

Stress and the Microbiota–Gut–Brain Axis in Visceral Pain: Relevance to Irritable Bowel Syndrome

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SUMMARY

Visceral pain is a global term used to describe pain originating from the internal organs of the body, which affects a significant proportion of the population and is a common feature of functional gastrointestinal disorders (FGIDs) such as irritable bowel syndrome (IBS). While IBS is multifactorial, with no single etiology to completely explain the disorder, many patients also experience comorbid behavioral disorders, such as anxiety or depression; thus, IBS is described as a disorder of the gut–brain axis. Stress is implicated in the development and exacerbation of visceral pain disorders. Chronic stress can modify central pain circuitry, as well as change motility and permeability throughout the gastrointestinal (GI) tract. More recently, the role of the gut microbiota in the bidirectional communication along the gut–brain axis, and subsequent changes in behavior, has emerged. Thus, stress and the gut microbiota can interact through complementary or opposing factors to influence visceral nociceptive behaviors. This review will highlight the evidence by which stress and the gut microbiota interact in the regulation of visceral nociception. We will focus on the influence of stress on the microbiota and the mechanisms by which microbiota can affect the stress response and behavioral outcomes with an emphasis on visceral pain.

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Introduction

Irritable bowel syndrome (IBS) is a complex heterogeneous disorder associated with abdominal visceral pain, constipation, diarrhea, or a combination of both [1]. It is the most common disorder seen by gastroenterologists and presents frequently with a

number of intestinal and nonintestinal comorbidities [2]. Notably, anxiety and depressive disorders account for 20–60% of these comorbidities [2,3]. The disorder is now viewed as one of altered gut–brain axis homeostasis [4–6]. A distinguishing feature of IBS is that symptoms, including abdominal pain, are often triggered or exacerbated during periods of stress [7,8]. Stress is defined as the

reaction by the body to a stimulus, either physical or psychological, that disrupts homeostasis. Stress has profound effects on the gastrointestinal (GI) tract including but not limited to alterations in intestinal motility [9], mucosal transport, gut barrier function [10–12], and visceral perception [13,14]. More recently, the role of the gut microbiota in the bidirectional communication along the gut–brain axis, and subsequent changes in behavior, has emerged [15,16]. Recent evidence proposes that stress can lead to long-term changes in the gut microbiota [17]; however, the importance of the gut microbiota and their role in visceral sensation and nociception remain to be further explored. The focus of this review will be to attempt to summarize a complex body of literature describing the relationship between stress and visceral pain and how the gut microbiota may interact through complementary or opposing factors to influence visceral nociceptive behaviors.

Stress

Stress was first described by Hans Selye almost 80 years ago as the general response of the body to any noxious stimulus [18]. Selye elucidated the role of the hypothalamic–pituitary–adrenal (HPA) axis in mediating the biological effects of stress on the host. At the same time, Walter Cannon coined the phrase “fight or flight” response [19] with much of his work building upon Claude Bernard’s description of homeostasis [20]. The HPA and sympathomedullary axes are the two stress response pathways in mammals. The HPA axis is slower-acting and adaptive, encompassing a network of anatomical constituents located both in the central nervous system (CNS) and in the periphery. The crucial components are the paraventricular nucleus (PVN) of the hypothalamus, the pituitary gland (anterior lobe), and the adrenal gland [21]. The HPA axis responds to a stressor by releasing corticotropin-releasing hormone (CRH) into the hypophyseal portal circulation, which travels to the anterior pituitary gland, where it binds to its respective receptor (CRH₁). This event leads to the production of pro-opiomelanocortin, which is subsequently cleaved within the pituitary corticotropes, to produce adrenocorticotrophic hormone (ACTH), which in turn is released into systemic circulation. ACTH targets the adrenal cortex to stimulate the production and secretion of glucocorticoids such as cortisol (humans) or corticosterone (rats and mice) [21]. Glucocorticoids are the main effector molecules of the HPA axis and, via binding to their intracellular receptors, function to regulate the physiological adaptations to stress [22,23]. Cortisol/corticosterone initiates negative feedback through binding to glucocorticoid (GR) and mineralocorticoid (MR) receptors [24,25] in the hippocampus, PVN, and anterior pituitary [26]. However, binding of cortisol/corticosterone to the amygdala promotes CRH expression and facilitation of the stress axis [27,28]. The HPA axis is under stringent regulation at both the neuronal and the endocrine levels; however, the body can also elicit maladaptive changes resulting in altered brain structure and function in response to chronic and uncontrollable stressors [29–31]. The sympathomedullary axis is responsible for the acute fight or flight response, which is driven by the activation of preganglionic sympathetic neurons located within the intermediolateral cell column of the thoracolumbar spinal cord. The projections of these preganglionic sympathetic neurons are pre- and

paravertebral ganglia. These project to terminal organs and to chromaffin cells of the adrenal medulla resulting in increased circulating levels of epinephrine (adrenal medulla) and norepinephrine (sympathetic nerves) [32]. The release and action of these catecholamines results in alterations in physiology such as increased heart rate, vasoconstriction, and mobilization of energy stores, to allow the host to adequately adapt to the stressor.

Visceral Pain

Visceral pain is a generic term that is applied to pain arising from the internal organs contained within the thorax and abdomen [33]. Acute visceral pain usually has an identifiable cause, such as infection or tissue damage, which can be typically treated with an appropriate therapeutic agent. In contrast, chronic visceral pain, such as other types of chronic pain, is long-lasting and can be difficult to treat with current pharmaceuticals. The lack of identifiable pathology in some types of chronic visceral pain has led to the use of the term functional gastrointestinal disorders (FGIDs) to describe 45 adult and pediatric disorders, including IBS, functional dyspepsia, infant colic and abdominal migraine, that affect discrete regions of the GI tract [34].

Within the GI tract, extrinsic nociceptors can respond to multimodal stimuli, depending on receptor expression, including stretch, pH, bacterial products, substances released from immune cells, and neurotransmitters released from the enteric nervous system or enterochromaffin cells [35]. The nociceptors have nerve endings throughout the layers of the GI tract (mucosal, submucosal, muscular), and their cell bodies are located in the dorsal root ganglion (DRG). The first synapse is in the superficial layers of the dorsal horn of the spinal cord. The nociceptive signal is then transmitted to the contralateral side of the spinal cord via decussating fibers, and pain signals reach the brain via the spinothalamic tract, which has a somatotopic arrangement in the anterolateral aspect of the spinal cord. Although vagal afferents were not previously thought to be involved in the mediation of visceral pain [36], recent evidence suggests a role for vagal transmission of anti-/pro-nociceptive signals, which bypasses the spinal cord [37–40]. Within the brain, the signal is then relayed to cortical areas for localization and to limbic areas for the emotional component of the pain response. Output from the cortical and limbic regions in response to the pain activates descending inhibitory circuitry within the brainstem that causes release of inhibitory neurotransmitters within the dorsal horn of the spinal cord. While the mechanisms responsible for chronic pain are still under investigation, and likely are dependent on the nature of the initiating stimulus, sensitization can occur at a number of different sites. These include but not limited to local mediators within the GI tract, remodeling of ascending afferents within the dorsal horn, hyperactivity of central pain circuitry, and/or loss of descending inhibition (Figure 1).

In the laboratory setting, GI hypersensitivity, or an exaggerated sensitivity, is typically measured in response to distension of the particular area of the GI tract. In the case of clinical studies, the subject has a balloon catheter inserted into the colorectal region and graded isobaric or isovolumetric distensions are performed with the individual reporting their perception of the stimulus. Thresholds can be determined for perception of the balloon, dis-

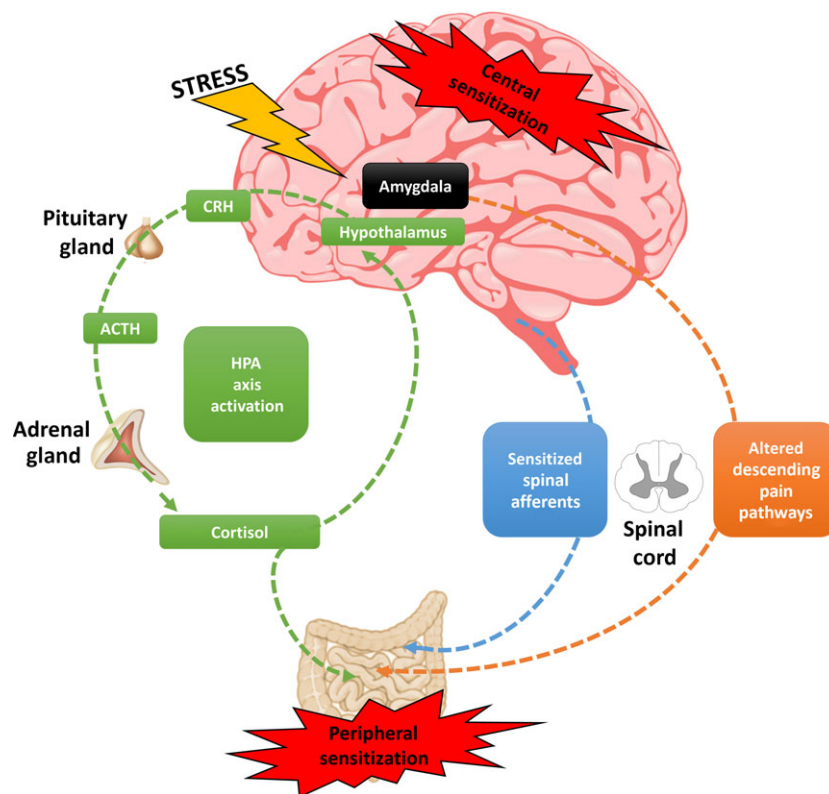


Figure 1 Central and Peripheral Pain Sensitization. Heightened pain perception can occur due to a combination of both central sensitization and peripheral sensitization. The hypothalamic–pituitary–adrenal (HPA) axis is activated by stress. In brief, the hypothalamus secretes corticotropin-releasing hormone (CRH) into the hypophyseal portal system for the activation of the anterior pituitary and subsequent release of adrenocorticotrophic hormone (ACTH) into the systemic circulation. In response to ACTH, the adrenal cortex releases cortisol (corticosterone in rodents), which can directly activate resident immune cells and extrinsic primary afferents within the gastrointestinal tract to promote peripheral sensitization. While cortisol binding to the hypothalamus promotes feedback inhibition of the stress response, cortisol binding to the amygdala facilitates further stress-induced secretion of CRH, promoting central sensitization of stress pathways. The amygdala also promotes CRH signaling in the brain stem to further promote central sensitization by altering descending inhibition within the spinal cord. Direct injury to the GI tract can lead to sensitization of spinal afferents (lower threshold for activation and/or longer lasting responses) that can persist following recovery from the injury.

comfort, and pain, with many patients with IBS reporting lowered thresholds for all stimuli compared to healthy volunteers, and hence, visceral hypersensitivity is apparent in a subset of patients with IBS [41,42]. In preclinical studies, the same experimental paradigm used in patients to assess visceral sensitivity has been adapted to rats and mice [43,44]. Graded colorectal distension (CRD) produces a visceromotor behavioral response (VMR) in rodents that is typically positively correlated with distension pressure/volume. The VMR can be quantified visually without additional instrumentation in freely moving animals, or as quantification of the electromyogenic (EMG) signal following implantation of electrodes in the overlying abdominal muscle, which requires the animal to be partially restrained to minimize movement artifact with the EMG signal. A nonsurgical assessment of VMR via manometric recordings using sensors within the colon to measure changes in intracolonic pressure has been established in mouse models [45–47]. This method has the advantage that animals do not have to be restrained while assessing colonic sensitivity; however, the disadvantage is that the measurements can

contain artifacts due to colonic contractions during normal colonic motility and unrelated to measures of colonic sensitivity. For further reading on rodent CRD, the reader is referred to a review on the assessment of visceral pain in rodent models by O'Mahony and colleagues [45].

Multiple techniques have been developed to produce acute and chronic colonic hypersensitivity as measured by an increase in VMR to distension. Exposing neonatal pups to adverse experiences (separation from the dam, direct colonic irritation/inflammation) can produce a heightened VMR in adulthood [48,49]. In adult animals, acute stressors (physical or psychological) typically produce transient increases in the VMR, while repeated homotypic or heterotypic stressors can produce a chronic hypersensitivity [48,49]. Similarly, active inflammation induced by chemical irritants or a pathogen causes acute hypersensitivity, with a subset of rodents developing a persistent hypersensitivity following recovery from the inflammation [48,49]. While each of these models can demonstrate an increase in VMR to distension, which has good construct validity for the clinical paradigm used to evalu-

ate visceral pain, one deficit with these preclinical models is the lack of the ability to monitor spontaneous visceral nociceptive behaviors. A study by Chen *et al.* aimed to develop an alternative approach to assess the affective responses of visceral pain. In an experimental model of rodent colitis, a conditioned place preference paradigm was established whereby animals were conditioned with intraluminal administration of 2% lidocaine hydrochloride or vehicle followed by confinement to an assigned choice compartment for 30 min. A clear preference was observed for the chamber paired with intracolonic lidocaine treatment in colitic rats, whereas no preference was seen in control animals [50]. Continuing to develop novel translationally relevant animal models will undoubtedly aid in our understanding of the heterogeneous pathophysiology of visceral pain.

Stress and Visceral Pain

Maladaptive stress responses have been associated with an array of pathologies including FGIDs, affective disorders, autoimmune disease, and hypertension [21,22,51–53]. Moreover, evidence suggests that stress can also have profound effects on pain processes [48,54–57] including visceral pain [49]. Here we will discuss evidence from the literature to show a centrally mediated mechanism of stress-induced visceral pain in IBS in both clinical and preclinical studies.

Evidence from Clinical Studies

The emergence and rapid advancement of imaging technologies has aided our understanding of the neurocircuitry underlying visceral hypersensitivity in patients with IBS [58]. Imaging studies have allowed us to visualize aberrant circuitry within the brain in regions involved in the stress response in patients with IBS. Silverman *et al.* were one of the first to show altered brain activation patterns using positron emission tomography (PET) in patients with IBS not only in response to actual and simulated rectal distension but also in the anticipation of rectal pain, specifically decreased activation within the anterior cingulate cortex (ACC) of patients with IBS compared to controls [59]. A similar study using functional magnetic resonance imaging (fMRI) [60] also showed altered activation patterns within specific brain regions. Again, altered ACC activity was observed in patients with IBS; however, in this study, increased activation within the ACC was observed. It is important to note that these two earlier studies showed opposite effects of rectal distension on ACC activity in patients with IBS, which may in part be explained by methodological procedures. Other studies have highlighted the important role of deactivation of brain areas and circuits in the normal processing of pain signals, with controls having greater frontal deactivation than patients with IBS [61]. This was in line with others showing that patients with IBS exhibited significant deactivation within the right insula, the right amygdala, and the right striatum [62]. Moreover, patients with IBS exhibit not only altered brain patterns to noxious rectal distension but also subliminal, liminal, and supraliminal distensions [63,64]. Taken together, it is becoming clear that patients with IBS process visceral stimuli differently to that of control subjects at a central level, with IBS patients showing altered activation and deactivation patterns to both nonnoxious and nox-

ious stimuli, which is characteristic of visceral hypersensitivity. Following on from these earlier studies, there has been an abundance of literature to support a role of altered central circuitry in IBS and we are now beginning to build a consensus on the numerous brain regions altered in IBS patients. The ACC is one specific brain region that has consistently been shown to distinguish patients with IBS from controls in imaging studies; however, other regions include the amygdala, insula, prefrontal cortex, thalamus, somatosensory cortex, posterior cingulate, hippocampus, periaqueductal gray, and cerebellum [65–69]. These studies also showed gender-related differences in brain activation patterns [65,70]. The progression and development of imaging tools such as structural MRI and diffusion tensor imaging has allowed us to understand the aberrant connectivity between these brain regions in patients with IBS [71–78]. We are now beginning to appreciate that different networks within the brain are altered in the IBS population, which may in part explain the heterogeneous nature of IBS symptomology [79]. These data implicate a “top-down” mechanism of altered discrete neurocircuits, mainly involving brain regions and nuclei implicated in nociception and affect, in the mediation and presentation of visceral hypersensitivity in IBS.

Taken together, imaging studies have provided strong evidence for altered neurocircuitry in the IBS population, which may underpin centrally mediated visceral pain as well as altered descending modulation of visceral pain in this patient population. The implications of such studies suggest that a cognitive or behavioral intervention may be of therapeutic benefit to patients with IBS [80] as has recently been shown using mindfulness-based techniques [81–84] and cognitive behavioral therapy [85–87]. Reverse translation models employing optogenetic technologies will also allow us to further delineate the circuitry underlying visceral hypersensitivity and allow for a more precise understanding of exact brain regions, nuclei, cell types, and mediators that are involved and are amenable to pharmacological manipulation for future drug discovery.

Evidence from Preclinical Studies

The majority of evidence implicating stress as a crucial player in the pathophysiology of visceral pain comes from experimental stress models, which have been developed to target critical periods throughout the life span to assess the vulnerability, potential triggers, and perpetuation influences of stress and the future development of visceral pain [47]. Here we will discuss the most commonly used animal models of early-life stress and adulthood stress-induced visceral pain.

Early-life psychological stressors in the form of maternal neglect or abuse (maternal separation, limited nesting, odor attachment learning), or physical stressors in the form of injury (colonic irritation) can enhance the susceptibility of individuals to develop altered visceral pain responses in adulthood, a key symptom of IBS [47]. The maternal separation, limited nesting, and odor attachment models are based on the premise that by altering the dam–pup relationship during sensitive HPA axis phases in the first 2 weeks of life, this will have long-lasting effects on the stress response and subsequent visceral pain sensitivity. Indeed, numerous independent research groups have consistently found heightened visceral sensitivity in these models [13,48,88–100].

Moreover, maternal separation has also been associated with altered intestinal permeability and motility [88,101–105]. Early-life stress in the form of physical stressors has also been shown to be a valid preclinical model of visceral pain. The neonatal GI tract is highly sensitive to both mechanical and chemical stressors resulting in a proinflammatory phenotype characterized by mucosal inflammation and tissue irritation. Animal models of daily irritation of the neonatal colon by mechanical irritation (CRD) or chemical irritation (mustard oil) have been shown to increase visceral pain behaviors from adolescence to adulthood [106–109]. The mechanisms by which these physical stressors exert their long-term effects on visceral sensation are varied with contributions from both local effects within the gut and spinal and supraspinal processes seen to induce plasticity and maintenance of the hypersensitive profile [107,110–112].

Life-threatening stressors, psychological stressors (acute and chronic stress), or physical stressors (intestinal infection or inflammation) during adulthood have all been implicated in the development of IBS. Water avoidance stress (WAS) and restraint stress are among the most widely used acute stress paradigms to model features of IBS preclinically. These stressors are based on an aversive surrounding environment. In the case of WAS, animals are placed on a raised platform surrounded by water for 1 h/day, whereas restraint-stressed animals are placed in a device, which restricts movement for 1–2 h/day. Both of these paradigms can be performed in an acute or repetitive manner. In recent studies, WAS has been described as a form of psychological stressor to assess modulation of visceral pain [46,51,92,113–115]; however, earlier work showed that WAS also leads to stress-related alterations in gut motor function [116,117]. Restraint stress for 2 h has also been shown to induce an immediate visceral hypersensitivity in male [118] and female Wistar rats [97]. Convergent reports suggest that daily stress predicts the intensity and severity of visceral pain [7,8,119–121]. In rat models, data show that a 1 h daily WAS for 7/10 consecutive days induced visceral hypersensitivity in male rats [113,114,122]. In mice, the data are inconsistent, showing visceral hyperalgesia [46], visceral analgesia [46] or to have no effect on visceral sensitivity [123] following WAS. Recently, a clinically relevant model of IBS has been described whereby animals undergo stress in the form of forced swim stress for 3 days as well as estradiol treatment and have existing chronic somatic pain (craniofacial muscle injury). This model displayed chronic visceral hypersensitivity that persisted for months and also exhibited other key features of IBS, specifically central sensitization [124]. Moreover, estrogen-dependent visceral hypersensitivity has also been developed as an animal model of visceral pain with clinical relevance due to the female preponderance seen in patients with IBS [125]. Other models such as the chronic psychosocial stress paradigm model the unpredictable nature of life's stressors with unpredictable and randomized sessions of social defeat and cage overcrowding for 19 days. This model has been shown to induce a heightened response to CRD [11,14,126] and anxiety- and depression-related behaviors [127]. As a model of corticosterone-induced pain targeting only the amygdala, implantation of corticosterone micropellets on the dorsal surface of the central nucleus of the amygdala has also been shown to increase colonic sensitivity to CRD [128]. Although patients with IBS report that psychological stress is a key factor in the onset and

exacerbation of symptoms, a significant proportion of IBS cases occur after an illness, particularly an infection of the GI tract. A transient *Trichinella spiralis* infection was shown to induce sustained visceral hypersensitivity in a mouse model [129,130]. Moreover, similar findings were reported in a rat model of *Nippostrongylus brasiliensis* infection [131]. Although the vast majority of human postinflammatory hypersensitivity symptoms are observed after bacterial infection (*Campylobacter*, *Shigella*, *Salmonella*, or *Escherichia coli* infections), there has been limited animal models of postinfectious visceral hypersensitivity [132,133]. Inflammation is one of the leading causes/mechanisms thought to underpin IBS and its associated symptomatology [134–137]. In animal models, acetic acid [138], mustard oil [139,140], zymosan [141,142], trinitrobenzenesulfonic acid (TNBS) [143,144], and dextran sulfate sodium [145] evoke visceral hypersensitivity associated with colonic inflammation.

Central Mechanisms of Stress-Induced Visceral Pain

Numerous mediators involved in the HPA axis response to stress have been implicated in the pathophysiology of IBS. Most notably, the role of CRH has been extensively investigated in preclinical studies [101,146–157]. Although we acknowledge that CRH has many peripheral functions [158], here we will briefly focus on the centrally mediated effects of CRH. Numerous reviews discuss this topic in more detail [156,159]. CRH is a 41-amino acid peptide with two endogenous G-protein-coupled receptors, CRH₁ and CRH₂ [160]. The receptors have a common signal transduction pathway coupled to Gs-adenylate cyclase; however, they have very different effects depending on localization and cell type [161,162]. One of the earliest demonstrations that CRH was involved in stress-related visceral pain was shown by Gué et al. [118] where intracerebroventricular administration of CRH recapitulated stress-induced visceral hypersensitivity in a similar manner to that induced by restraint stress. Moreover, they also showed that antagonism of CRH receptors using α -helical CRF₉₋₄₁ reversed both the CRH- and the stress-induced visceral pain [118]. There have been limited studies since then to specifically elucidate the central role of CRH and its receptors on visceral hypersensitivity with many studies showing changes in expression but lack pharmacological interventions [163–168]. We have shown that stress-induced visceral pain is associated with increased CRH expression in the central nucleus of the amygdala (CeA) and that oligonucleotide knockdown of CRH within the CeA reverses corticosterone- and stress-induced visceral hypersensitivity [169]. Moreover, we have shown in a model of high anxiety that CP 376395, a specific CRH₁ antagonist administered to the CeA, reversed visceral hypersensitivity [170]. Others have also implicated the CeA as a critical nucleus involved in the mediation of CRH effects on visceral sensitivity. Su et al. demonstrated that direct CRH administration to the CeA increased visceral pain, while CP 376395 attenuated this effect [171]. Furthermore, intrahippocampal administration of CRF₉₋₄₁ or JTC-017, a specific CRH₁ antagonist, attenuated visceral pain [172]. There has been an abundance of studies showing peripheral administration of CRH₁ antagonists, which cross the blood–brain barrier, have positive effects in animal models of visceral pain [92,146,148,155];

however, to date, no CRH₁ antagonist has shown evidence of efficacy in patients with IBS, and further research is required to understand the relationship of CRH and its receptors on visceral pain.

The Gut Microbiota

The gut microflora has recently emerged as one of the most fascinating entities in modern biomedical research. The gut microbiome has been implicated in a whole host of physiological functions from energy metabolism to psychiatric well-being [173–175]. The abundance of microbiota residing in the human intestine is estimated as 10^{14} microorganisms, which amounts to three times the number of human cells in the body. The bulk of the intestinal microbiota is of bacterial origin that varies in stability, diversity, and number throughout development, from birth to old age [176–178]. Technological advances in the methods used to identify and quantify the gut microbiome have allowed us to better understand its complexity, not only population complexity but also functional complexity. Sequencing-based approaches have rapidly developed and are reviewed in many recent publications [179–182]. The human GI tract is dominated by two phyla: Firmicutes and Bacteroidetes, together with members of Actinobacteria, Verrucomicrobia, Proteobacteria, Fusobacteria, and Cyanobacteria phyla [183]. The relative abundance of microbial populations stabilizes after the first 3 years of life [184] and appears to be relatively stable throughout adult life, but can be altered during disease states. Specifically, disorders directly affecting the GI tract such as inflammatory bowel disease [185] and celiac disease [186] have been shown to exhibit microbial dysbiosis. Moreover, dysbiosis has also been implicated in other nonintestinal disorders such as autoimmune diseases [187], allergy, asthma, metabolic syndrome, cardiovascular disease, and obesity [188]. Although there is a general consensus that an overall decrease in the diversity of the microbiota populations present within the GI tract is associated with disease states, as well as reduced *Lactobacillus* and *Bifidobacterium* species, no specific dysbiotic signature has emerged across studies.

Stress and the Gut Microbiota

The origin and development of a “healthy” gut microbiota starts in early life, which is also designated as an important neurodevelopmental time window. This provides an opportunity for the colonizing microbiota to influence immature systems such as the pain pathways in the CNS and make a permanent impact. This is abetted by the bidirectional communication provided by the microbiota–gut–brain axis [189,190]. During early life, this axis is also developing and is in itself open to modification by the gut microbiota, which has been shown in several studies [191,192].

Over 40 years ago, Tannock and Savage demonstrated that both environmental stress and dietary stress were capable of markedly altering the gut microbiota in mice, affecting factors that regulate the localization and population levels of microorganisms along the GI tract [193], possibly allowing for the establishment of pathogenic bacterial species [193,194]. Studies have shown that both prenatal and postnatal stress after birth can impact on microbial colonization [195]. Furthermore, appropriate bacterial coloniza-

tion postnatally impacts on pain pathways, as germ-free mice display impaired ability to respond to inflammatory pain [196], and we have shown that treatment with antibiotics in early life is associated with visceral hypersensitivity [191]. Also, adult mice exposed to a social disruption stressor showed an altered gut microbiota as well as increased circulating levels of cytokines [197]. In particular, this stress led to a decrease in *L. reuteri*, an immunomodulatory species of bacteria. Social stress increases the risk of inflammation-related diseases, promoting proinflammatory gene expression and monocyte differentiation [190,198]. Therefore, inflammatory alterations leading to an altered gut microbiota can enhance the ability of enteric pathogens to colonize the intestine [199]. It has also been shown that both acute stress and repeated stress affect levels of intestinal secretory IgA, impacting intestinal homeostasis, inflammatory response, and possibly dysbiosis [200].

Stress can affect the gut microbiota not only through the immune system but also leads to changes in catecholamine levels, which also have a significant impact on the gut microbiota [201]. Moreover, stress affects recovery from bacterial infections due to the fact that gut bacteria respond to neurotransmitters and other stress-related mediators [201]. Animal models of IBS have shown altered gut microbial populations [90,202,203], and recovery of the IBS-like symptoms has occurred upon probiotic administration [203–205]. Recently, it has been shown that the gut microbiota is necessary for the expression of the anxiety-like and depressive-like behaviors induced by maternal separation as germ-free mice separated from their mothers in early life did not show the typical phenotype induced by this early-life stress [206]. Maternal separation stress also appears to alter the gut environment, which can potentially lead to changes in the bacterial population [207].

Hence, it is possible that stress changes the internal environment of the GI tract through immune, neurochemical, and physical mechanisms to make it a less habitable space for certain bacteria, yet leads to the enhancement of more pathological species. This can potentially increase pain and pain signaling mechanisms from the GI tract contributing to visceral hypersensitivity.

The Gut Microbiota and Visceral Pain

Evidence from Clinical Studies

In recent years, the role of the gut microbiota in the pathophysiology of IBS has been investigated with numerous independent research groups showing divergent gut microbiota populations in IBS patient cohorts, when compared to healthy controls [208–214]. This topic has been the focus of many recent reviews [4,10,215–217]. Patients with IBS were shown to have reduced *Bacteroides* and *Parabacteroides* sp. when compared to healthy volunteers [218]. Other groups have also shown similar findings with decreased abundance of *Bacteroides/Prevotella* group and *Veillonella* genus and increased *Lactobacillus*, *Bacillus*, *Bifidobacteria*, *Clostridium*, and *Eubacterium rectale* [219]. However, conflicting results have emerged with *Bacteroidetes* phylum significantly increased in patients with IBS [220,221]. Moreover, others have demonstrated that subtypes of IBS cluster by microbiota composition revealing certain subgroups of patients with IBS display normal-like micro-

biota composition compared with healthy controls, while others were characterized by an increase in *Firmicutes*-associated taxa and a depletion of *Bacteroidetes*-related taxa [208].

Recent evidence suggests that probiotic interventions appear to be beneficial for patients with FGIDs [222–224], further implicating a role of the gut microbiota in the pathophysiology of FGIDs. Symprove, a probiotic containing *Lactobacillus rhamnosus* NCIMB 30174, *L. plantarum* NCIMB 30173, *L. acidophilus* NCIMB 30175, and *Enterococcus faecium* NCIMB 30176, was shown to significantly improve overall symptom severity in patients with IBS [225]. Moreover, *Bifidobacterium bifidum* MIMBb75 was shown to alleviate global IBS symptomology, as measured on the Likert scale, and also significantly improve symptoms such as pain/discomfort, distension/bloating, urgency, and digestive disorder. In addition, probiotic intervention also improved the quality of life of patients with IBS [226]. Furthermore, another multispecies probiotic cocktail containing *Bifidobacterium longum*, *B. bifidum*, *B. lactis*, *Lactobacillus acidophilus*, *L. rhamnosus*, and *Streptococcus thermophilus* also showed positive effects over placebo in terms of relief from IBS symptoms [224]. More recently, studies are suggesting that probiotic treatment itself does not alter the gut microbiota populations, indicating a more complex relationship between the microbiota and IBS pathophysiology [227–229]. Moreover, there is now increasing evidence that alterations in the gut microbiota can modulate both peripheral and central nervous systems functions, thus altering neurochemistry and subsequently behavior [230–232]. This is of particular relevance to visceral pain as both peripheral sensitization and central sensitization are thought to play significant roles in the onset and development of visceral hypersensitivity [233].

Evidence from Preclinical Studies

Manipulation of the gut microbiota through the use of probiotic and prebiotic treatments has shown that visceral hypersensitivity can be reversed in preclinical models [222]. A mixture of 8 probiotic bacterial strains (VSL#3) was shown to have protective effects against development of visceral hypersensitivity driven by neonatal maternal separation [234]. Moreover, the same cocktail of probiotics was shown to prevent visceral hypersensitivity induced by inflammation via intracolonic instillation of 4% acetic acid when given prophylactically [235]. *Bifidobacterium* species, particularly *Bifidobacterium infantis* 35624, has been shown to be particularly effective at ameliorating visceral hyperalgesia in both stress-induced visceral hypersensitivity and colitis [236–238]. *Lactobacillus* species have also displayed efficacy in visceral pain models [89,239–241]. Indeed, *Lactobacillus rhamnosus* CNCM I-3690 was shown to exhibit protective effects on intestinal barrier function in a mouse model of increased colonic permeability by restoring barrier integrity and increasing the levels of tight junction proteins, occludin, and E-cadherin [242]. The modulation of the intestinal barrier by the gut microbiota has been the focus of recent reviews [243,244]. Furthermore, antibiotic-induced visceral hypersensitivity again underpins a role of the gut microbiota in the pathophysiology of visceral pain, which appears to be dependent on the time of exposure [191,239]. When animals are exposed to antibiotic treatment in early life, they subsequently develop visceral hypersensitivity in adulthood [191]. Further evi-

dence has also shown that antibiotic treatment in adulthood attenuated visceral pain-related responses elicited by intraperitoneal acetic acid or intracolonic capsaicin [245], or indeed increased visceral sensitivity to CRD [239]. Interestingly, rifaximin, a semisynthetic, nonabsorbable antibiotic, has also shown positive effects in the treatment of IBS [246–254] and received FDA approval in early June 2015 for the treatment of diarrhea predominant IBS. Finally, the concept of fecal microbiota transplantation as a potential treatment for FGIDs has recently been the topic of numerous reviews [255–257]. Preclinical evidence has shown that visceral hypersensitivity could be transferred to rats by transplantation with IBS fecal microbiota [258]. These findings add to the growing literature that microbiota dysfunction may be a key player in the pathophysiology of IBS and may lead to future novel therapeutic interventions.

Peripheral Mechanisms of Stress-Induced Visceral Pain

The clinical literature suggests that altered microbial populations are evident in patients with IBS; however, the implications of such changes remain to be elucidated. The metabolic profile of the gut microbiota is also altered in patients with IBS [213,259–266], which may in part explain changes in symptomology. Numerous independent groups have shown changes in the metabolites produced by the gut microbiota, including bile acids [266], organic acids such as acetic acid and propionic acid [213], volatile organic metabolites [264], fecal proteases [263], formate, glucose, lactate, pyruvate [260], amino acids (alanine, pyroglutamic acid [262], tyrosine, lysine, leucine [260]), phenols (hydroxyphenyl acetate, hydroxyphenyl propionate [262]), polyunsaturated fatty acids (PUFAs) [267], and short-chain fatty acids (SCFAs) [268]. These metabolites themselves can act as signaling molecules to exert effects locally within the gut but also have the potential to elicit effects distant to the site of production. The gut microbiota are also involved in the production of a range of neuroactive compounds including neurotransmitters: gamma-aminobutyric acid (GABA), serotonin, norepinephrine, and dopamine.

To date, there have been only a limited number of studies investigating the direct interactions of the gut microbiota and its metabolites on pain and nociceptive processes. Formyl peptides have been shown to directly stimulate primary afferent nerves [269], while other bacterial products such as lipopolysaccharide (LPS) can directly activate colonic DRG neurons [270]. The mechanism of therapeutic effects of probiotic interventions has also been investigated with *Lactobacillus reuteri*, demonstrating inhibitory effects on lumbar DRG neurons [240]. In a recent study by Cenac *et al.* [267], the authors investigated alterations in PUFA content in colonic biopsies from patients with IBS. PUFAs are known to be endogenous agonists of transient receptor potential (TRP) channels, which are key in nociceptive signaling. TRPV4 in particular is involved in numerous processes associated with visceral hypersensitivity, including protease-, serotonin-, and histamine-induced visceral pain [271,272]. In this study, they show increased levels of 5,6-EET, a TRPV4 agonist, in IBS colonic biopsies, which correlated with increased pain scores [267]. They also show that this specific PUFA activates mouse sensory neurons

in vitro and that 35% of human DRG neurons express TRPV4, implicating it as a critical channel in the mediation of visceral pain.

Proteases produced by the gut microbiota and activated mast cells have been implicated in the pathophysiology of visceral hypersensitivity [273–278]. Proteases exert their effects through proteinase-activated receptors (PARs), which are found on both enteric neurons and extrinsic nerves that innervate the gut and have been implicated in the mediation of pain [279]. Despite this, the role of mast cells and their mediators, histamine, tryptase, and chymase, to serve as therapeutic targets using mast cell stabilizers remains controversial due to other sites of action outside of the gut [280].

Many patients with IBS report that their diet is a key contributor to their symptoms [281]. Specific dietary interventions such as the low fermentable substrate diet (LFSD) and the FODMAP diet, which are known to alter the gut microbiota profile [282], have shown beneficial effects with reduced abdominal pain in patients with IBS both in childhood and adulthood [283–286]. Short-chain fermentable carbohydrates increase luminal H₂ and CH₄ production, which increases gas production and bloating in patients with IBS leading to luminal distension and increased small intestinal water volume, which may worsen abdominal pain [287].

Finally, a novel area of speculation is the role of SCFAs in the communication of the gut microbiota with the brain. SCFAs produced in the gut, such as butyrate, are known to have histone deacetylase (HDAC) inhibitor activity. Thus, epigenetic processes such as histone acetylation may also be altered by the gut microbiota (reviewed in [288]). Moreover, there is evidence to suggest that epigenetic changes at the level of the spinal cord are involved in early-life stress-induced visceral pain [100], adulthood stress-induced visceral pain [115], and estrogen-induced visceral pain [289]. Indeed, supraspinal epigenetic mechanisms have also been implicated in stress-induced visceral hypersensitivity [113,290].

The Microbiota–Gut–Brain Axis

From the evidence discussed above, it is apparent that IBS is a multifaceted disorder with both central and peripheral factors at play; thus, it is most commonly described as a biopsychosocial disorder of the gut–brain axis. The gut–brain axis encompasses a number of fundamental elements, including the CNS, the autonomic nervous system (ANS) (sympathetic and parasympathetic), the enteric nervous system (ENS), the neuroendocrine (HPA axis), and neuroimmune systems, and more recently has expanded to include the gut microbiota, which fulfill key roles in bidirectional communication thus leading us to now refer to it as the microbiota–gut–brain axis [15,17,291–296]. This axis is pivotal in maintaining homeostasis and is involved in the control of a plethora of physiological functions including motor, sensory, autonomic, and secretory functions of the gastrointestinal tract to regulate an array of processes from energy metabolism to mood regulation [296,297]. The network of communication throughout the axis is facilitated by an extensive neuronal web of afferent fibers projecting from peripheral tissues to higher-order processing centers in

cortical CNS structures and efferent projections from the CNS to the smooth muscle in the intestinal wall [4].

As mentioned previously, the spinal cord is fundamental in mediating sensory signals between peripheral organs and the integrative cortices within the brain. Sensitization of peripheral afferents plays a key role in the peripheral sensitization leading to visceral hypersensitivity in patients with IBS. This occurs as a result of local inflammatory processes as well as alterations in sensory motor gut function [298]. Indeed, clinical evidence has shown low-grade mucosal inflammation [134] and enhanced intestinal permeability [299] in patients with IBS, which may be responsible for the local sensitization and facilitation of visceral pain responses. The process of peripheral sensitization includes a plethora of different receptor types that include the TRPV family, PARs, cholecystokinin receptors, serotonin receptors, cannabinoid receptors, as well as an array of ion channels including ATP-gated ion channels, voltage-gated sodium and calcium channels, and acid-sensing ion channels [298]. The ligands for these receptors such as luminal contents, epithelial metabolites, immune mediators, lipids, and gut hormones are found within the GI tract. Binding of these endogenous ligands to their respective receptors leads to the release of neurotransmitters such as acetylcholine, somatostatin, substance P (SP), neurokinin A, and calcitonin gene-related peptide, and a cascade of events that are all associated with pain signaling and neurogenic inflammation [300].

Moreover, the CNS and GI tract are in constant bidirectional communication through the vagus nerve and its branches. The essential role of the vagus nerve in the microbiota–gut–brain axis signaling has been shown previously [301,302], in preclinical models whereby vagotomy prevented the anxiolytic effects of probiotic treatment, an effect thought to be mediated by altered GABAergic receptor expression [232,303,304].

Gut hormones released from the enteroendocrine cells such as cholecystokinin (CCK), glucagon-like peptide (GLP), peptide YY (PYY), and serotonin are mediators that sense the local environment in the gut and respond appropriately. They are involved in many functions including digestion and protective processes. Indeed, CCK has been implicated in visceral pain in both preclinical and clinical studies [38,305–307]. Serotonin is predominantly known for its role in the brain, where it functions as a neurotransmitter; however, approximately 95% of serotonin in the body is contained within the gut, specifically, in the enterochromaffin cells of the mucosa and in the nerve terminals of the ENS neurons [295]. Serotonin's peripheral functions involve regulation of GI motility, secretion, and sensory perception [308,309]. Its central functions include the regulation of mood, cognitive functioning, and central processing of sensory signals involved in pain processes [310–312]. Thus, it is apparent that serotonin signaling is a key linker in communication along this axis [313], dysfunction of which may underlie the pathological symptoms present in both GI and mood disorders, and may also explain the high comorbidity of these disorders [3]. Moreover, therapeutic compounds that modulate serotonergic neurotransmission, such as tricyclic antidepressants (TCAs) and selective serotonin reuptake inhibitors (SSRIs), have been shown to be effective in the treatment of not only affective disorders but also GI disorders such as irritable bowel syndrome (IBS) [314–316].

The immune system plays an important intermediary role in the dynamic equilibrium that exists between the brain and the gut [317]. It is well known that the HPA axis, ANS, and ENS all have direct interactions with the immune system [318–322]. The concept of the “leaky gut” again underpins the important bidirectional communication between the periphery and the CNS and the significant role the immune system plays. This phenomenon is thought to underlie disorders of the microbiota–gut–brain axis as well as CNS disorders such as major depressive disorder (MDD) [323] and alcohol addiction [324]. It is thought that stress can lead to alterations in epithelial barrier integrity, which can become compromised, thus increasing intestinal permeability and consequently translocation of Gram-negative bacteria across the mucosal lining. This allows humoral and cellular mediators to directly interact with immune cells and the ENS [325] leading to the activation of an immune response characterized by increased production of inflammatory mediators such as IL-6 and IFN- γ [326]. Moreover, Toll-like receptors have been shown to play a key role in IBS pathophysiology both in preclinical [327,328] and in clinical studies [329]. Microglia, the immune cells of the CNS, have also been shown to be altered by the gut microbiota, in particular microglia maturation and function [330].

Taken together, several routes of communication have been proposed to understand the communication between the intestine, including the microbiota, and the brain, some of which have been summarized here. The high comorbidity between gastrointestinal disorders including IBS and stress-related psychiatric symptoms such as anxiety [331–333] are further evidence that perturbation of the microbiota–gut–brain axis leads to alterations in the stress response and overall behavior including pain sensitivity [334,335].

Summary and Future Directions

In this review, we have attempted to highlight for the reader the mechanisms that facilitate both stress and nociception. Furthermore, we have provided evidence from the literature for the microbiota–gut–brain axis and the role that it plays in visceral nociception. An important key message is the need for more research focusing on the overlapping mechanisms linking stress and visceral hypersensitivity with alterations in the gut microbiome (Figure 2). The exact routes by which the microbiota can exert direct effects on visceral pain and vice versa remain to be explored, but potential targets include altered cognitive processes and epigenetic mechanisms [288,336]. We anticipate that future research will likely lead to novel therapeutic compounds targeting the microbiota–gut–brain axis to treat patients with functional bowel disorders such as IBS whose visceral pain is exacerbated during periods of stress. The complex and multilayered communication between the gut microbial population and the CNS has far-reaching implications not least in the area of visceral pain and comorbid stress disorders. Being able to target one element of the axis in order to alleviate symptoms at both ends is a huge therapeutic leap. Studies on the modulation of the gut microbiota via prebiotic/probiotic treatment have shown a very positive light on the potential for this therapy in comorbid visceral pain and stress disorders [235–237,337]. Also of potential therapeutic value is the possibility of fecal transplantation. Studies have shown that condition and behavior are transferrable via transplantation of feces [291,338,339]. Future wide-scale clinical validation of such interventions in visceral pain disorders is now warranted.

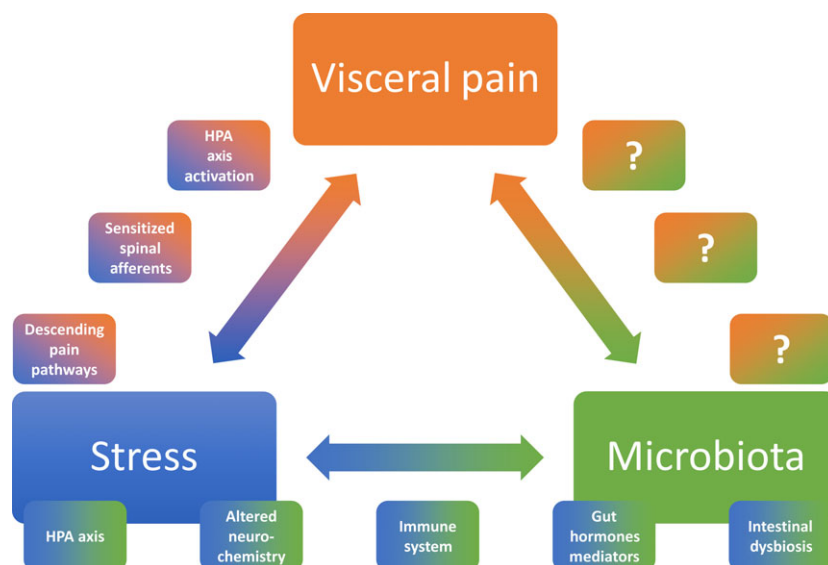


Figure 2 Summary Figure. The mechanisms by which stress can lead to heightened pain perception are varied and primarily occur through 3 distinct routes: (1) hypothalamic–pituitary–adrenal (HPA) axis activation, (2) sensitized spinal afferents, and (3) altered descending pain pathways. Stress and the gut microbiota are also known to interact bidirectionally, with stress causing intestinal dysbiosis, which subsequently alters HPA axis functioning. Many systems and mediators are involved in this complex network including altered neurochemistry of the central, peripheral, and enteric nervous systems, altered immune system functioning, and perturbed local production of gut hormones and mediators. What remains to be fully investigated are the exact pathways by which the microbiota can exert direct effects on visceral pain processes and vice versa.

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Conflict of Interest

The authors declare no conflict of interest.

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