the body (standard deviation=14%), and the mean unpleasantness rating for the most intense spontaneous pain site was 22% (standard deviation=17%).

In many cases the stimulus-intensity response curves of the ascending and descending series did not overlap. Furthermore, the direction and magnitude of hysteresis exhibited no discernible pattern. At the three representative points (10, 20 and 40% pain intensity), the ascending and descending series did not differ to a statistically significant degree. There were no significant differences in pain sensitivity between the three test sessions. Therefore, for most analyses, the data of the ascending and descending series of all three test sessions were pooled.

Thermal sensitivity of the IBS group was significantly higher in all three areas tested (calf, forearm, and cheek): the temperatures necessary to reach pain levels of 10%, 20%, and 40% were significantly lower (p<.001) in IBS patients compared to the control group. The data indicate that sensitization was not limited to symptomatic dermatomes (L4-S2) but extended across the body, including the face (Figure 2-2). The results do not suggest that the presence of disease (IBS) leads to a more pronounced sensitization in lower segments (no sensitization gradient). Figure 2-2 shows that in both groups pain sensitivities of the upper and lower extremities were similar. The face of control subjects appears to be slightly less sensitive (higher stimulus temperature needed) than the other
locations; however, this trend is not statistically significant. Also, the difference between IBS and control groups did not depend on the evoked pain intensity level, i.e., the degree of sensitization of IBS patients was similar near threshold (10%) and at higher intensities.

The idea that the higher pain ratings of IBS subjects could be due to abnormal skin temperature regulation was considered. Pre- and post-stimulation skin temperature data collected with an infrared scanner speak against this possibility. The pre-stimulation skin temperatures and stimulation-induced temperature changes did not differ to a statistically significant degree at any of the stimulation sites between the two groups (Table 2-1).

Lastly, no correlation was found between IBS subjects’ pain sensitivity of any of the three test sites and their ratings (intensity and unpleasantness) of spontaneous pain.

**Discussion**

The results support hypothesis 1, which states that IBS patients have higher somatic heat pain sensitivity compared to healthy pain free individuals, and this confirms what others have found (Verne et al., 2001; Verne et al., 2003a). Pain sensitization of IBS patients may not be limited to heat but extend to nociceptive cold: exaggerated sensitivity to cold was reported in a study where the stimulus consisted of immersion of the nondominant hand into 4°C cold water, and where the diagnosis of IBS was based upon the Rome II criteria (Bouin et al., 2001). In contrast, a group of investigators that used modified Manning criteria (Manning et al., 1978) to define their IBS group failed to confirm this finding (Whitehead et al., 1990). Their IBS group exhibited reduced tolerance to visceral distension but not to a stimulus consisting of immersion of the hand into ice water. The authors did not report the temperature of the water or whether or not it was stirred to prevent thermal layering. Likewise, Zighelboim and coworkers failed to find cold hypersensitivity in a hand immersion experiment using a water temperature of
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They used a cohort of IBS patients (Rome criteria) with minimal or no recent symptoms and considered the possibility that the absence of somatic sensitization may have been due to insufficient severity of the disease. It could be argued that the disagreement resulted from the fact that Zighelboim’s 12 °C water immersion stimulus was not rated as painful but as of “moderate discomfort”, while Bouin’s 4 °C immersion stimulus elicited pain. In summary, at least two possible factors that might explain the different results of the cited studies must be considered: (1) the mechanism that renders IBS patients more sensitive may be effective below pain threshold only for visceral stimuli but be specific to the nociceptive range for somatic stimuli; (2) multiple disease mechanisms may lead to the same diagnosis of IBS, and the diagnostic criteria used may not adequately discriminate between them. Therefore, the results of similar studies may differ due to different representation of subgroups in the samples used. In this case, thermo-cutaneous nociceptive testing might be useful for discriminating between subgroups of IBS patients and have the potential to evolve into a diagnostic tool.

Our data suggest that pain sensitization of IBS patients is similar along the entire segmental axis and independent of the magnitude of disease-related spontaneous pain. These results can be interpreted as a pain sensitization or disinhibition phenomenon that is caused by a systemically or diffusely acting factor, and not by a localized vicious cycle. These findings are in disagreement with an earlier IBS study that reported a higher degree of sensitization on the foot than on the hands (Verne et al., 2001). The study used the same selection criteria for IBS patients as the present study, however, the thermal stimulus consisted of hot water immersion of hand and foot. This methodological difference offers a possible explanation for the difference in results: immersion of the
foot leads to a larger stimulated area than immersion of the hand, and it cannot be ruled out that IBS subjects are more sensitive to spatial summation. Furthermore, water immersion leads to stimulation of hairy and glabrous skin, and the relative surface area of the two skin types may be different for the immersed hand and foot. Thermal layering is difficult to avoid with fluid immersion stimuli, and it may be different on hairy and glabrous skin. The use of contact stimulation in the present study eliminated these potentially confounding factors: the skin surface temperature was precisely maintained by a feedback-controlled Peltier device; the uncertainty of the actual skin surface temperature was reduced by sampling the temperature during each stimulus; the stimulated skin was of the hairy type only, not a mix of hairy and glabrous skin as was the case in the water immersion study, and the stimulated area was identical in size at all locations. The probing of sensitivity, by including the face, extended over a larger segmental area facilitating reliable detection of potential gradient effects.

Our findings of spatially diffuse, widespread pain sensitization of IBS patients are incompatible with hypothesis 2, which states that sensitization of IBS patients is caused by a vicious pain cycle that is most effective in close segmental proximity to the area of spontaneous pain. However, the results are consistent with the clinical observation that many IBS patients, in addition to intestinal symptoms, are prone to widespread pain problems that can range from muscle pain to headaches (Whorwell et al., 1986). An unanswered question is whether the mechanism of sensitization targets the central nervous system or peripheral tissues. Our results combined with the data of others may shed some light on the issue: studies that used electrical stimuli to the hands or gut (and thus bypassed the peripheral receptor level) failed to find sensitization or --to the contrary
-- reported elevated detection (Accarino et al., 1995) and pain thresholds in IBS patients (Cook et al., 1987). This raises the question of whether abnormal pain sensitivity of IBS patients is the net effect of two antagonistic processes: a central inhibitory process (possibly DNIC) and a diffuse (possibly endocrine) sensitization mechanism affecting peripheral receptors (visceral and cutaneous). A testing method that bypasses receptors (electrical stimulation) would be expected to reveal the central inhibitory effect, while a receptors-mediated stimulation method would provide a measure of the net effect of both mechanisms.

The finding of uniformly increased pain ratings, independent of stimulus location or intensity requires us to examine the alternate hypothesis of a generalized shift in rating bias of IBS patients, rather than a change in the sensory signal that reaches the conscious level. This issue has not been specifically addressed by this and the cited studies, however, preliminary insights may be possible based upon the literature. It appears that the exaggerated sensitivity of IBS patients is specific to certain stimulus characteristics: sensitization has been demonstrated with nociceptive hot (Verne et al., 2001) and nociceptive cold (Bouin et al., 2001) but not by electrical (Cook et al., 1987; Accarino et al., 1995) and not by uncomfortable yet non-painful cold (Zighelboim et al., 1995) stimuli to the skin. This stimulus-specificity speaks against the notion of a mere rating bias or hypervigilance as an explanation for higher intensity ratings of stimuli by IBS patients, as compared to healthy individuals. The fact that ratings of visceral and cutaneous stimuli return to normal when IBS patients are treated with rectal lidocaine (Verne et al., 2003b) or systemic fentanyl (Lembo et al., 2000) further suggests that the
higher ratings of IBS patients are not due to a non-specific shift in rating bias but to the result of pain sensitization.

The picture that emerges from this discussion prompts us to propose that diffuse sensitization to painful visceral and somatic stimuli is a characteristic of at least a subpopulation of IBS patients, and it appears that the sensitization mechanism involves the peripheral (receptor) level.

Table 2-1. Skin temperature (°C) of each stimulation site

<table>
<thead>
<tr>
<th></th>
<th>CHEEK mean</th>
<th>CHEEK stdev</th>
<th>FOREARM mean</th>
<th>FOREARM stdev</th>
<th>CALF mean</th>
<th>CALF stdev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-stim temp</td>
<td>Ctrl 32.8</td>
<td>1.0</td>
<td>Ctrl 31.3</td>
<td>1.3</td>
<td>IBS 32.9</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>IBS 32.9</td>
<td>0.6</td>
<td>IBS 31.0</td>
<td>0.7</td>
<td>IBS 31.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Stim-induced increase</td>
<td>Ctrl 4.9</td>
<td>1.0</td>
<td>Ctrl 7.1</td>
<td>1.9</td>
<td>IBS 4.4</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>IBS 4.4</td>
<td>1.0</td>
<td>IBS 7.5</td>
<td>1.2</td>
<td>IBS 6.5</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Note: The skin temperature of each stimulation site was measured with an infrared scanner immediately before and after each stimulus series in order to determine whether IBS and control subjects had systematically different baseline skin temperature, and whether their skin exhibited different heat storage characteristics. Neither the baseline temperatures nor the stimulation-induced temperature increase of the two groups were different to a statistically significant degree.
Figure 2-1. Comparison of stimulus-intensity response curves of control and IBS groups, obtained at three segmentally widely spaced stimulus locations. The curves were obtained by fitting a two-parameter regression function to the pooled data of all three test sessions of all subjects within each group.
Figure 2-2. Comparison (groups and stimulus locations) of heat pain sensitivity represented by the group mean temperature needed to elicit pain of 40% (top chart), 20% (intermediate), and 10% (bottom) on the visual analog intensity scale.

First, a two-parameter regression function was fitted to the pooled ascending and descending stimulus-intensity response data of each individual subject. The regression function was used to obtain the 10, 20 and 40% data points for each subject. Subsequently, the stimulus temperature group means and standard deviations were calculated for the three respective pain intensity levels.
CHAPTER 3
CUTANEOUS PAIN SENSITIZATION CHARACTERISTICS IN IRRITABLE BOWEL SYNDROME

Introduction

Irritable bowel syndrome (IBS) leads to episodic bowel dysfunction, intestinal discomfort and occasionally pain. Furthermore, patients with IBS, when compared with healthy individuals, appear to be more pain prone in non-visceral areas of the body (Whorwell et al., 1986). This is consistent with experimental findings of exaggerated sensitivity to painful stimuli in non-visceral tissues of IBS patients. Recent work suggests that somatic hyperalgesia is not limited to dermatomes that have known convergent projections with symptomatic visceral tissues: sensitization extends evenly along the segmental axis and affects the face as much as the lower extremities (Rodrigues et al., 2005). This pronounced and widespread somatic sensitization, which has been found even during periods of symptom remission, may offer an explanation for the frequent non-visceral pain conditions in areas of the body far removed from the bowel (e.g., headaches) of IBS patients (Whorwell et al., 1986). Widespread somatic sensitization is not unique to IBS: it has been reported for myofascial pain syndrome (MPS) and fibromyalgia syndrome (FMS) as well (Maixner et al., 1998; Staud et al., 2001). It may help explain why these patient groups tend to be pain prone across the entire body. The factors that lead to this sensitization are not well understood. The following questions to date have not been answered satisfactorily: (1) do the mechanisms that induce, maintain, and reverse these sensitized states differ between subgroups of
patients? And consequently, (2) is the demonstration of somatic sensitization of diagnostic relevance, i.e., is it a differentiating feature for certain subgroups of patients that will require a special therapeutic approach to prevent or reverse widespread chronic pain?

A first step toward answering these questions is to define and categorize the sensitization phenomena seen in these patient groups. A second step, which promises to reveal insights regarding the underlying mechanisms and thus point to therapeutic targets, is to test how these phenomena respond to specific pharmacological probes. The present study is limited to the first step and uses IBS as a model for studying persistent and widespread somatic sensitization phenomena. The basic methodological approach of the study is “signal tracking”. It is a commonly used method for the diagnosis of faulty electronic circuitry, however, it may hold promise in a biological setting as a diagnostic tool in diseases involving functional changes within the pain processing system. Signal tracking injects a defined signal (“stimulus”) and measures the input-output relationship of the processor. Even though the mechanisms of disease-related and stimulus-evoked pain may not always completely overlap, signal tracking may reveal abnormalities that also contribute to chronic pain. Diagnostic criteria derived from this approach are based upon amplitude- and temporal aspects of the input-output function that is detected at strategic points of the circuit. The first step is to create a library of input-output functions found in normal and diseased populations. Interpretation of a sufficiently large data pool, obtained with a battery of stimulus profiles and with outputs sampled at different locations of the system, may allow dividing the patient pool into therapeutically relevant subsets. Mechanistic insights can be expected from a second step where subgroup-
specific psychophysical phenomena will be studied in the presence of specific pharmacological probes.

A number of investigators have focused on the first step by using a variety of visceral and somatic stimuli and different types of psychophysical response measures on IBS patients and matching controls. Studies that measured sensitivity took snapshots of the amplitude aspect of the input-output relationship while others recorded how sensitivity changes over time, i.e., they measured sensitization. Studies of visceral sensitivity analyzed pain responses to rectal and jejunal mechanical or electrical stimuli. Investigations of somatic sensitivity measured pain evoked by cutaneous electrical or thermal stimuli. Visceral sensitivity was increased in some but not all patients that met the diagnostic criteria for IBS (Zighelboim et al., 1995; Mertz et al., 1995). Somatic sensitivity was abnormally high in most IBS patients when stimuli were receptor-mediated and painful (heat, nociceptive cold) but not when they bypassed the receptor level (electrical) or were non-painful (Cook et al., 1987; Accarino et al., 1995; Rodrigues et al., 2005). The ability to discriminate mechanistically may be enhanced by tests that include temporal aspects of the pain processor’s input-output relationship (rate of temporal integration). A recent study (Munakata et al., 1997) used individual stimuli to sample rectal sensitivity and found hyperalgesia only in a subset of IBS patients. However, when repetitive sigmoid stimulation was used to compare the degree of temporal integration of IBS and healthy subjects, all IBS- but no control subjects developed hyperalgesia throughout the course of the prolonged stimulus series. Thus, it appears that an abnormal induction process of transient visceral sensitization goes hand in hand with the clinical diagnosis of IBS in most cases, while persistent changes in baseline
visceral sensitivity may be a defining feature of only a subset of IBS patients. The present study continues this work by analyzing and comparing the characteristics of cutaneous nociceptive temporal integration in samples of IBS patients and matched healthy control subjects.

Temporal integration has been studied by others in MPS and FMS (Maixner et al., 1998; Staud et al., 2001) using thermal wind-up paradigms. Sensitization was inferred from the increase in pain ratings throughout series of brief thermal stimuli of equal intensity. The duration of these experiments was short to avoid unacceptably high pain intensities or tissue damage. It was therefore a method appropriate for analyzing feedback-based sensitization phenomena that occur early during stimulus exposure. Traditional wind-up experiments are likely to reveal phenomena related to the early induction phase of persistent states of sensitization. They do not provide much information about the mechanisms responsible for long-term maintenance of sensitization. The present study focuses on the conditions needed in IBS patients and healthy subjects to maintain an already established sensitized state and on thermoregulatory consequences of prolonged thermal nociceptive stimulation. This will include addressing specific questions like (1) how does a sensitized state, induced by pulsed thermal stimulation, respond to changes in pulse duration or interval; and (2) are the stimulus conditions required for maintaining a sensitized state different depending on the presence or level of disease-related pain?
Methods

Subjects

The subjects were the same as those used for the experiments described in Chapter 2. Recruitment and study procedures were approved by the University of Florida Institutional Review Board. Written informed consent was obtained from all participants. The criteria for members of the control group required (1) no significant spontaneous pain anywhere in the body, (2) no ongoing pharmacotherapy with narcotics or antidepressants, (3) no disease that might significantly affect pain perception or unduly increase risk of injury (e.g., neurological disorders, serious psychiatric disorders, diabetes, hypertension, serious cardiovascular disorders, and chronic pain diseases such as fibromyalgia syndrome). The criteria for the disease group required a diagnosis of ongoing IBS based upon the Rome II criteria (Thompson et al., 1999), supplemented by additional criteria: absence of other diseases (including other chronic pain diseases), risk factors, and ongoing drug treatments, as described for the control group. Patients with any condition where spontaneous pain—according to the definition of the American College of Rheumatology (Wolfe et al., 1990)—was widespread were excluded from the study. This ruled out the participation of subjects that met the ACR diagnostic criteria for fibromyalgia syndrome. Initial screening consisted of blood pressure measurement, completion of a health questionnaire and—for IBS patients—a clinical diagnosis by a physician. Considering that all IBS patients (n=8, all diarrhea-predominant) were females of childbearing age, only female individuals of the same age bracket were recruited for the control group (n=10). Subject recruitment did not control for menstrual phase and for whether or not contraceptives were taken. There is no reason to believe that menstrual phase was not randomly distributed within groups and systematically
different between groups. Synchronization of menstrual phases is most likely among females that live together, e.g., among college roommates (McClintock 1971). Participants of our study were recruited from diverse social settings and had no close contact with each other. Test sessions were scheduled independently of whether or not IBS patients had an acute exacerbation of symptoms on the day of testing. Some but not all of the patients reported spontaneous pain or discomfort during some of the sessions. All subjects were right-handed.

**Pain Measurement**

Spontaneous clinical pain and experimental pain (induced by thermode) were measured with an electronic version of a visual analog scale (Price DD and Harkins SW 1987). The electronic visual analog scale (eVAS) consisted of a low-friction sliding potentiometer of 100 mm travel. The left endpoint of the scale was identified as "no pain", while the right endpoint was defined as "intolerably intense pain". There were no divisions between these two anchors. The position of the slider was electronically converted into a pain rating between 0 and 100%. The slider automatically returned to the left ("no pain") position when so required by the protocol. The eVAS was mounted into the surface of a small inclined desk positioned to facilitate precise operation with minimal fatigue. The custom-built testing system integrated all inputs (temperature process value, eVAS signal) and outputs (stimulus temperature control, stimulus timing) and allowed automated execution of test protocols with preprogrammed parameters, including limits for temperature and pain intensity.

**Response-Dependent Stimulation Method**

Thermal stimuli were administered with a flat copper contact thermode of 23x23mm in size. The thermode was electronically held at the desired temperature by a
Peltier thermoelectric device. It was brought into light skin contact of reproducible force by solenoid activation. Series of brief contacts were administered by periodically turning the solenoid on and off. The duration of each contact, the interval between contacts (Inter-Stimulus Interval, ISI) and the number of contacts of each series was preprogrammed in the stimulator control software, allowing fully automated data collection. Unlike most pain tests, the stimulation / data acquisition system used in this study did not define the stimulus as the dependent variable and the subject’s rating of the pain as the dependent variable. Similarly to a method described by Gracely et al., it reversed this arrangement by linking the subject and the stimulator in a closed proportional control loop (Figure 3-1) (Gracely et al., 1988). A pain intensity setpoint was defined, and an algorithm in the stimulator control software calculated the deviation of the patient’s actual pain rating from the setpoint as well as the derivative of this error. These data were the basis for automatic adjustments of the stimulus temperature to maintain an average pain rating that equaled the setpoint. The methodology has much in common with an autopilot that keeps altitude or course of an aircraft near the desired setpoint. Response-dependent stimulation (REDSTIM) allows prolonged stimulation without the risk of an escalation of pain intensities to intolerable levels. Furthermore, it is more likely to engage clinically relevant mechanisms, because –as is the case in chronic pain—it maintains low to midrange signal intensities over prolonged periods of time.

**Testing Protocol**

**Measurement of spontaneous pain**

At the beginning of all experimental sessions, subjects were asked to shade the locations of spontaneous pain on an anatomical diagram and to rank these sites according to pain intensity. Subsequently, the intensity of disease-related pain of the upper (head,
neck, shoulder, upper back, arms, hands) and lower (low back, bowel, legs, feet) parts of
the body were rated on the eVAS. The subjects were then asked to rate the
unpleasantness of the single most intense clinical pain. All these ratings were required to
be below 5% (on the 0-100% eVAS scale) for subjects to be admitted to the control
group.

Measurement of skin temperature

Skin temperature at the stimulation site and the corresponding contralateral site was
measured with an Exergen Dermatemp infrared temperature scanner model DT-1001
(Exergen Corp., Watertown, MA, USA) before and immediately after each stimulus
series.

Thermal stimulation

Thermal stimulation was conducted during three separate and identical daily
sessions. Each session included thermal tests designed to collect snapshots of pain
sensitivity. These protocols and data are presented in chapter 2. The present chapter
focuses on experiments that used prolonged series of stimuli and REDSTIM
methodology. During each session a separate REDSTIM experiment was conducted on
the thenar eminence of each hand.

Experiment 1. The thenar eminence of the left hand was the site for an experiment
that used four series of 25 brief thermal contact pulses each. The interval between stimuli
(ISI) was 3 sec throughout the experiment. The only difference between the series was
stimulus pulse duration. It was 1.0 sec for the pulses of series 1 and 3, 0.8 sec for series 2
and 4 (Figure 3-2 A, Table 3-1). As was discussed earlier, REDSTIM methodology
automatically adjusted stimulus temperature in order to maintain a constant average pain
intensity level throughout the experiment (35% on the visual analog scale). The goal was
to assess how much the stimulus temperature (series average) would have to change to compensate for the change in pulse duration from one series to the next. The eVAS slider automatically returned to the “no pain” position at the end of the fourth series.

**Experiment 2.** It began 3 minutes after the end of the first experiment and used the thenar eminence of the right hand as the stimulation site. Like the first experiment it consisted of four series of 25 thermal pulses each, without interruption between series. Consistent with REDSTIM methodology, the temperature was automatically adjusted from pulse to pulse in order to maintain a constant average pain intensity rating (35%) throughout the experiment. Duration of all stimulus pulses for the entire experiment was 0.9 sec. ISI, however, was subject to change from series to series. It was 2.5 sec during the first and third series, 3.5 sec during the second and fourth series (Figure 3-2 B, Table 3-1). The goal was to determine how much the stimulus temperature (series average) would have to change to compensate for the change in ISI from one series to the next.

**Induction phase.** The temperature needed to maintain the pain intensity setpoint under given circumstances varies from individual to individual and even more so between the IBS and control groups. The stimulator software was designed to find the individually appropriate temperature automatically during an induction phase that preceded series 1 of each experiment using the pulse duration and inter-stimulus interval parameters of the first series. Since these parameters were different for the induction phases of the two experiments, it is important to be aware that the cumulative stimulus duration per ten seconds was larger for experiment 2 (3.6 seconds of stimulation compared to 3.3 seconds). Therefore, the induction phase of the second experiment was expected to be shorter. However, past the induction phase, the cumulative stimulus duration was equal
for series 1 through 4 (90 seconds) The induction series began with a 43°C pulse, which was never perceived as painful by any subject. The temperature then increased from pulse to pulse in 1°C increments until the pain intensity rating reached 10% on the electronic visual analog scale. Thereafter, the temperature continued to rise at a reduced rate (0.5°C / pulse) if the pain intensity did not continue to increase over a period of 3 consecutive pulses. The temperature remained unchanged from pulse to pulse if pain intensity continued to increase across triplets of pulses. During the induction phase, the thermode temperature could either remain the same or increase; it could not decrease. The induction phase ended when the pain intensity rating first reached the setpoint of 35% on the eVAS. At that point, temperature modulation became bidirectional, in proportion to the deviation from setpoint, and the tests consisting of 4x25 pulses, as described above, began.

**Transition phase.** Preliminary experiments had shown that an abrupt large change of pulse duration or ISI from one series to the next could lead to a large deviation from the pain intensity setpoint. To minimize such fluctuations a transition period of 4 pulses was interposed between series. The total change of pulse duration or ISI between series was divided up between the 4 transition pulses, resulting in small, almost unnoticeable steps and preventing sudden changes in pain intensity.

**Oscillations around setpoint.** A well tuned proportional control system is expected to approach a new setpoint with a damped oscillation which eventually transitions into a new steady state. It soon became clear that in the case of REDSTIM, oscillations around the setpoint are poorly damped and often continue indefinitely. The most plausible explanation is the effect of “offset analgesia” (Grill and Coghill 2002):
when the stimulation control algorithm reduces the thermode temperature in order to return a high pain rating to setpoint it triggers an “offset analgesia” event and pain intensity drops disproportionately to near zero levels. This prompts the REDSTIM system to incrementally increase temperature again. The subject, while in “offset analgesia”, will not perceive a corresponding pain increase. When the “offset analgesia” ends (after about 3 - 5 seconds) the pain rating will suddenly “catch up” and often overshoot the setpoint. The above-setpoint error will trigger a new drop in stimulus temperature and mark the beginning of a new cycle of the pain intensity oscillation. This phenomenon could be minimized but not completely eliminated by proper choice of gain and time constants within the control algorithm. The oscillations had the advantage that they blinded the subject regarding the setpoint and possibly led to a more realistic model of chronic pain, which often fluctuates in intensity. The REDSTIM method, unlike most traditional pain tests, provided the opportunity to monitor the subject’s rating reliability by comparing the mean eVAS ratings across a series with the setpoint. A mean rating error >4% was grounds for exclusion of the series from data analysis.

**Data Analysis**

The following factors were included in the analysis:

1. **Group** (IBS patient, healthy control)
2. **Day** (each test was repeated on three different days)
3. **Time** (skin temperature was measured before and after stimulation)

The following response variables were used:

**Skin temperature:** Pre-stimulus skin temperature (a) at stimulation site and (b) at corresponding contralateral site; (c) Skin temperature recorded after end of stimulus series at stimulation site and (d) at corresponding contralateral site.
Stimulus temperature needed to elicit a given pain intensity during induction phase: (e) temperature when pain rating first reached 10% (“near pain threshold”); (f) temperature at the end of the induction phase, i.e., when pain rating first reached set point (35%). This was the point where the computer began modulating the thermode temperature in order to maintain an eVAS score of 35% (“midrange pain intensity”, presumed to be representative of intensities frequently experienced clinically).

Average stimulus temperature needed to maintain pain intensity setpoint: (g) for series 1; (h) for series 2; (i) for series 3; (k) for series 4.

The data were analyzed using a repeated measures ANOVA model.

Results

Skin Temperature

Skin temperature was measured at the thenar eminence of both hands prior to thermal stimulation on each of the three identical daily test sessions. The temperature did not differ to a statistically significant degree between IBS and control groups and between sessions. Repeated measures ANOVA revealed no significant group-by-day interactions. Furthermore, no significant correlation was found between pre-stimulus skin temperature at the stimulation site and the average thermode temperature needed to maintain the pain intensity near the 35% setpoint during any of the four stimulus series on any of the three daily test sessions (correlation coefficients ranged from 0.31 to -0.09).

Skin temperature was measured at the thenar eminence on the side opposite to stimulation before and after stimulation (ANOVA variable “Time”) in order to assess contralateral thermoregulatory responses that might be triggered by prolonged stimulus exposure. Both IBS and control groups (ANOVA variable “Group”) underwent three identical daily sessions (ANOVA variable “Day”). The data collected during experiment
1 (prolonged stimulation on left hand, skin temperature measurement on right hand) were analyzed with repeated measures ANOVA. Significance was obtained for “Group” (F (1, 16) = 4.895, P = 0.042), “Time” (F (2, 16) = 14.325, P = .002) and the “Group x Time” interaction (F (2, 16) = 6.690, P = .020). Similarly, for experiment 2 (prolonged stimulation on right hand, skin temperature measurement on left hand) “Time” (F (2, 16) = 9.076, P = .008) and the “Group x Time” interaction (F (2, 16) = 5.589, P = .031) reached significance.

These findings suggest that the IBS and control group did not differ in their baseline skin temperatures, i.e., the skin temperature before stimulation onset. Furthermore, the data suggest that the skin temperature of the palm of the hand drops when the corresponding location on the opposite side is subjected to prolonged thermal stimulation. The temperature drop is smaller in the IBS group than in the control group. In other words, IBS patients, when compared to healthy control subjects, appear to have a reduced autonomic thermoregulatory response to a prolonged thermal stimulus on the opposite side of the body (Table 3-2 and Figure 3-3).

Clinical Pain

There were no significant correlations between the ratings of disease-related pain (intensity and unpleasantness) at the most painful site on the day of testing and the average stimulus temperatures for any of the three daily sessions and for either of the two experiments.

Induction of Stimulus-Induced Sensitization

In experiments 1 and 2 the thenar eminence of the left and right hand respectively was exposed to a series of thermal contact stimuli of incrementally increasing temperatures until a pain intensity of 35% was reached on the electronic visual analog
scale. The response variables derived from this portion of the experiment were thermode
temperature at the time when the pain intensity first reached 10% and when it reached
35%. A mixed model ANOVA included between-subjects factors (“Group”) and
within-subjects factors (“Day”; “Temperature @ 10%” pain intensity”; “Temperature @
35% pain intensity”). In both experiments a significant main effect of “Group” and
“Temperature” was found. In other words, for IBS subjects, when compared with healthy
controls, lower temperatures were needed to reach the 10% and 35% pain intensity levels
respectively (Figures 3-4A and 3-5A). Thus, IBS patients emerge as more sensitive to
thermal nociceptive stimuli in this experimental setting, as has been the case in other
types of tests (see chapter 2). No significant “Group” x “Temperature” interaction was
found in either of the two experiments, suggesting that the temperature increase needed to
boost pain intensity from 10% to 35% was not significantly different between groups.
This finding does not permit conclusions regarding sensitization rate because the
experiment did not control for the number of stimuli that were presented between the
10% and 35% pain intensity criterion. Under certain circumstances, as described in the
methods section, the stimulator maintained a constant temperature across consecutive
stimuli during the induction phase.

**Maintenance of Stimulus-Induced Sensitization**

It was argued that the average temperature required for maintaining the average
pain rating near the setpoint of 35% depends in part on the duration of the stimulus pulses
and the length of the intervals between pulses. The temperature changes needed to
compensate for modifications in pulse duration and ISI were measured in experiment 1
and 2 respectively. Each experiment was repeated 3 times on different days. A mixed
model ANOVA with “Group” as a between subjects factor and “Day” & “Average
temperature to maintain 35%” as within subject factors was used for analysis. Numerical
details can be gleaned from table 3-3 and figures 3-4B,C, 3-5B. As a group, IBS
patients—under given stimulation conditions—require a lower temperature to maintain
the pain intensity setpoint of 35% than their healthy counterparts, i.e., they are more
sensitive to thermal nociceptive stimulation of the palm of the hand than controls in both
experiments. The IBS group, however, exhibits much larger variability of sensitivity than
the control group: some IBS subjects are no more sensitive than healthy controls while
others are much more sensitive. Few IBS patients of our sample reported disease-related
pain, and a statistically significant correlation between thermal sensitivity and disease-
related pain was not found.

In a number of experimental settings it was noted that exposure to a prolonged
thermal nociceptive stimulus leads to sensitization, but the rate of sensitization flattens
during the course of stimulus exposure. In the present experiments, the temperature
required to maintain the pain intensity setpoint of 35% may be considered a measure of
pain sensitivity: for more sensitive subjects a relatively lower temperature suffices to
maintain 35% pain intensity. The existence of an initially rapid sensitization that slows
as the stimulus series progresses is evident when early and late series with the same
stimulus parameters are compared: i.e., series 1 with series 3 and series 2 with series 4
(Figure 3-4). The average stimulus temperature (inversely related to pain sensitivity) of
both groups dropped between series 1 and 3 and between series 2 and 4 (to a statistically
significant degree in experiment 1) suggesting that sensitization takes place over the
course of stimulation (Table 3-4). In both groups the drop was significantly larger
between series 1 and 3 than between series 2 and 4 (Table 3-4). This is consistent with
the notion that stimulus-induced sensitization is relatively small once stimulation has progressed beyond series 1. Sensitization, as indicated by a drop in stimulus temperature between series 1 and 3, was significantly larger (p=.008) for the IBS group (1.5°C) than for the control group (0.8°C). Sensitization between series 2 and 4 was similar for both groups (compensatory temperature change of 0.4°C and 0.5°C respectively). These results suggest that the difference in stimulation-induced sensitization between the two groups is most visible early on during the prolonged stimulation experiment.

A main objective of experiments 1 and 2 was to assess the time constants of processes that maintain the stimulation-induced sensitized state. The responsiveness of the thermally induced sensitized state to small changes in stimulus pulse duration and ISI was measured. Between series 2, 3 and 4 the switch in stimulus parameters occurred once in each order. Therefore, the average change for the two transitions controls for order effect. Series 1 was not included in this analysis to minimize the global effect of prolonged stimulation on sensitivity. This confounding effect diminishes after the first series, as was pointed out earlier. The 0.2 sec change in stimulus duration (experiment 1) resulted in a smaller compensatory change in stimulus temperature (0.4°C) in the IBS group than in the control group (0.8°C). This group difference did not reach statistical significance (p=.180). It appears that the temperature change needed to compensate for modifications in stimulus pulse duration was inversely related to thermal pain sensitivity, as measured during the induction phase or in experiments using 3 second stimuli (see chapter 2). This relationship is illustrated in the scatter plot of figure 3-6 (for numerical details see Table 3-5).
In contrast, the temperature change needed to compensate for a 1.0 sec change in ISI (experiment 2) did not differ to a statistically significant degree between the IBS and control group (Table 3-5). In summary, changing stimulus pulse parameters required a compensatory temperature change in both groups and both experiments. However, the groups differed only to a significant degree when the temperature change was induced by a modification in pulse duration (Figure 3-4) but not when it was induced by lengthening or shortening the interval between pulses (Figure 3-5). It can not be ruled out that a group difference would have emerged if a larger ISI change had been chosen.

**Discussion**

Pain complaints of IBS patients are not restricted to the viscera, and often include areas very distant from the gut such as the head and neck (Whorwell et al., 1986). Earlier research did not support the notion that IBS patients exhibit increased somatic pain sensitivity by failing to reveal any sensitivity differences between IBS patients and healthy controls (Cook et al., 1987; Whitehead et al., 1990; Accarino et al., 1995; Chang et al., 2000). The results and the putative underlying causative factors were discussed in chapter 2. For many years these results and conclusions went undisputed until recently, when new results with improved pain testing methodology have contradicted the idea that IBS is strictly a visceral disorder (Verne et al., 2001; Bouin et al., 2001). These tests have shown cutaneous thermal hypersensitivity in dermatomes in close segmental proximity to the gut (i.e. the calf) as well as in segmentally remote areas (hand, forearm, and cheek). This study’s results support the presence of pain hypersensitivity in remote areas (hands) demonstrated by the lower temperatures necessary to reach 10% & 35% pain intensity and to maintain a pain level of 35% for series 1 through series 4 for each experiment. Even though the IBS group was more sensitive than the control group, there were some
IBS patients that were no more sensitive than any of the control subjects. Based on the vicious pain cycle idea, the large variability within the IBS group was thought to be a consequence of the intensity of the clinical pain symptoms. It was expected that individuals with the most clinical pain would be the most sensitive. This turned out not to be the case. The lack of correlation between sensitivity and clinical pain does not support the idea that clinical pain is the driving force of peripheral hypersensitivity. However, it can be argued that the sensitized state is so stable that disease related pain needs to be present only occasionally to maintain it. Also, the stimuli used in many studies may be too brief to engage disease-critical mechanisms and it may be necessary to use prolonged stimuli for the assessment of the mechanisms that contribute to the establishment of these plastic changes. The introduction of the response-dependent stimulation method is an attempt to challenge the pain processing system with longer stimulation exposure times. The results suggest that even with these longer stimuli, experimentally induced sensitization is not an adequate model of disease related states of sensitization. The time constants of experimentally induced sensitization are short while disease-related sensitization is very stable over time. This may be the reason why long term sensitization could not be induced in the control group during three days of testing.

Spatial distribution of somatic sensitization is another characteristic that needs to be considered during the discussions regarding the etiology of the sensitized state. Since pain hypersensitivity has been demonstrated to extend across the entire body, it is unlikely that the sensitization is maintained by a single pain generator in the gut. Therefore, a mechanism with a very long time constant, that is spatially diffuse, e.g. endocrine/cytokine based, must be considered.
We chose to monitor pre and post test skin temperature as a measure of autonomic nervous system function. The contralateral skin temperature drop in response to thermal stimulation was smaller in IBS patients than in healthy controls, suggesting a deficit in autonomic reactivity.

In conclusion, the prolonged testing was unable to reveal any obvious sensitization changes in the IBS group. In addition, the multiple days of testing could not induce a vicious pain like sensitization in the control group. The spatial and temporal characteristics of sensitization in IBS patients provide little support for a positive nociceptive feedback based mechanism of sensitization.

Table 3-1. Experimental parameters

<table>
<thead>
<tr>
<th>Experiment 1</th>
<th>Series 1</th>
<th>Series 2</th>
<th>Series 3</th>
<th>Series 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of Stimuli</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Stimulus Duration</td>
<td>1.0 sec</td>
<td>0.8 sec</td>
<td>1.0 sec</td>
<td>0.8 sec</td>
</tr>
<tr>
<td>Inter-Stimulus Interval</td>
<td>3.0 sec</td>
<td>3.0 sec</td>
<td>3.0 sec</td>
<td>3.0 sec</td>
</tr>
<tr>
<td>Temperature</td>
<td>Modulated to maintain 35% pain intensity</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Experiment 2</th>
<th>Series 1</th>
<th>Series 2</th>
<th>Series 3</th>
<th>Series 4</th>
</tr>
</thead>
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<td>Number of Stimuli</td>
<td>25</td>
<td>25</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>Stimulus Duration</td>
<td>0.9 sec</td>
<td>0.9 sec</td>
<td>0.9 sec</td>
<td>0.9 sec</td>
</tr>
<tr>
<td>Inter-Stimulus Interval</td>
<td>2.5 sec</td>
<td>3.5 sec</td>
<td>2.5 sec</td>
<td>3.5 sec</td>
</tr>
<tr>
<td>Temperature</td>
<td>Modulated to maintain 35% pain intensity</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3-2. Pre- and post-stimulation skin temperatures (°C) on the palm of the non-stimulated hand

<table>
<thead>
<tr>
<th>Baseline</th>
<th>End of Experiment 1</th>
<th>Baseline</th>
<th>End of Experiment 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBS</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Baseline</td>
<td>32.4 (1.0)</td>
<td>32.1 (0.8)</td>
<td>32.3 (0.9)</td>
</tr>
<tr>
<td>Control</td>
<td>32.2 (1.0)</td>
<td>30.7 (0.8)</td>
<td>32.2 (1.0)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>32.0 (1.0)</td>
<td>31.2 (0.9)</td>
<td>31.0 (0.9)</td>
</tr>
</tbody>
</table>
Table 3-3. Experiment 1 and 2 involved series of thermal stimuli to the palm of the right and left hand respectively. REDSTIM methodology was used to maintain an average pain intensity of 35%. Exp. 1: the stimulus pulse duration was varied from series to series while ISI remained at 3 seconds. Exp. 2: the stimulus pulse duration was held constant while the ISI varied from series to series. The table shows the mean thermode temperature (in °C) needed to maintain a 35% pain intensity under the different stimulus timing conditions.

<table>
<thead>
<tr>
<th>Exp. 1</th>
<th>Series 1 Stim Dur = 1.0s</th>
<th>Series 2 Stim Dur = 0.8s</th>
<th>Series 3 Stim Dur = 1.0s</th>
<th>Series 4 Stim Dur = 0.8s</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean (stdev)</td>
<td>Mean (stdev)</td>
<td>Mean (stdev)</td>
<td>Mean (stdev)</td>
</tr>
<tr>
<td>IBS</td>
<td>47.5 (2.5)</td>
<td>46.5 (2.9)</td>
<td>46.0 (2.5)</td>
<td>46.4 (2.9)</td>
</tr>
<tr>
<td>Control</td>
<td>50.7 (2.5)</td>
<td>51.0 (2.9)</td>
<td>49.9 (2.6)</td>
<td>50.5 (2.9)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exp. 2</th>
<th>ISI = 2.5s</th>
<th>ISI = 3.5s</th>
<th>ISI = 2.5s</th>
<th>ISI = 3.5s</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBS</td>
<td>46.7 (2.8)</td>
<td>47.4 (3.2)</td>
<td>46.1 (2.8)</td>
<td>47.0 (3.2)</td>
</tr>
<tr>
<td>Control</td>
<td>50.2 (2.8)</td>
<td>51.0 (3.1)</td>
<td>49.8 (2.8)</td>
<td>50.7 (3.1)</td>
</tr>
</tbody>
</table>

Table 3-4. Long term sensitization induced by prolonged stimulus exposure. The temperature change from the first to the second series of identical stimulus timing parameters is shown. A temperature drop (negative value) is indicative of an increase in pain sensitivity.

<table>
<thead>
<tr>
<th>Exp 1</th>
<th>Series 1 - 3</th>
<th>Series 2 - 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBS</td>
<td>-1.5°C</td>
<td>-0.4°C</td>
</tr>
<tr>
<td>Control</td>
<td>-0.8°C</td>
<td>-0.5°C</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exp 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>IBS</td>
<td>-0.7°C</td>
<td>-0.3°C</td>
</tr>
<tr>
<td>Control</td>
<td>-0.5°C</td>
<td>-0.3°C</td>
</tr>
</tbody>
</table>

Table 3-5. REDSTIM experiment; pain intensity setpoint 35%: temperature change needed to compensate for change in stimulus duration or ISI respectively.

<table>
<thead>
<tr>
<th>IBS</th>
<th>Stimulus Duration</th>
<th>Inter-Stimulus Interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>°C per .2sec change</td>
<td>°C per 1sec change</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>1.1</td>
</tr>
<tr>
<td>Control</td>
<td>0.8</td>
<td>1.1</td>
</tr>
</tbody>
</table>
Figure 3-1. The principle of response-dependent stimulus modulation (REDSTIM): subject and stimulator both are components of a closed proportional control loop. The value of the modulated stimulus parameter (thermode temperature) required to maintain the target pain intensity level serves as response variable.
Figure 3-2. REDSTIM parameters: (A) In Experiment 1 the stimulus duration switched from 1.0 sec (series 1 and 3) to .8 sec (series 2 and 4). (B) In experiment 2 the Inter-Stimulus Interval (ISI) switched from 2.5 sec (series 1 and 3) to 3.5 sec (series 2 and 4). The temperature was modulated in a pain rating dependent manner to maintain an average pain intensity near a predetermined setpoint (35%). The change in temperature needed to compensate for the change in stimulus duration or ISI served as response variable.
Figure 3-3. Contralateral palm skin temperature data before stimulation onset (Pre) and after the end of the stimulus series (Post) for experiments 1 and 2. Thermal stimulation induced a significant contralateral skin temperature drop in the control- but not the IBS group.
Figure 3-4. Experiment 1: Sensitivity to changes in stimulus duration. Following an induction phase, 4 series of 25 stimuli each were administered to the thenar eminence of the left hand. ISI was 3 sec for the induction phase and all series. Stimulus duration changed from series to series. The test was repeated on three different days. (A) Temperature needed to induce 10% and 35% pain levels during the induction phase of the REDSTIM test. (B) The average temperature needed to maintain a pain intensity of 35% for series 1 through 4. (C) Average REDSTIM temperatures for the three days of testing.
Figure 3-5. Experiment 2: Sensitivity to changes in Inter-Stimulus Interval. Following an induction phase, 4 series of 25 stimuli were administered to the thenar eminence of the left hand. Stimulus duration was 0.9 sec for the induction phase and all series. ISI changed from series to series. The test was repeated on three different days. (A) Temperature needed to induce 10% and 35% pain levels during the induction phase of REDSTIM. (B) Average temperature needed to maintain a pain intensity of 35% for series 1 through 4.
Figure 3-6. REDSTIM experiment; pain intensity setpoint 35%; Relationship between sensitivity to changes in stimulus pulse duration (y-axis) and pain sensitivity (x-axis). Pain sensitivity is related to the temperature needed to elicit 35% pain intensity as determined in an experiment using brief stimuli to the right forearm (see page 21 for details). Control subjects are represented by dark squares, IBS subjects by open squares.
CHAPTER 4
THE EFFECT OF TOPICAL LOCAL ANESTHETICS ON SOMATIC PAIN SENSITIZATION IN IRRITABLE BOWEL SYNDROME

Introduction

Irritable bowel syndrome is a common gastrointestinal disorder that consists of abnormal bowel movements that can be associated with abdominal pain. Often the pain symptoms are not limited to the gut but expand to remote regions such as the neck and head during the course of the disease. The question arises whether IBS is characterized by pathophysiological mechanisms that are initially regional (in the gut), and nociceptive signals emanating from the symptomatic viscera induce somatic sensitization across the body. It is critical to ask whether a temporary interruption of nociceptive signals from the gut allows somatic pain sensitivity of IBS patients to return to normal. In the event a relationship between the presence of nociceptive signals from the gut and somatic pain sensitivity cannot be demonstrated one needs to consider the alternate possibility that IBS is a regional manifestation of a pathophysiological mechanism that affects the entire body to begin with. According to this theory the primary problem would be a generalized state of sensitization that affects visceral and somatic tissues alike and makes it more likely that subsequent local insults develop into chronic pain problems. The present study concentrates on the first hypothesis which states that widespread somatic sensitization of IBS patients is maintained by a regional nociceptive focus, i.e., by nociceptive signals from the symptomatic viscera. If the presence of visceral pain or at least of nociceptive drive emanating from visceral tissues is a critical factor for somatic sensitization one
would expect that the degree of somatic sensitization correlates with the presence / absence or frequency / intensity of spontaneous visceral pain. Furthermore, one would expect that silencing the presumed visceral nociceptive focus with rectally administered topical local anesthetics would lead to a reduction in somatic sensitization.

At least two studies (conducted on cohorts of IBS patients and patients with myofascial pain syndrome respectively) have used local anesthetics in an attempt to silence a nociceptive focus that was presumed to maintain a sensitized state (Fillingim et al., 1998; Verne et al., 2003). Both have demonstrated a reduction in pain hypersensitivity in remote areas following anesthetic intervention, therefore providing support for the idea that a “vicious pain cycle” is an important factor in the maintenance of hypersensitivity. However, other reports have put this view into question: topical rectally administered lidocaine failed to reduce pain sensitivity in some studies (Lembo et al., 1994) and “triggerpoint” injections with saline or simply an empty needle were no less effective in reducing pain in myofascial pain patients than lidocaine injections (Garvey et al., 1989). The disagreement between studies clearly suggests that questions remain regarding the mechanism that maintains widespread somatic sensitization in regional pain disorders.

The present study addresses a number of questions that have not been answered conclusively by previous investigators. These questions are the following: (1) Is the somatic hypersensitivity that is typical for many IBS patients nociceptive-specific or does it extend to the perception of non-nociceptive stimuli? (2) Can the changes in somatic sensitivity that follow rectal lidocaine administration conclusively be explained by the topical anesthetic effect of the drug, or must systemic effects be considered as a possible
mechanism? A partially related question is: (3) Does the effect of lidocaine on somatic pain sensitivity require that the subjects have IBS or can it be demonstrated also in healthy pain-free individuals? (4) Is the effect of lidocaine more pronounced in the segmental proximity of the presumed visceral pain focus or is it similar across the entire body? (5) Is it possible that an unidentified placebo effect could explain the results of previous studies, considering that it cannot be ruled out that some subjects might detect the active drug by its numbing effect or its effect on the perception of the experimental stimuli? The present study makes an attempt to address these questions with improved methodology.

**Methods**

**Subjects**

Recruitment and study procedures were approved by the University of Florida Institutional Review Board. Written informed consent was obtained from all participants. The criteria for membership in the control group required (1) no significant spontaneous pain anywhere in the body, (2) no ongoing pharmacotherapy with narcotics or antidepressants, (3) no disease or condition that might significantly affect pain perception or unduly increase risk of injury (e.g., neurological disorders, serious psychiatric disorders, diabetes, hypertension, serious cardiovascular disorders, pregnancy, and chronic pain diseases such as fibromyalgia syndrome, (4) no prior complications or allergies with the local anesthetic lidocaine. The criteria for the disease group required a diagnosis of ongoing IBS based upon the Rome II criteria (Thompson et al., 1999), supplemented by additional criteria: absence of other diseases (including other chronic pain diseases ), risk factors, and ongoing drug treatments, as described for the control group. Patients with a history of widespread pain (e.g., individuals with fibromyalgia
syndrome) were excluded from the study. Initial screening consisted of blood pressure measurement, completion of a health questionnaire and--for IBS patients--a clinical diagnosis by a physician. Subject recruitment did not control for menstrual phase and for whether or not contraceptives were taken, but the respective information was recorded. There is no reason to believe that menstrual phase was not randomly distributed within groups and systematically different between groups. Synchronization of menstrual phases is most likely among females that live together, e.g., among college roommates (McClintock 1971). Participants of our study were recruited from diverse social settings and had no close contact with each other. Test sessions were scheduled independently of whether or not IBS patients had an acute exacerbation of symptoms on the day of testing. Some but not all of the patients reported spontaneous pain or discomfort during some of the sessions. All subjects were right-handed.

**Pain Measurement**

Spontaneous clinical pain and experimental pain (induced by thermod) were measured with an electronic version of a visual analog scale (Price DD and Harkins SW, 1987). The electronic visual analog scale (eVAS) consisted of a low-friction sliding potentiometer of 100 mm travel. The left endpoint of the scale was identified as "no pain", while the right endpoint was labeled as "intolerably intense pain". There were no divisions between these two anchors. The position of the slider was electronically converted into a pain rating between 0 and 100%. The slider automatically returned to the left ("no pain") position when so required by the protocol. The eVAS was mounted into the surface of a small inclined desk positioned to facilitate precise operation with minimal fatigue. The custom-built testing system integrated all inputs (temperature process value, eVAS signal) and outputs (stimulus temperature control, stimulus timing)
and allowed automated execution of test protocols with preprogrammed parameters, including limits for temperature and pain intensity.

**Testing Protocol**

**Daily Protocol**

Participants began each daily session of the non-consecutive seven day study by mapping and rating their spontaneous clinical pain. Pain testing began with a prolonged thermal stimulus to the thenar eminence of the right hand. The stimulus was controlled as a function of the pain intensity rating and maintained a 25% average pain intensity for two minutes. Subsequently, pain sensitivity was probed with brief thermal contact stimuli at three different locations on the right side of the body: on the lateral aspect of the calf (dermatome S1; same spinal segment as visceral symptomatic area), the volar forearm (dermatomes C6, T1) and the cheek (V2, V3). Testing concluded with a series of non-painful warm stimuli on the hypothenar eminence of the left hand. Stimulus locations were reproducibly defined relative to anatomical landmarks. Anesthetic intervention occurred on three days of the study prior to testing as explained in detail below.

**Measurement of spontaneous pain**

At the beginning of all experimental sessions, subjects were asked to shade the locations of spontaneous pain on an anatomical diagram and to rank these sites according to pain intensity. Subsequently, the intensity of disease-related pain of the upper (head, neck, shoulder, upper back, arms, hands) and lower (low back, bowel, legs, feet) parts of the body were rated on the eVAS. The subjects were then asked to rate the unpleasantness of the single most intense clinical pain. All these ratings were required to be below 5% (on the 0-100% eVAS scale) for subjects to be admitted to the control
group. Furthermore, individuals with widespread pain (in more than 2 quadrants of the body) were excluded from the study.

**Phone survey and questionnaires**

Participants were asked to call a phone survey system everyday throughout the study to report sleeping habits, bowel movements, and the presence of clinical pain in different areas of the body. In addition to completing a health questionnaire on the first day of testing, the participants were given three psychological assessment forms (BECK depression, State Trait Anxiety Inventory, and the Symptom Checklist for somatization) which were filled out and returned on the third day of testing.

**Drug Administration**

A gastroenterologist digitally applied a jelly containing 2% lidocaine (AstraZeneca) or a similar volume of placebo (KY Jelly) rectally and orally in all IBS and control subjects prior to testing on three of the seven daily testing sessions (Table 4-1). The lidocaine dose was between 150 and 300 mg per application. The application was the responsibility of the same clinician that had performed the procedure in an earlier study (Verne et al., 2003). Pain testing began within 15 minutes after administration, when the lidocaine effect was expected to peak (Deboer et al., 1979). The order of anesthetic and placebo were not random: the double placebo treatment (oral and rectal administration of KY Jelly) was always first in order to allow within-subject control in the event that the anesthetic had an irreversible therapeutic effect (Table 4-1). The subjects were not informed about the order and expected effects of the treatments in order to minimize rating bias. Sessions that included lidocaine administration were always followed by a testing day without lidocaine application to facilitate detection of prolonged drug effects.
Measurement of skin temperature

Skin temperature at the stimulation site and the corresponding contralateral site was measured with an Exergen Dermatemp infrared temperature scanner model DT-1001 (Exergen Corp., Watertown, MA, USA) before and immediately after each stimulus series.

Thermal Nociceptive Stimulation Experiments

Thermal stimuli were administered with a flat copper contact thermode of 23x23mm in size. The thermode was electronically held at the desired temperature by a Peltier thermoelectric device. It was brought into light skin contact of reproducible force by solenoid activation. A thermistor in the center of the thermode, very close to the surface, sampled the temperature during each skin contact to record potential temperature deviations resulting from the skin's heat sink effect. The stimulator assembly was mounted on an adjustable arm (comparable to those that support dental x-ray machines) for convenient and stable positioning for any desired stimulation site.

Sensitivity to prolonged stimuli

Traditional methods for studying central pain sensitization have used short series of brief thermal stimuli of constant magnitude while measuring the change in pain intensity rating throughout the series to define sensitization (Maixner et al., 1998; Staud et al., 2001). These methods did not provide insight into pain modulation stages of late onset because the stimulation series had to be short to prevent the pain ratings from reaching unacceptably high levels. Furthermore, discrimination between mechanisms of different activation thresholds was not possible because the pain intensity induced by the heat pulses was not constant, but progressively increased throughout the series. The custom-built thermal contact stimulator used in this study did not have the limitations of the
traditional methods that defined the stimulus as an independent variable and the subject's rating of the pain as the dependent variable. This method, similar to a method described by Gracely et al., reversed this arrangement and linked the subject and the stimulator in a closed negative feedback loop (Gracely et al., 1988). The thermode temperature was continuously adjusted in proportion to the deviation of the rating response on the eVAS from a predetermined setpoint in order to maintain an average pain intensity rating near the setpoint (Response-Dependent Stimulation method, REDSTIM).

The objective of the REDSTIM test was to measure how the presence of disease affected the characteristics of sensitization and adaptation during prolonged thermal stimulation, as well as how silencing the nociceptive focus with topical lidocaine altered these stimulus-induced phenomena. The test induced and maintained an average pain intensity level of 25% for two minutes by modulating the thermode temperature (dependent variable). The temperature needed to maintain the desired pain intensity under given test conditions varied from individual to individual and was very different between the IBS and control groups. The stimulator software was designed to find the individually appropriate temperature automatically during the induction phase of the experiment. The induction series began with a thermode temperature of 34°C which was never perceived as painful by any subject. The temperature then increased in 0.6°C increments every second until the pain intensity rating reached 10% on the electronic visual analog scale. Thereafter, the temperature continued to rise at a reduced rate (0.3°C / second) if the pain intensity did not continue to increase over a 3 second period. The temperature remained unchanged when the pain intensity continued to increase after passing the 10% level. During the induction phase, the thermode temperature could either
increase or remain the same but it could not decrease. The induction phase ended when
the pain intensity rating first reached the setpoint of 25% on the eVAS. At that point, the
two minute test began and temperature modulation became bidirectional, in proportion to
the deviation from setpoint. Clamping the average pain level at a tolerable level for a
prolonged period of time allowed testing of slowly responding pain modulation
mechanisms. During this test (the first of the session) it was impossible for the subject to
distinguish active drug from placebo by its effect on experimentally-induced pain
because the average pain intensity remained the same, regardless of the treatment
condition. By maintaining the pain-intensity at a constant level, a potential decrease in
pain sensitivity was detected by an increase in thermode temperature (unknown to the
subject) and not by the pain rating. Once pain intensity had first reached 25%, thermode
temperature and eVAS rating were sampled once per second for 120 seconds. The 10
samples from 51 -60 seconds were used for analysis, because during this window the pain
intensity was reliably maintained near setpoint by all the subjects. Later in the test some
subject failed to properly attend to the rating task and this could lead to an unacceptably
large deviation of the running average of pain intensity from setpoint. Stimulus
temperature can be used to predict changes in pain sensitivity only when average pain
intensity remains near setpoint during all treatment conditions.

Sensitivity to Brief Stimuli

The relationship between stimulus temperature and pain intensity was assessed by
rating series of stimuli across a pain intensity range from threshold to at least 45%. The
theremode was preheated to the desired temperature before it contacted the skin. Contact
duration was short (3 seconds), and the intervals between stimuli were relatively long (30
seconds) to minimize sensitization. The temperature was set to 43 °C (below pain threshold for all tested subjects) for the first stimulus, and increased in 0.7 °C increments from one pulse to the next until a pain rating of 45% was reached (ascending temperature series). At that point, a descending temperature series of equal length was initiated. The series ended with the starting temperature of the ascending series (i.e., 43 °C).

Terminating the ascending series upon reaching the 45% pain intensity level minimized discomfort for the subjects yet assured that the clinically most relevant intensity range was equally represented in both groups, regardless of their pain sensitivity. The inclusion of disease-typical suprathreshold intensities was important considering the possibility that some modulatory mechanisms might be active only within a certain signal intensity range. The pain intensity was rated retrospectively within 5 seconds of the end of each stimulus, at which time the eVAS slider automatically returned to the left endpoint.

It could be argued that randomized stimulus intensities should have been used to minimize expectation-based response-bias. We chose to use the ascending and descending intensity series instead in order (1) to avoid uncomfortably high pain intensities and anxiety in subjects with unexpectedly high pain sensitivity and (2) to avoid unpredictable distortion of the stimulus response function due to effects of stimulus history on pain sensitivity (Lamotte and Campbell, 1978; Grill and Coghill, 2002).

**Non-painful warm stimulation experiments**

Non-painful warm stimuli (temperature range 36-42°C) were administered to the hypothenar eminence of the left hand. The thermode was brought into light skin contact while at 34°C. This baseline temperature was held for 30 sec before the thermode was ramped (ramp speed 3°C / sec) to the stimulus temperature of 36, 38, 40 or 42°C. The
stimulus was terminated after 30 sec by removing the thermode from the skin. The
stimuli were delivered in a quasi random order (36, 42, 38, 40, 40, 38, 42, 36°C) with a 1
minute interval between stimuli. The subjects were instructed to rate the intensity of the
warm sensation continuously throughout the thermode contact period. The endpoints of
the eVAS were defined as “neutral sensation which is neither warm nor cold” and “very
warm, bordering the painful”. The scale had no intermediate anchor points. Intensity
ratings typically increased early during the stimulus to a peak after which the rating
tended to decline. The mean eVAS rating over the thirty second stimulus duration served
as the response variable (total warmth). The data for tests conducted at the same
temperature were averaged to generate average stimulus intensity response functions for
the non-painful warm range. Comparisons were made between the IBS and control
groups to determine whether sensitization of IBS patients was specific to the nociceptive
range or extended into the non-painful range. The latter finding would leave rating bias
rather than sensitization as a possible explanation for the differences between the groups.

Data Analysis

Repeated measures ANOVA was used for statistical analysis. A total of 13 factors
and variables were considered (a through m). (a) Group: IBS patient, healthy control. (b)
Day: baseline (day 2), average baseline (average of days 2, 5 and 7), double placebo (day
3), rectal lidocaine (day 4), oral lidocaine (day 5). Skin temperature: pre-stimulus skin
temperature (c) at stimulation site and (d) at corresponding contralateral site, (e) skin
temperature recorded after end of stimulus series at stimulation site and (f) at
corresponding contralateral site. Pain sensitivity measures obtained with prolonged
nociceptive stimuli: (g) thermode temperature when pain rating first reached 10% (“near
pain threshold”), (h) temperature at the end of the induction phase, i.e., when pain rating
first reached set point (25%) and (i) the average temperature needed to maintain setpoint throughout the window from 51-60 sec of the REDSTIM test. *Sensitivity measures obtained with brief nociceptive stimuli:* a two-parameter regression function (*pain intensity* = \[100/ \{1 + \exp (-A-B* \text{temperature})\}\]) was used to estimate the stimulus-intensity response relationship of each subject (sensitivity); three representative pain intensity points, i.e., (j) 10% (near threshold), (k) 20%, and (l) 40% on the stimulus-intensity response curve were used for analysis. *Sensitivity measures obtained with non-painful warm stimuli:* (m) the total warmth elicited by each stimulus.

**Results**

**Clinical Characteristics of Subjects**

11 diarrhea-predominant female IBS patients (age range 18-52, mean age: 26.4 years) and 11 healthy female controls (age range 20-54, mean age: 26.4 years) successfully completed the study. There was no statistically significant difference in age, pretest blood pressure, or heart rate between the groups. The majority of IBS patients did report clinical pain at one time or another throughout the time period of their involvement in the study, but not always on the days of testing.

**Psychometric Characteristics of Subjects**

The subjects completed three psychological assessment forms: State Anxiety Trait Inventory (STAI), Beck Depression, and Symptom Checklist (somatization measure). The groups did not significantly differ in the Beck Depression or Symptom Checklist assessments. However, the IBS patients scored significantly higher on the STAI, suggesting that they were more anxious. These increased anxiety scores did not correlate with any psychophysical pain measures or treatment effects.
**Skin Temperature**

Skin temperature was recorded prior to and at the conclusion of each test on both the test site and corresponding contralateral site to indirectly determine whether differences in spontaneous and stimulus-induced thermoregulation exist between groups. The groups did not differ in baseline skin temperature on any site. Furthermore, the contralateral skin temperature changes induced by series of *brief* (3 sec) heat stimuli were similar in the IBS and control group. These findings are in agreement with results of a study described in chapter 3. When the REDSTIM method was used to maintain a 25% average pain intensity for 2 minutes the contralateral skin temperature of the control group remained at 31.4°C while it drifted up slightly (by 0.8 °C) in the IBS group. The contralateral skin temperature change did reach statistical significance and did not correlate with any of the other variables (see below), including the type and location of drug intervention.

In an earlier study (Chapter 3), where an average pain intensity level of 35% was maintained with pulsed stimuli for over 7 minutes, the thermoregulatory difference between the groups was statistically significant as the contralateral skin temperature dropped by 1.5 degrees in the control group but only by 0.3 degrees in the IBS group. Possible explanations for the much more pronounced thermoregulatory group difference in the earlier study may be the longer stimulation period (approximately 100 seconds of thermode contact accumulated over more than 7 minutes of stimulation) and the higher average thermode temperature. The thermode temperature was higher because stimulation used to maintain the pain intensity setpoint was pulsed rather than continuous and the setpoint was higher (35%).
No-treatment (Baseline); Sensitivity to Prolonged Nociceptive Stimuli

During three of the seven daily test sessions pain sensitivity was assessed in the absence of any lidocaine or vehicle treatment (Table 4-1). During these baseline sessions the thenar eminence of the right hand was exposed to a prolonged thermal contact in which the thermode temperature was incrementally increased until a pain intensity of 25% was reached on the electronic visual analog scale. An average intensity level of 25% was then maintained for 2 minutes, using the REDSTIM feature of the stimulator. The thermode temperatures at the time when the pain intensity first crossed the 10% and 25% marks were significantly lower for the IBS group during all baseline sessions (Figure 4-2), but the temperature difference between these marks was the same for both groups. This suggests that IBS patients are more sensitive to thermal nociceptive stimuli than healthy controls. It may be tempting to use the temperature difference between the 10% and 25% mark to make inferences regarding the sensitization rate. However, this would be incorrect because the time needed to increase pain intensity from 10% to 25% depended on how the pain rating changed during the induction phase and thus was not the same for all tests.

A close inspection of the data collected during the phase where the average stimulus intensity was to be maintained at the 25% pain intensity level revealed that a number of subjects were unable to attend to the rating task for much longer than approximately 1 minute. Inattentiveness could lead to an unacceptably large deviation from the pain intensity setpoint of 25% and thus invalidate the average thermode temperature as an indicator of pain sensitivity. In all cases the pain rating remained within 4 percentage points of setpoint during the first minute. In the interest of optimal validity only data collected during the first minute were used for analysis and group
comparisons. Over this period, the running temperature average was calculated with a window of 10 seconds (1 sample/sec). In both groups the running temperature average increased progressively. The increase was steepest initially and flattened later (Figure 4-1). This suggests that pain sensitivity decreased during this phase of prolonged stimulation.

**No-treatment (Baseline); Sensitivity to Brief Nociceptive Stimuli**

The test was conducted on day 1 of the seven day protocol and was comprised of an ascending and descending temperature series (stimulus contact duration 3 sec, ISI 30 sec). Pain intensity, in combination with the thermode temperature used to evoke the pain, allowed inferences regarding pain sensitivity. The data of the ascending and descending series were pooled for analysis because the direction and magnitude of the series hysteresis exhibited no discernible pattern. The temperatures necessary to reach pain levels of 10%, 20%, and 40% were significantly lower at all test sites in IBS patients compared to the control group (Table 4-3). This suggests that the difference between IBS and control groups did not depend on the evoked pain intensity level, and sensitization was not limited to dermatomes in segmental proximity to the symptomatic area but extended across the body, including to the face (Table 4-3, Figure 4-1B). These results corroborate earlier work on a different cohort of subjects in which group differences in sensitivity likewise was neither intensity- nor site-specific (chapter 2).

**Control for Placebo- and Systemic Treatment Effects**

The main goal was to determine whether the vicious pain cycle that presumably started the expansion of sensitization in non-visceral areas of the body can be stopped. Administered rectally, lidocaine was expected to silence the pain focus by its topical anesthetic effect allowing neurons receiving convergent input from visceral and somatic
tissues to return to a normal sensitivity level. On a different day topical lidocaine was given orally to control for potential systemic and placebo effects. Rectal and oral administration were expected to lead to similar degrees of absorption into the systemic circulation. In addition to the usual vehicle control tests, the oral lidocaine experiment was useful as an enhanced placebo control because it was usually detectable as an active compound by its numbing effect. Lidocaine administered rectally was identified by some of the subjects by a numbing sensation in the anal region. The recognition as an active drug is known to lead to an enhanced placebo effect. Every session involved administration of cream in both locations simultaneously, i.e., rectally and orally. The subjects were told that they would receive lidocaine and vehicle throughout the series of tests. However, they were not informed about the location assignment of each compound during any of the tests and where lidocaine was supposed to have an effect on pain sensitivity. A prolonged or even irreversible desensitizing effect of rectal lidocaine could not a priori be ruled out. This would have made it impossible to obtain within-subject control data if the rectal lidocaine test was the first test of the series. Therefore, unbeknownst to the subjects, the double oral and rectal placebo test always preceded the rectal lidocaine test. This aspect of the protocol made blinding of the investigators more difficult. Therefore, measures were taken to minimize the transmission of bias from investigators to subjects: (1) the investigator administering the treatment was never present during data collection. (2) a prerecorded standardized video sequence instead of direct communication between investigator and subject was used for instruction regarding the pain rating task; (3) non-verbal communication between investigator and subject was minimized by an arrangement where the subject was always facing away from the
investigator and by an equipment rack providing separation; (4) the experimental setup did not require the investigator to hold the thermode to the subject’s skin but was limited to pressing a start button to initiate the fully automated protocol; (5) the protocol included a test where the average pain intensity was held constant by response-dependent stimulus control, denying the subject potentially reinforcing feedback from changes in perceived pain intensity.

**Treatment Effect on Sensitivity to Non-painful Warm Stimuli**

Since previous sensitivity tests (chapter 2) revealed a similar shift in pain ratings at all sites and intensities, it was necessary to rule out the possibility of a generalized rating bias. Therefore a test was included where the participants were asked to rate non-painful warm stimuli. The intensity ratings of warm sensation, elicited by 36°C and 42°C stimuli, did not statistically differ between the IBS and control groups (Figure 4-1C). However, differences began to emerge at the highest temperature (42°C), which elicited a painful sensation in 2 IBS subjects. This temperature is known to potentially lead to nociceptor activation but not necessarily to pain (Lamotte et al., 1983).

The first nociceptive stimulus of a series tends to elicit a higher pain rating than subsequent stimuli of the same intensity. The “novelty effect” reportedly is of prolonged duration and site-specific (A.Gallez et al., 2003). The data obtained with non-painful warm stimuli suggest a similar effect: in both groups the warm rating elicited by the first 36°C stimulus was consistently higher than the rating following the second and third 36°C stimulus. Thus, the analysis was conducted without the data obtained with the first stimulus. It was found that for either group the treatments (placebo or lidocaine) failed to have a statistically significant effect on the perception of non-painful warm stimuli.
Treatment Effect on Sensitivity to Prolonged Nociceptive Stimuli

A comparison of the results of the three no-treatment control tests revealed that pain sensitivity measures slightly decreased from one session to the next. This baseline drift was not statistically significant. For all baseline-to-treatment comparisons baseline was defined as the average of the three no-treatment sessions (“average baseline”).

The results include (1) a comparison of pain sensitivities measured during treatment (lidocaine or placebo jelly) sessions with the average baseline sensitivity and (2) a comparison of pain sensitivities measured after the administration of lidocaine (orally or rectally) with the data collected after applying placebo jelly. Under all treatment conditions, IBS patients as a group, when compared to their healthy counterparts, were significantly (p<0.001) more sensitive to thermal nociceptive stimulation, i.e., the temperatures needed for eliciting any given pain intensity level were lower (Table 4-4). Furthermore, the data suggest that the IBS and control group were affected equally by the treatments, and that the effect of rectal and oral lidocaine application did not differ to a statistically significant degree. This finding weakens the notion that generalized sensitization of IBS patients is primarily maintained by a localized nociceptive focus.

Treatment Effect on Sensitivity to Brief Nociceptive Stimuli

Treatment effect analysis (placebo vs. rectal and oral lidocaine) did not reveal any Group x Treatment x Site interactions, suggesting that the treatments did not affect the groups differently at any site (Figure 4-3). In other words, if any treatment, including rectal lidocaine, affected pain sensitivity it did so similarly in both groups. This finding is inconsistent with the hypothesis that lidocaine affects pain sensitivity of IBS patients by silencing a visceral nociceptive focus.
Relationship Between Disease-Related Pain and Treatment Effect

The hypothesis of this study implies that a lidocaine effect on somatic sensitization is contingent upon the presence of an active rectal nociceptive focus which presumably is responsible for maintaining the sensitized state through positive feedback amplification. According to the hypothesis, sensitization can also be demonstrated in asymptomatic regions due to convergence of afferent pathways. The following logical consequences follow this argument: (1) topical rectal lidocaine is expected to reduce somatic sensitivity more in patients with a significant amount of visceral pain, less so in patients with less visceral pain and have little or no effect in healthy pain-free individuals; (2) topical oral lidocaine is not expected to have an effect on pain sensitivity given the fact that none of the subjects had any oral pain; (3) the effect of topical rectal lidocaine is expected to be more pronounced in the segmental proximity of the nociceptive focus in the rectum, i.e., be minimal at the facial test site. The results did not support the above argumentation.

IBS patients typically do not have visceral pain constantly, and many of the IBS subjects of this study did not experience visceral pain during some of the test sessions. However, most IBS patients have visceral pain periodically, and likewise the majority of our IBS sample reported in the daily telephone survey having had episodes of abdominal pain at some time during the study period. It can be argued that a low level of nociceptor activity may not always elicit a conscious pain perception but suffice to maintain a sensitized state. No correlation was found between lidocaine effect and clinical symptoms (pain intensity and unpleasantness) during or between sessions in the lower or upper part of the body.
Discussion

Clinicians are often frustrated when confronted with patients complaining about persistent pain for which an obvious peripheral etiology cannot be found, as is the case with irritable bowel syndrome. Complicating the matter is the observation that the hypersensitivity in IBS patients (which had previously been thought to be limited to the viscera) appears to extend to somatic tissues. The discovery of widespread somatic sensitization in IBS patients is consistent with reports that these patients have a higher propensity for a number of remote pain symptoms such as back pain, migraine headaches, and muscle pain. The predictability of treatments for many chronic pain diseases is not likely to improve until the mechanisms leading to general sensitization are understood. The goal of this research was to test the hypothesis that sensitization is a direct consequence of the continued presence of regional nociceptive signals which presumably maintain a positive feedback cycle of sensitization ("vicious pain cycle" hypothesis). It is conceivable that feedback-based sensitization could expand to large parts of the body once it has reached a sufficiently central component of the pain system that receives widespread convergent inputs. In this case one could argue that the "vicious pain cycle" begins with a focal pain problem which progressively expands the symptomatic territory by sensitizing distant areas, rendering the patients prone to developing spontaneous pain in an extended area. Based on the results of an earlier study by Verne et al., we hypothesized that local anesthetic blockade of regional clinical pain would interrupt the presumed positive feedback cycle, normalize central pain processing and reverse remote allodynia/hyperalgesia (Verne et al., 2003). Unexpectedly, the results of the present study did not support the notion that clinical pain or a rectal nociceptive focus are indispensable for maintaining a sensitized state in somatic areas.
In agreement with previous work (Verne et al., 2001; Bouin et al., 2001), thermal testing revealed that the temperature needed to elicit the midrange pain intensity levels tended to be approximately 2 degrees lower for the IBS group compared to the healthy control group. However, this difference was independent of the site tested, and the presence / absence or intensity of spontaneous pain. The lack of a relationship between segmental distance from the spontaneous pain site and degree of somatic sensitization and the lack of a correlation between thermal pain sensitivity and clinical pain are not consistent with the vicious cycle theory. However, one could argue that low-level nociceptive activity that is not sufficient to elicit a conscious pain experience may suffice to maintain feedback sensitization. This argument is partially addressed by the rectal lidocaine experiment where one could assume that nociceptive activity, whether perceived or not, is suppressed. Again the results did not speak in support of the “vicious pain cycle” hypothesis because not only was the lidocaine effect small but it was the same for the IBS and the pain-free control group and independent of whether it was given rectally or orally. These findings are in agreement with those of others: Lembo et al. tested rectal sensitivity of IBS patients following rectal lidocaine and discovered that it did not affect perception of rectal distensions (Lembo et al., 1994). Similarly, Sabate et al. have shown that lidocaine had no effect on the sensations elicited by rapid phasic rectal distension and only a minor effect when the rate of distension was slow (Sabate et al., 2000). The question regarding the causes for the different results becomes more intriguing when one considers that the same highly trained gastroenterologist was responsible for lidocaine administration in both studies. Therefore, methodological differences other than the rectal application techniques are among the likely suspects. (1)
In Verne’s et al. earlier study the subjects may not have been adequately blinded regarding the treatment because they may have identified lidocaine by its effect on thermal pain early on during the simple pain tests. Memory-based comparisons of perceived pain intensities are more likely to lead to recognition of the active drug when the testing protocol is simple (only one stimulus was used for all cutaneous pain tests). This recognition may have affected the ratings later in the test, resulting in a placebo effect. (2) In the earlier study the pain intensity levels induced in the control group were lower than those of the more sensitive IBS group. This may have left little room for a lidocaine effect in the control group (the present study’s comparisons were made at the same pain intensity level for both groups and similar lidocaine effects were found). (3) The lidocaine effects reported in the earlier study may not have been limited to the rectal area but may have been systemic. Verne’s et al. blood test to rule out systemic lidocaine levels may not have been sensitive enough to detect the lowest biologically effective levels. A number of studies have demonstrated significant reductions in pain with doses below or slightly above the 1 microgram / milliliter concentration (Brose and Cousins, 1991; Groudine et al., 1998; Mallon and Thomas, 2000; Koppert et al., 2004). Systemic lidocaine is known to have an attenuating effect on pain responses in neuropathic conditions of human and animal models, as well as in human post-operative abdominal pain (Bach et al., 1990; Mao et al., 1992; Koppert et al., 1998). Interestingly, intravenous lidocaine has been shown to reduce secondary mechanical hyperalgesia without affecting sensitivity thresholds (Dirks et al., 2000), a result which parallels the absence of a lidocaine effect found in the non-nociceptive tests of the present study. It, therefore, cannot be ruled out that levels of lidocaine below the detection threshold of
common blood assays might have an attenuating effect on pain. Consequently, in the present study it was considered prudent not to rely on such blood tests but to include an oral lidocaine experiment as a control for possible systemic effects instead.

In conclusion, some of our results confirm what has been shown before, i.e., that IBS patients have increased somatic thermal pain sensitivity. However, the present lidocaine experiments were unable to reproduce the results of an earlier lidocaine study, even though it shared one collaborator. In our sample of subjects lidocaine failed to reduce the difference in pain sensitivity between the IBS and healthy control group. This could lead to rejection of the theory that sensitization is maintained primarily by nociceptive drive from the symptomatic area. The nociceptive feedback or “vicious pain cycle” theory is also weakened by the absence of a correlation between degree of somatic pain sensitization and spontaneous visceral pain and by the fact that thermal hyperalgesia of IBS patients failed to decline with increasing segmental distance from the symptomatic area. Nevertheless, even this cumulative evidence against the feedback hypothesis should not absolve us from considering alternate explanations for the absence of a lidocaine effect in our study. (1) The decay time constant of feedback-based sensitization may be so slow that the short duration of the rectal lidocaine effect does not suffice for the hyperalgesia to measurably decline. (2) Rectal lidocaine administration may not reach more proximal portions of the gut that -- in many patients --may be more important in maintaining sensitization than the distal rectum. In this case a potential therapeutic topical effect of digitally administered lidocaine would be limited to a subgroup of IBS patients with a primary nociceptive focus in the distal digitally accessible part of the gut.
In combination, this and other studies have raised enough questions regarding the pain focus based sensitization theory that it may be time to seriously consider other mechanisms. For instance, one could argue that widespread somatic sensitization is not a consequence of IBS-related visceral nociceptive inputs but predates the onset of the symptoms that led to the diagnosis of IBS. Events that occurred much earlier in life, such as damaging influences to the CNS or to the immune system (e.g., minor traumatic brain injury, toxic exposure or infection) could have induced plastic changes in the pain processing system and led to permanent widespread hyperalgesia. Indeed, symptom-free control subjects are occasionally encountered that are as sensitive in thermal pain tests as the most sensitive IBS patients. It will be important in future studies to collect a thorough disease and trauma history from birth to the present from these individuals because it may provide clues regarding the cause of their sensitization. Once a widespread sensitized state is established, it will take only a small local insult to start a regional (e.g., IBS, MPS) or generalized (FMS) pain condition. In other words, preexisting changes in the pain processing system may have rendered these patients pain prone long before the spontaneous symptoms that define these pain diseases emerged. This argument finds support in animal models. It has been possible to induce lifelong visceral and somatic hyperalgesia in rats with visceral insults early in life. In these models sensitization appears to persist even after visceral pathology associated with the insult is no longer visible. The issue could be put to rest by conducting a longitudinal study on large samples of symptom-free subjects. The predisposition theory would move to center stage when it turns out that hyperalgesia can be demonstrated in a subpopulation of healthy subjects and that these individuals have a higher likelihood of developing pain conditions later in
life. The most clinically pressing question would then be how sensitization from an early life insult can be prevented from becoming permanent.

Table 4-1. Treatment and stimulation schedule. (N = no treatment occurred, TS = thermal stimulation session)

<table>
<thead>
<tr>
<th>Day</th>
<th>Treatment</th>
<th>Rectal</th>
<th>Oral</th>
<th>Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 1</td>
<td>Training</td>
<td>N</td>
<td>N</td>
<td>TS</td>
</tr>
<tr>
<td>Day 2</td>
<td>Baseline 1</td>
<td>N</td>
<td>N</td>
<td>TS</td>
</tr>
<tr>
<td>Day 3</td>
<td>Treatment 1</td>
<td>PLACEBO</td>
<td>PLACEBO</td>
<td>TS</td>
</tr>
<tr>
<td>Day 4</td>
<td>Treatment 2</td>
<td>LIDO</td>
<td>PLACEBO</td>
<td>TS</td>
</tr>
<tr>
<td>Day 5</td>
<td>Baseline 2</td>
<td>N</td>
<td>N</td>
<td>TS</td>
</tr>
<tr>
<td>Day 6</td>
<td>Treatment 3</td>
<td>PLACEBO</td>
<td>LIDO</td>
<td>TS</td>
</tr>
<tr>
<td>Day 7</td>
<td>Baseline 3</td>
<td>N</td>
<td>N</td>
<td>TS</td>
</tr>
</tbody>
</table>

Table 4-2. REDSTIM experiment of day 1. Continuous thermal stimulus applied to thenar eminence of right hand of Control- and IBS subjects. Thermode temperatures during induction phase at the time when pain intensity first reached 10% and 25% respectively.

<table>
<thead>
<tr>
<th>Pain Intensity</th>
<th>10% avg</th>
<th>10% stdev</th>
<th>25% avg</th>
<th>25% stdev</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>45.8</td>
<td>0.8</td>
<td>46.7</td>
<td>0.8</td>
</tr>
<tr>
<td>IBS</td>
<td>43.5</td>
<td>0.8</td>
<td>44.2</td>
<td>0.8</td>
</tr>
<tr>
<td>p value</td>
<td>0.008</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4-3. Series of brief (3 sec) stimuli applied to right cheek, forearm, and calf of Control- and IBS subjects on day 1 (baseline) of the seven day protocol. Data of ascending and descending temperature series were pooled. Temperatures (in °C) needed to elicit 10, 20 and 40% pain intensity respectively.

<table>
<thead>
<tr>
<th>Cheek</th>
<th>Forearm</th>
<th>Calf</th>
</tr>
</thead>
<tbody>
<tr>
<td>avg temp</td>
<td>avg temp</td>
<td>avg temp</td>
</tr>
<tr>
<td>st. dev.</td>
<td>st. dev.</td>
<td>st. dev.</td>
</tr>
<tr>
<td>Control</td>
<td>IBS</td>
<td>p value</td>
</tr>
<tr>
<td>49.2</td>
<td>47.5</td>
<td>0.01</td>
</tr>
<tr>
<td>1.5</td>
<td>1.3</td>
<td>2</td>
</tr>
<tr>
<td>48.6</td>
<td>47.3</td>
<td>0.009</td>
</tr>
<tr>
<td>1.4</td>
<td>1.53</td>
<td>2</td>
</tr>
<tr>
<td>48.6</td>
<td>46</td>
<td>0.0071</td>
</tr>
<tr>
<td>1.7</td>
<td>3.8</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Table 4-4. Temperatures necessary to induce 10% and 25% pain intensities during the REDSTIM induction phase for the various testing days.

<table>
<thead>
<tr>
<th>Avg Baseline</th>
<th>Double Placebo</th>
<th>Rectal Lidocaine</th>
<th>Oral Lidocaine</th>
</tr>
</thead>
<tbody>
<tr>
<td>10%</td>
<td>10%</td>
<td>10%</td>
<td>10%</td>
</tr>
<tr>
<td>Control</td>
<td>IBS</td>
<td>Control</td>
<td>IBS</td>
</tr>
<tr>
<td>46.1</td>
<td>46.7</td>
<td>46.5</td>
<td>46.8</td>
</tr>
<tr>
<td>43.6</td>
<td>44.2</td>
<td>43.9</td>
<td>44.2</td>
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<tr>
<td>46.8</td>
<td>47.3</td>
<td>46.9</td>
<td>44.7</td>
</tr>
<tr>
<td>44.2</td>
<td>44.7</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>43.6</td>
<td>43.9</td>
<td>45</td>
<td>45.3</td>
</tr>
</tbody>
</table>
Figure 4-1. No-treatment pain tests: (A) The temperature needed to maintain pain intensity near the 25% setpoint was sampled once/sec for a continuous stimulus (REDSTIM) of 2 minute duration (not counting induction phase). The running temperature average, using a window of 10 samples, is shown for the first 60 sec of the test. (B) The temperature needed to elicit 20% pain intensity with a 3 second thermal stimulus on three body sites (error bars represent standard deviation) (C) Average eVAS ratings for non-painful warm stimuli of 30 seconds duration on the left volar forearm.
Figure 4-2. Comparison of no-treatment (baseline) thermal pain responses of the Control and IBS group. The data obtained with an ascending and descending temperature series of thermal pulses of 3 sec duration were pooled. The stimuli were applied to the right calf. The height of the columns represents the stimulus temperature corresponding to the 20% pain intensity level. Pain tests without preceding lidocaine or placebo treatment took place on three different days. The data of the first session (dark columns) and the average of all three sessions (open columns) are shown.

Figure 4-3. Comparison of treatment effects on thermal pain responses of the Control and IBS groups. The data obtained with an ascending and descending temperature series of thermal pulses of 3 sec duration were pooled. The stimuli were applied to the right calf. The height of the columns represents the stimulus temperature corresponding to the 20% pain intensity level. The error bars represent the standard deviation. The data of the three no-treatment days were averaged ("Average Baseline" columns).
CHAPTER 5
NEONATAL VISCERAL INFLAMMATION INCREASES PAIN BEHAVIOR IN A RODENT MODEL OF IRRITABLE BOWEL SYNDROME

Introduction

Irritable bowel syndrome (IBS) is a common gastrointestinal disorder and is characterized by recurring abdominal pain associated with diarrhea and/or constipation. IBS is estimated to affect up to 20% of the U.S. population. Even though the pathophysiology of IBS is unclear, visceral hypersensitivity is an accepted biological marker of the disorder (Mertz et al., 1995; Naliboff et al., 1997; Verne et al., 2001). The hypersensitivity was thought to be limited to the gut, but patients with IBS frequently complain of pain in body regions somatotopically distinct from the gut, suggesting that central hyperalgesic mechanisms may be involved (Mayer and Raybould 1990; Mayer and Gebhart 1994). Interestingly, several studies have shown that IBS patients demonstrate hyperalgesia to nociceptive stimuli applied to cutaneous areas of the extremities (Verne et al., 2001; Bouin et al., 2001; Verne et al., 2003a; Rodrigues et al., 2005). These results suggest that visceral and somatic changes in pain processing overlap. In order to determine mechanisms and evaluate treatments, a valid animal model of IBS must be used.

Al-Chaer developed a model where neonatal colonic mustard oil injection induced colitis that was transient but colonic hypersensitivity that was permanent. The hypersensitivity was inferred from the abdominal withdrawal reflex response to colonic stimulation (Al Chaer et al., 2000). It is intended to be a model of a subset of IBS patients
who develop their symptoms after experiencing an infectious colitis (McKendrick and Read 1994). In addition to revealing significant increases in the rodent abdominal withdrawal reflex, the model also demonstrated increases in firing of viscerosomatic convergent neurons in spinal segments L6-S1 in response to rectal distension and somatic nociceptive pinch (Al Chaer et al., 2000). Together these results suggest an increase in both visceral and somatic sensitivity, although one could argue that reflex- and electrophysiological measures are not necessarily a valid reflection of (cortically mediated) pain perception. An assay that measures a cortically mediated response may be a better predictor of cerebral manifestations of pain than reflexes or neuronal firing patterns in the spinal dorsal horn. Therefore, the present study used learned operant escape behavior from a thermal stimulus as a measure of somatic pain sensitivity. The overall purpose of our current study was to see if rats that were neonatally treated with mustard oil developed a lifelong increase in thermal sensitivity to nociceptive stimulation of the paws. A demonstration of increased sensitivity to nociceptive stimulation of the paws would parallel the cutaneous thermal hypersensitivity seen in human IBS patients and thus contribute to the validation of the model.

Methods

Induction of Colitis:

A total of 22 neonatal pathogen-free female Long Evans rats were treated for 14 days, between the ages of 8 and 21 days, with either intracolonic injections of saline (n=11) or mustard oil (0.2 mL, 5%) (n=11). Mustard oil produces a transmural colitis that resolves within four weeks (Al Chaer et al., 2000). The animals were provided free access to food and water throughout treatment and testing. All procedures were approved by the University of Florida’s Institutional Animal Care and Use Committee and the
Operant Escape Testing

Behavioral testing was initiated 2 months following colonic injections with mustard oil or saline. Thermal pain sensitivity was measured in an operant escape test (Figure 5-1). It is a shuttle-box-like test that measures escape behavior induced by thermal nociceptive stimulation (Mauderli et al., 2000). One side of the operant escape apparatus consisted of a thermally controlled surface and the opposite side of an escape platform which was at a neutral temperature. The animals were free to move between the two areas that were partially separated by a hanging septum (i.e., the animals could escape the hot stimulus by stepping on the escape platform). The escape platform compartment was brightly lit (3000ft candles), while the thermal stimulus compartment was relatively dark. Bright light is aversive to rodents and was used to minimize avoidance behavior. Avoidance is defined as preemptive utilization of the neutral floor, independent of whether the thermal floor elicits pain or not. The thermal floor was either at a neutral (36°C) or a nociceptive (44°C) temperature. The inclusion of a neutral temperature allowed detection of potential avoidance behavior by providing information about the relationship between stimulus intensity and response. The duration of each trial was 15 minutes during which multiple escape responses could be observed. The operant test minimized stress and the possibility of thermal injury to the paws as the animals were unrestrained and free to escape the stimulus at any time. This learned situation-specific escape response requires a higher level of neural processing than innate reflexive behaviors such as withdrawal or licking of the paw.
Data collection was preceded by a training period of approximately 2-3 weeks. Training began at low temperatures with the light off, to facilitate acclimation to the test environment. The temperature was increased to 36, 42, 44, and 47°C over a week to allow the animal to learn to escape from the thermal plate to the thermally neutral platform. Once the rat demonstrated stable escape behavior, training continued with the light on. Once test-retest comparisons suggested that the learning curve had reached a plateau, data collection commenced. Tests were conducted approximately 3 times a week for 2 months. Each test session consisted of two phases of 15 minutes duration each. During the first phase of 15 minutes the thermal plate temperature was either 36°C (skin temperature) or 44°C. The 36°C conditioning phase allowed the paws of the animals to equilibrate to a standard temperature, and exploratory behavior could subside prior to the second phase. A 44°C conditioning phase allowed slowly-responding pain modulation systems to be activated prior to the second phase of the test. During the second phase escape behavior from either a 36°C or 44°C floor stimulus was measured. The response variables were: (1) frequency of movement between the plate and escape platform, (2) latency of each escape event, and (3) duration of each escape event. The animals shuttled back and forth between the thermal plate and the escape platform. An escape latency decrease (spent on thermal plate prior to escape) was interpreted as an increase in pain sensitivity. An increase in escape duration (time spent on the escape platform) was considered to be an indicator of the intensity of stimulus-related after-sensations. The behavior could gradually change over the course of the 15 min test, e.g., due to pain adaptation. Therefore, certain analyses focused on only the first four escape events.
Place Preference Testing

The place preference test consisted of two compartments with different floor temperatures: compartment 1 had a heated floor (45°C), compartment 2 had a nociceptively cold surface (10°C). The number of crossings and duration of time spent on each side were recorded for each 15 minute trial. The rats quickly learned to apportion their time according to the relative aversiveness of the two plates. Unlike in the escape test, bright light was not used as a behavioral driving force in the place preference assay.

Paw Temperature Measurement

At the conclusion of 8 weeks of operant testing, each rat underwent a test to measure paw temperature regulation in response to thermal heating. After induction of general anesthesia (Nembutal), the animals’ core temperature was stabilized at 37.5 °C with a heating blanket and monitored with a rectal probe. Temperature sensors were placed on both forepaws, one hind paw and the tail to record thermoregulatory responses in areas that were not directly stimulated. In order to improve accuracy, thermally conductive paste was applied on the paw-side of the thermistors while the other side was covered with insulation material. This assembly was secured to the paws with tape. The remaining hind paw was exposed to a 10 minute 44°C contact heat stimulus. Throughout the stimulus and the subsequent 10 minute recovery period, the core, tail, and paw temperatures were recorded in 15 second intervals. After a 15 minute break, the procedure was repeated in mirror-image fashion, i.e., with the other hind paw serving as stimulation site.

Protocol

Thermal operant testing began two months following colonic injections. Five different test protocols, each repeated 8 times over the course of two months, were used.
The number of days between sessions varied, but the order of escape and place preference tests was kept constant (Table 5-1).

**Statistical Analysis**

Escape numbers, latencies, durations and temperature changes were analyzed with a repeated measures ANOVA model. The first four escapes for the operant escape assay and place preference test were used for the within and between group comparisons.

**Results**

**Temperature Effect on Operant Escape**

In order to assure that the measured responses represented escape rather than avoidance, tests were conducted above and below pain threshold (i.e., 36°C and 44°C) (Table 5-1). Avoidance behavior can be recognized when the stimulus-response function is flat, i.e., when the responses to 36 and 44°C are similar. The temperature of 44°C is known to excite nociceptors (Tillman et al., 1995a; Tillman et al., 1995b), while 36°C neither activates nociceptors nor elicits pain. For both test conditions, the animals cycled back and forth between the plate and platform, responding to the conflicting motivations of escaping thermal pain and bright light. The total number of platform occupancies was slightly increased for the suprathreshold floor temperature of 44°C compared to the neutral 36°C floor temp. Within-group analysis showed that both groups spent a significantly larger amount of time on the escape platform when it was at 44°C as opposed to 36°C (p<0.005). Analysis of consecutive escape events revealed that the temperature effect on escape duration is most visible during the second, third, and fourth escapes and less so during the first escape of the trial when the drive to explore is likely to still be a significant force. Baseline escape latencies and escape platform dwell times
for the 36°C and 44°C tests following a 36°C conditioning phase are presented in Figure 5-2 A,B and Table 5-2.

Within-group analysis of escape latency in tests that followed a 36°C phase 1 conditioning stimulus revealed as expected that escape latency became shorter when the temperature increased (p<0.009) from 36 to 44°C (Figure 5-2C and D). Therefore, dwell times on thermal plate and escape platform both exhibited a relationship with stimulus temperature and thus may be suitable to infer the perceptual intensity during the stimulus or the sensory after-effect that remains after escaping.

Within-group analysis was also used to evaluate test responses to 36°C and 44°C following a 44°C conditioning stimulus. Table 5-2 and Figure 5-3 demonstrate that escape latency and duration again were temperature-dependent.

In summary, the operant behavior in response to a thermal stimulus under all test conditions exhibited the expected relationship with stimulus intensity.

**Treatment Effect on Operant Escape**

The latency and duration of the first escape event of each trial did not differ significantly between treatment and control groups under any of the test conditions. Tests conducted at a non-nociceptive 36°C produced similar escape durations for the group with a history of chemically-induced colitis and the control group, regardless whether the test was preceded by a 36°C or 44°C conditioning stimulus. Treatment-specific effects were seen when the test temperature was 44°C, however, only when a neutral (36°C) conditioning stimulus preceded the test. In this case, the treatment group spent more time (p=0.001) on the escape platform for escape events 2-4 (Figure 5-4A, Table 5-3). A timeline (Figure 5-5) shows the onset and duration of the first four consecutive escape responses. When the plate temperature in the operant test was 44°C, a preceding 44°C
conditioning stimulus prolonged escape platform dwell times only for the control group but not for the treatment group (Figure 5-4 A, B). It appears that in the treatment group the transient colitis had already saturated the sensitization mechanism, allowing no room for further sensitization by the thermal conditioning stimulus.

**Place Preference Test**

In this test the animals had a choice between a nociceptive cold 10°C and a nociceptive hot (45°C) stimulus. Normal female Long Evans rats appear to perceive these two stimuli as approximately equally aversive (Vierck et al., 2002). Indeed, the control group spent 50% of the time on each side, confirming the previous results (Figure 5-6). In contrast, the group with a history of colitis exhibited a cold preference by spending more time on the 10°C side (65%) and less time on the 45°C side (35%)(p=.05) during the first four escapes. This suggests that colitis marked the onset of sensitization to somatic nociceptive heat stimuli.

**Paw Temperature Regulation**

The goal of this experiment was to compare autonomic reactivity of the two groups by measuring the effect of thermal nociceptive stimulation of one hind paw on the temperature of the three non-stimulated paws, body core and tail. (Figure 5-7 and 5-8). The treatment group’s initial paw temperature was lower than the control group, but not significantly. In order to compensate for baseline differences, temperature changes relative to baseline were used for analysis. Figure 5-7C demonstrates a significant group difference in the skin temperature response for the non-stimulated hind paw (p=0.006), while figure 5-7A illustrates that for the left fore paw the change failed to reach the significance criterion (p=0.061). The significant difference between the groups on the right hind paw was visible from 5 minutes after onset to the end of the 10 minute 44°C
stimulus. There was no significant difference in temperature changes between the two groups for the right fore paw, tail, or core. These results suggest that normal animals respond to a thermal nociceptive paw stimulus with an initial skin temperature reduction on the contralateral paw. In treated animals this response appears to be blunted. This effect could not be duplicated when the experiment was repeated in mirror image fashion (stimulus applied to right hind paw) 15 minutes later (Figure 5-8). It appears the delay between experiments was too short and did not allow the thermoregulatory of the systems to recover. The treatment group appears to be lacking thermoregulatory responsiveness to begin with.

Discussion

The chief pain complaints of IBS patients are typically localized in the gut. However, it is known that hyperalgesia can be demonstrated in somatic tissues across widespread areas of the body where no spontaneous pain is reported. It has been argued that feedback-based sensitization mechanisms and convergence between visceral and somatic afferent projections lead to sensitization of somatic tissues. The results of the three preceding chapters provided little support for this notion and so the alternate hypothesis of a sensitized state that precedes the onset of IBS had to be given more consideration. Little local pathology is needed in a hyperalgesic individual to trigger and maintain a chronic pain disease like IBS. The question arises what can cause a widespread hyperalgesic state that renders the patient pain-prone. The present study tested the theory that an insult early in life, e.g., a severe gut insult, may have induced plastic changes within the pain processing system that resulted in hyperalgesia. A rodent model of this scenario where the late effects of transient neonatally induced colitis are studied was developed by AlChaer and coworkers (Al Chaer et al., 2000). The neonatal
colonic irritation model thus far has been used mostly to study long-term changes in 
visceral sensitivity, changes in somatic withdrawal reflexes, and neurophysiological 
changes (Al Chaer et al., 2000; Lin and Al Chaer, 2003). The present research project 
expanded this work by measuring sensitivity changes with an operant test that may be the 
best rodent model of the human conscious pain experience thus far. The combined 
results of these studies suggest that a visceral insult experienced very early in life can 
have lasting consequences on visceral and somatic pain sensitivity. The studies do not 
allow to conclusively reject the vicious cycle theory, i.e., the notion that continued 
occlusive activity in a region of the body is necessary to maintain the body-wide 
sensitized state because they did not investigate whether spontaneous nociceptive firing 
persists indefinitely after the neonatal visceral insult. However, the fact that 
histopathological changes in the gut are no longer seen once the treated animals reach 
adulthood suggests that the insult does not leave a persistently inflamed area that would 
be a likely origin of continued nociceptive input. Furthermore, attempts to induce 
persistent visceral or somatic hypersensitivity with mustard oil administered rectally in 
adult rats were unsuccessful: sensitization was elevated for no more than approximately 
one week after rectal treatment and then returned to normal (Al Chaer et al., 2000).

Human psychophysics data (chapter 4) suggested that somatic sensitization of IBS 
patients is specific to the nociceptive range of stimulus intensities. Interestingly, the 
sensitization of neonatally treated rodents likewise was specific to the nociceptive range: 
non-nociceptive somatic stimuli failed to elicit abnormally high activity levels in dorsal 
horn cells with viscerosomatic convergent inputs. The responses of the same neurons 
were enhanced in treated animals when noxious pinching and deep tissue stimulation of
cutaneous skin was administered within the appropriate dermatome (Al Chaer et al., 2000). Similarly, in the present study a group difference in sensitivity was obtained only with nociceptive (44°C) but not with non-nociceptive (36°C) stimuli. The different conditioning stimuli (44 or 36°C) did not have an effect on the animals’ ability to distinguish between the non-nociceptive and nociceptive temperatures during the subsequent operant test.

The group differences were limited to the variable “escape duration”, i.e., the time spent on the escape platform before returning to the thermal plate. The variable “escape latency”, i.e., the time the heat stimulus was tolerated before an escape response took place, did not produce significant differences between treated and naïve animals. In other words, treated animals did not escape sooner from the 44°C plate than healthy controls but -- once they had escaped -- spent relatively more time on the escape platform. This suggests that treatment may primarily affect the rate of recovery from the pain elicited by the thermal plate. The place preference results, likewise, are compatible with the notion that the heat-induced pain experience lingers on longer in animals with a history of a neonatal visceral insult. There are numerous mechanisms that may cause an increase in the duration of pain sensation in the rodent paw. The central neurons’ ability to reset to baseline activity levels could be compromised due to a deficit in inhibitory control. In this case though, it would be expected that the animals would escape sooner because they would reach the escape threshold faster, unless one assumes that negative-feedback-based inhibitory systems react so slowly that they do not manifest themselves until late into the heat exposure period. A persistent up-regulation of neuromodulators such as substance P in the aftermath of the neonatal visceral insult must be considered as an
alternate mechanism. Substance P is known to increase duration of neuronal afterdischarges (Kellstein et al., 1990; Budai and Larson, 1996). Indeed, substance P levels reportedly are increased in the cerebro-spinal fluid of IBS patients (Dong et al., 2004). A role of substance P as a causative factor for sensitization of IBS patients is quite plausible when one considers that 80% of intestinal neurons release this transmitter (De Felipe et al., 1998).

In addition to visceral and somatic sensitization, IBS appears to be associated with changes in thermoregulation. The treated and naïve rodent group did not differ in baseline paw temperatures, but regulation of contralateral paw temperature in response to a thermal paw stimulus appeared to be blunted in treated animals. It has been proposed that that skin temperature regulation is inversely related to sympathetic activity (Mcallister et al., 1990). Therefore, one could hypothesize that sympathetic regulation may be one of the targets of plasticity induced by neonatal injury.

In conclusion, Al Chaer’s model appears to mimic a number of features that characterize IBS patients clinically and in the context of psychophysics tests. The neonatally treated rodents have increased thermal sensitivity to nociceptive temperatures on the paw which persist long after the visceral symptoms cease. Al Chaer demonstrated that the same treatment to adults does not result in the visceral or cutaneous hypersensitivity. Together these results suggest that visceral insults can induce permanent changes in pain processing and autonomic function only during an early postnatal window of plasticity.
Table 5-1. Testing Schedule. Each escape test session (1-4) in the operant apparatus consisted of 2 phases of 15 minutes each. A conditioning phase (phase 1) was followed by the data collection phase (phase 2). The table shows the temperature assignments of the four escape test sessions. The place preference test (session 5) measured relative time spent on a 10 and 45°C plate.

<table>
<thead>
<tr>
<th>Testing Schedule</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Phase 1 and 2:</strong> 15 minutes each</td>
<td></td>
</tr>
<tr>
<td><strong>Test 1</strong></td>
<td><strong>Test 2</strong></td>
</tr>
<tr>
<td>Phase 1 36°C</td>
<td>Phase 1 44°C</td>
</tr>
<tr>
<td>Phase 2 44°C</td>
<td>Phase 2 36°C</td>
</tr>
</tbody>
</table>

Table 5-2. Effect of plate temperature (36 vs. 44°C) on escape duration (sec) and escape latency group means (sec). The change in stimulus temperature from 36 to 44°C lead to a statistically significant change in escape duration and latency for the first four escape events of the trial in both groups, regardless of whether the conditioning stimulus (phase 1) was 36 or 44°C.

<table>
<thead>
<tr>
<th>Control group; Escape duration (sec)</th>
<th>Treatment group; Escape duration (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escape 1</td>
<td>Escape 2</td>
</tr>
<tr>
<td>Phase 1 36°C</td>
<td>Phase 2 36°C</td>
</tr>
<tr>
<td>Phase 1 44°C</td>
<td>Phase 2 36°C</td>
</tr>
<tr>
<td>26.7</td>
<td>31.3</td>
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<tr>
<td>30.6</td>
<td>65.1</td>
</tr>
<tr>
<td>36.3</td>
<td>49.4</td>
</tr>
<tr>
<td>Control group; Escape latency (sec)</td>
<td>Treatment group; Escape latency (sec)</td>
</tr>
<tr>
<td>Escape 1</td>
<td>Escape 2</td>
</tr>
<tr>
<td>Phase 1 36°C</td>
<td>Phase 2 36°C</td>
</tr>
<tr>
<td>Phase 1 44°C</td>
<td>Phase 2 36°C</td>
</tr>
<tr>
<td>120.4</td>
<td>56.9</td>
</tr>
<tr>
<td>63.1</td>
<td>44.8</td>
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<td>99.2</td>
<td>67.1</td>
</tr>
<tr>
<td>30.5</td>
<td>48.6</td>
</tr>
</tbody>
</table>

Table 5-3. Effect of treatment on escape duration (sec) and escape latency (sec) group means. The treatment group differed from the control group to a statistically significant degree only when the test temperature (Phase 2) was nociceptive (44°C) and the preceding conditioning stimulus (Phase 1) was non-nociceptive (36°C).

<table>
<thead>
<tr>
<th>Phase 2 36°C; Escape duration (sec)</th>
<th>Phase 1 44°C; Escape duration (sec)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Escape 1</td>
<td>Escape 2</td>
</tr>
<tr>
<td>Phase 2 36°C</td>
<td>Phase 2 44°C</td>
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<td>Phase 2 44°C</td>
<td>Phase 2 44°C</td>
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<tr>
<td>115.7</td>
<td>86.4</td>
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<tr>
<td>63.1</td>
<td>44.8</td>
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</table>
Figure 5-1. The Rodent Operant Assay uses a shuttle box with two compartments. The floor of one compartment consists of a thermal plate which is held at the desired test temperature by internal water circulation. The floor of the other compartment is thermally neural (escape platform). A bright light (aversive to rodents) turns on when the escape platform is occupied.
Figure 5-2. Effect of Phase 2 test temperature (open squares: 36°C; black squares: 44°C) on Escape Duration and Latency group means shown for the first four escape events of the 15 minute trial. The Phase 1 conditioning stimulus was 36°C in all cases.

Figure 5-3. Effect of Phase 2 test temperature (open squares: 36°C; black squares: 44°C) on Escape Duration and Latency group means shown for the first four escape events of the 15 minute trial. The Phase 1 conditioning stimulus was 44°C in all cases.
Figure 5-4. Effect of Treatment (open squares: control group; black squares: rectal mustard oil) on Escape Duration (upper charts) and Latency (lower charts) group means shown for the first four escape events of the 15 minute trial. The Phase 1 conditioning stimulus was 36°C (left charts) and 44°C respectively.

Figure 5-5. Sequential representation of the first four escape events on the time axis. The group means of the escape latencies, i.e., the times spent on the thermal plate (black) and the escape duration, i.e., the times spent on the escape platform (white) are shown. C= Control group; Trt=Treatment group. The thermal plate was at 44°C for all tests. The three horizontal bars represent tests following different conditioning stimuli (36 and 44°C respectively).
Figure 5-6. Place Preference Test. Group means (pooled first four crossing events) for time spent on the 10°C and 45°C sides. Error bars represent the standard deviation.

Figure 5-7. Skin temperature regulation. Group means of temperature recorded at four sites during a 10 min thermal stimulus (44°C) to the left hindpaw and for 10 minutes after the end of the stimulus. Recording sites: (A) left forepaw; (B) right forepaw; (C) right hindpaw; (D) rectum.
Figure 5-8. Skin temperature regulation. Group mean of temperature recorded at four sites during a 10 min thermal stimulus (44°C) to the right hindpaw and for 10 minutes after the end of the stimulus. Recording sites: (A) left forepaw; (B) right forepaw; (C) left hindpaw; (D) rectum. This experiment followed the test shown in Figure 5-7 after a delay of 15 minutes and the stimulation and recording sites were arranged as a mirror image of the preceding experiment.
Localized or widespread pain complaints in somatic and visceral tissues are typical for a number of chronic pain conditions. For instance, IBS, which is considered a visceral disease and diagnostically defined by visceral symptoms, is often associated with pain far removed from the gut, e.g., the incidence of headaches, back pain, etc, is higher than average. Similar findings occur in patients with masticatory myofascial pain syndrome (MPS) as they present with episodes of pain not only in masticatory muscles but also in non-facial areas. Even when the clinical complaints are confined to a localized area (e.g., the gut in IBS patients or the face in masticatory MPS), widespread pain sensitization has been experimentally demonstrated in many of these patients (Maixner et al., 1998; Verne et al., 2001; Bouin et al., 2001; present work). A sensitized state may offer an explanation why these patients are generally more pain-prone anywhere in the body considering that in the presence of hyperalgesia it takes a smaller tissue insult to reach pain threshold. It is well known that prolonged exposure to a nociceptive stimulus leads to an increase in pain sensitivity at least at the location of the stimulus and—over time— even beyond. In fact, short lasting sensitization (wind-up) has been induced in an experimental setting with brief repetitive thermal and mechanical stimuli (Vierck et al., 1997; Maixner et al., 1998). It cannot be ruled out that a disease-related nociceptive focus can do the same, and due to its persistent nature, over time lead to states of sensitization that are longer lasting than wind-up and extend to more distant areas of the body. The question addressed by this body of work is whether the
widespread sensitization or pain-proneness of IBS patients originates in the symptomatic areas that diagnostically define the disease and gradually spreads from there (vicious pain cycle). The data presented in chapters 2-4 suggest that a key role for the vicious pain cycle as a basis for remote sensitization in IBS patients must be questioned. Other possible mechanisms must be given serious consideration.

The first finding that put the vicious pain cycle hypothesis into question was that remote pain sensitization did not occur in a gradient fashion (IBS patients were expected to be most sensitive on the foot and less sensitive on the cheek compared to control subjects), but instead the sensitization was equal along the entire segmental axis of the body. Interestingly, the IBS patient’s thermal hypersensitivity was nociceptive-specific but independent of the pain intensity level at which the test was conducted and similar regardless whether brief or prolonged test stimuli were used.

The vicious pain cycle hypothesis was weakened further when it was found that at least in the samples of this project, the IBS group’s thermal pain sensitivity was independent of the magnitude of disease-related spontaneous pain. Since the hypersensitivity induced by the disease are very stable: i.e., episodes without constant clinical pain are not associated with appreciably lower sensitivity levels, it appears that disease-related sensitization of somatic tissues of IBS patients is maintained by an extremely long term mechanism. Granted, the lack of a relationship between disease-related pain and experimentally measured sensitization does not necessarily eliminate the vicious cycle theory as a viable hypothesis because it is known that low level activity of nociceptors may not always induce consciously perceived pain. It cannot be ruled out that nociceptors are firing in the viscera of IBS patients that do not currently have pain.
This made it necessary to include an experiment where nociceptor activity (whether consciously perceived as pain or not) was silenced with topical local anesthetics. It turned out that rectally administered topical anesthetic affected sensitization only minimally and similarly in IBS and control subjects. According to the vicious pain cycle hypothesis, IBS patients only but not healthy controls (who do not have a nociceptive focus) should have been affected by the rectal lidocaine treatment. Again, this finding by itself does not allow discounting completely the feedback-based sensitization theory because one could argue that in the present sample of subjects the presumed nociceptive focus was located proximal to the rectal area that is digitally accessible for topical drug administration.

However, the fact that none of the results of the present series of studies provide direct or indirect support for the nociceptive focus-based sensitization theory must at least convince us to consider alternate hypotheses. The new theory should be able to accommodate the findings that--at least in a subpopulation--sensitization is widespread across the body, largely independent of intensity and unpleasantness of clinical pain and not responsive to topical lidocaine intervention when control experiments for systemic and placebo effects are included in the protocol. A possible alternate hypothesis could postulate that widespread sensitization is not the result of IBS-related regional nociceptive activity but that the hyperalgesia predates onset of IBS and facilitates the development of the chronic pain disease. In other words, according to this theory, widespread sensitization would be a primary factor that makes the individual pain-prone across the body and thus susceptible to developing a chronic pain condition, such as IBS or MPS, upon a minor regional insult. In this context it is interesting to note that some
IBS patients had normal sensitivity while a small number of healthy control subjects exhibited hypersensitivity when probed with experimental stimuli. The heterogeneity within the IBS group found in most psychophysics studies should not come as a surprise when one considers that the diagnostic criteria for the disease are symptom-based only and pain sensitivity of somatic tissues is not one of the criteria. The observation that “healthy” control subjects can be hyperalgesic prompts the questions (1) whether the hyperalgesic IBS subgroup is more likely to develop a generalized pain disorder such as fibromyalgia than the subgroup without cutaneous sensitization; (2) whether the hyperalgesic subgroup and the subgroup with normal sensitivity to thermal nociceptive stimuli are characterized by different disease mechanisms and thus require different treatments? If the latter turns out to be the case, psychophysics tests similar to those used in this project may ultimately be of diagnostic value. Lastly, the question arises whether control subjects with widespread cutaneous hyperalgesia are prime candidates for developing a chronic pain disease later in life? Longitudinal studies to conclusively answer this question are lacking. However, animal experiments, such as the one described in chapter 5, suggest that visceral insults early in life can result in lifelong visceral (Al Chaer et al., 2000) and somatic (chapter 5) sensitization. This, in combination with the lack of convincing support for the vicious cycle hypothesis (chapters 2, 3, and 4) suggests that our alternate hypothesis deserves serious consideration in the design of future studies: the widespread pain sensitization seen in a large number of chronic pain patients (including many IBS patients) is not induced by nociceptive drive from a localized symptomatic region but may have preexisted before the onset of the chronic pain syndrome. As possible causes for the generalized sensitized
state one must consider a plasticity-inducing injury or infection earlier in life (consistent with the findings of the rodent study described in chapter 5), genetic factors, or a combination of the two. The plastic changes responsible for sensitization could be widespread and affect large parts of the somatosensory central nervous system. Alternatively, they could affect a central component of the CNS where nociceptive signals from a large part of the body converge. Lastly, it is possible that the plasticity leads to diffuse widespread sensitization because it targets the neuroendocrine or cytokine system. Longitudinal long-term studies in large populations of initially healthy subjects and experiments in animal models where the late consequences of transient neonatal and postnatal insults can be studied under controlled conditions will provide the answers.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

I was born in Providence, RI on March 7, 1978. By the age of one, I moved to Smithfield, RI. I attended Smithfield High School from 1992-1996. In August 1996, I began my collegiate work at Assumption College in Worcester, MA. I graduated in May 2000 and received a Bachelor of Arts. The following August I started the Interdisciplinary Program in Biomedical Sciences at UF, to work on my Ph.D. in neuroscience.