Mechanisms of Stress-induced Visceral Pain

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Evidence suggests that long-term stress facilitates visceral pain through sensitization of pain pathways and promotes chronic visceral pain disorders such as the irritable bowel syndrome (IBS). This review will describe the importance of stress in exacerbating IBS-induced abdominal pain. Additionally, we will briefly review our understanding of the activation of the hypothalamic-pituitary-adrenal axis by both chronic adult stress and following early life stress in the pathogenesis of IBS. The review will focus on the glucocorticoid receptor and corticotropin-releasing hormone-mediated mechanisms in the amygdala involved in stress-induced visceral hypersensitivity. One potential mechanism underlying persistent effects of stress on visceral sensitivity could be epigenetic modulation of gene expression. While there are relatively few studies examining epigenetically mediated mechanisms involved in stress-induced visceral nociception, alterations in DNA methylation and histone acetylation patterns within the brain, have been linked to alterations in nociceptive signaling via increased expression of pro-nociceptive neurotransmitters. This review will discuss the latest studies investigating the long-term effects of stress on visceral sensitivity. Additionally, we will critically review the importance of experimental models of adult stress and early life stress in enhancing our understanding of the basic molecular mechanisms of nociceptive processing.

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Key Words
Amygdala; Early life adversity; Irritable bowel syndrome; Models, animal; Visceral pain

Introduction

Irritable bowel syndrome (IBS) is a complicated condition with an unknown etiology. The IBS patient population is heterogeneous with subpopulations of IBS with diarrhea, IBS with constipation and a mixed population with episodes of diarrhea and constipation with symptoms differing from one patient to the next and with severity of symptoms ranging from debilitating to mild to moderate.1-6 Risk factors for IBS include being female and exposure to chronic stress.7-11 A common feature of IBS is that for many patients, their symptoms are worsened by stress and overlap with other stress disorders, including anxiety, depression, and post-traumatic stress disorder.12-15 In support of IBS being a stress-sensitive disorder, there is experimental evidence showing abnormal hypothalamic-pituitary-adrenal (HPA) axis reactivity in IBS patients as shown by a heightened HPA response to a corticotropin-releasing hormone (CRH) challenge, and an overall increase in hourly cortisol secretion in IBS compared to controls.16-18 Another interesting observation is that some patients with IBS, especially female IBS patients, can recall repetitive exposure to adversity in their childhood.19,20 In this review we will discuss the hypothesis that visceral pain disorders result from abnormalities of the brain-gut axis due to “multiple hits” which include external risk factors such as abuse history and chronic psychological stress in adulthood which together are capable of sensitizing the stress response system to cause alterations in no-

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cceptive processing and the development of IBS pathophysiology. Taken together, the complex clinical phenotype makes recapitulating IBS in rodent models extremely challenging. However, an aim of this review will be to describe how the use of rodent models of adult stress, early life stress (ELS), and a dysregulated HPA axis has improved our understanding of stress in the pathophysiology of IBS.

Pathophysiology of Irritable Bowel Syndrome: Activation of the Brain-Gut Axis

Evidence from clinical and basic research points to dysregulation of the bidirectional communication between the brain-gut axis in IBS. The brain-gut axis represents a dynamic interplay between central circuits and peripheral mechanisms. The messengers of this complex circuit include neural, endocrine, metabolic, and immune mediators that are activated by central factors such as stress (Fig. 1). Although IBS is not an inflammatory disorder, the immune system plays a role in the pathogenesis of IBS in at least a subset of patients and likely contributes to the etiology of IBS symptoms. A subset of IBS patients display a low grade chronic inflammation, and there is also evidence that following an enteric infection and combined with stressful life events, there is an increased risk of developed post-infectious IBS. Clearly, the immune system and inflammatory processes are modulated by the HPA and autonomic nervous systems. In support, IBS patients also show increased number and reactivity of mast cells. Data from peripheral blood mononuclear cells, from IBS patients show that cytokines levels including TNF-α, IL-1β and IL-6 are increased in IBS. It is well recognized that proinflammatory cytokines can alter neurotransmitter release within the enteric nervous system to alter motility, secretion, and epithelial permeability via tight junction dysregulation to alter visceral sensitivity. There is also evidence for alterations in microbiota balance in IBS and alterations in the bidirectional communication between the gut microbiota, the CNS, and the enteric nervous system.

Visceral Hypersensitivity in Irritable Bowel Syndrome

An important aspect to enhancing our understanding of IBS pathophysiology is the knowledge that IBS patients exhibit visceral hypersensitivity characterized by hyperalgesia and allodynia in a subset of IBS patients ranging from 33-90% of patients with IBS depending on the study. Although the cause of visceral hypersensitivity is unknown, clinical studies have shown that chronic stress worsens IBS symptomatology and ELS serves as a risk factor for IBS. The sympatho-medullary and the HPA axes are activated by exposure to stress; the sympatho-medullary axis releases epinephrine from the adrenal medulla, to allow the organism to “fight” or “flee” from a threat, whilst activation of the HPA axis releases cortisol (or corticosterone [CORT] in rodents) from the adrenal cortex to mobilize reserves of glucose with the goal of replenishing the expended sympaho-medullary system. Activation of the HPA axis by stress releases CRH from the paraventricular nucleus of the hypothalamus into the hypophyseal portal circulation. CRH then binds to a CRH type-1 receptor in the anterior pituitary stimulating the production and the release of adrenocorticotropic hormone (ACTH) from the pituitary gland into the systemic cir-
Mechanisms of Stress-induced Visceral Pain

Circulation. The circulating ACTH binds to receptors in the adrenal cortex to stimulate production of CORT, which binds to a cortisol binding globulin, prior to being released at target organs throughout the body. A pivotal role for CORT release from the HPA axis in response to stress is the initiation of feedback-inhibition through binding to the mineralocorticoid receptor (MR) and the GR at multiple nuclei, such as the hippocampus, the paraventricular nucleus of the hypothalamus, and anterior pituitary. In contrast to the feedback-inhibition, when CORT binds at the amygdala there is an increase in CRH release and the subsequent facilitation of the stress axis (Fig. 2).

Amygdala Facilitation of the Hypothalamic-Pituitary-Adrenal Axis

Neuroimaging studies in IBS patients have shown that there is altered brain activation in response to a nociceptive stimulus suggesting central sensitization. In particular, the amygdala has been found to consistently demonstrate altered activation to visceral stimulation in IBS patients. The amygdala is a brain nucleus that is important for the integration of the body’s neurophysiologic responses to stress, as well as modulating the perception of anxiety, which is increased in IBS. The amygdala, and more specifically the central nucleus of the amygdala (CeA), is a key brain nucleus involved in the facilitation of the stress response. Specifically, CORT acting via GR and MR mediated mechanisms in the CeA drives the HPA and autonomic components of the stress axis. Since stress worsens visceral pain in IBS patients, an overarching goal of our laboratory has been to investigate the mechanisms within the CeA that are involved in stress-induced nociception. To address this goal we have employed a series of rodent models. In one model, micropellets of CORT are implanted bilaterally on the dorsal CeA via stereotaxic surgery. The CORT micropellets are a pharmacological tool to model CeA dysfunction induced by a chronic stressor. We initially showed that CORT micropellets on the CeA increase colonic sensitivity to colorectal distension (CRD) in the absence of histological damage to the amygdala. In subsequent experiments, we demonstrated that following amygdala implants of

Figure 2. Amygdala-mediated mechanisms for stress-induced colonic hypersensitivity. The body’s neuroendocrine stress response (hypothalamic-pituitary-adrenal [HPA] axis) is initiated at the hypothalamus and causes the synthesis and release of corticotropin-releasing hormone (CRH) onto the anterior pituitary. Upon binding of CRH to its type 1 receptor, the pituitary gland secretes adrenocorticotropic hormone (ACTH) into the peripheral blood circulation leading to production of cortisol in humans or corticosterone (CORT) in rodents. Following release into the circulation, CORT binds to the glucocorticoid receptor (GR) to initiate negative feedback of the HPA axis at the level of the hypothalamus and the hippocampus. In contrast, CORT binding to GR at the amygdala leads to facilitation of HPA axis, thus promoting persistent stress activation. In this review, we discuss our recent data showing that stress activation or implantation of a CORT micropellet at the amygdala produces persistent colonic hypersensitivity to balloon distension along with decreased GR expression and increased CRH expression. Our findings have also revealed that a decrease in GR expression could be due to increased methylation (Me) of the GR promoter along with decreased acetylation (Ac) of histone 3 (H3) at the GR promoter. The persistent decrease in GR expression decreases the binding of GR to a negative GR response (nGRE) element in the CRH promoter, thus removing GR-induced repression of CRH expression. The loss of the repression of the CRH promoter induced a persistent increase in CRH expression, and inhibiting the increase in CRH expression reversed the stress-induced colonic hypersensitivity.
CORT there are profound and persistent effects on gene expression in the CeA including a downregulation of GR and an upregulation in CRH (Fig. 2). These findings suggest that elevating CORT induced prolonged changes to GR and CRH homeostasis in the CeA that persist despite depletion of the source of CORT. To advance these studies, we aimed to determine the relationship between CRH expression in the CeA and visceral hypersensitivity. We tested the hypothesis, using a loss-of-function approach, that preventing the increase in CRH expression would inhibit the visceral nociceptive behaviors induced by elevated amygdala CORT. We sought to investigate whether CRH expression within the CeA is a key regulator of heightened visceral nociceptive behaviors. We showed that CRH knockdown in the CeA using antisense oligodeoxynucleotide (ASO) targeting CRH in the CeA, but not non-targeting random sequence oligodeoxynucleotide, inhibits visceral hypersensitivity. However, the role of GR and/or MR expression in the regulation visceral nociceptive behaviors remained to be determined. Thus, we tested our next hypothesis that, within the CeA, selective knockdown of GR in the absence of the CORT micropellets could promote colonic hypersensitivity. We found that GR knockdown in the CeA using oligodeoxynucleotide of ASO targeting GR, but not random sequence, in stress-naive rats induced colonic hypersensitivity, mirroring the effect of the CORT micropellet. The GR mRNA expression was knocked down 67-88% which is comparable to the decrease in expression with CORT-implanted on the CeA and treated with vehicle-induced decrease in GR expression. In summary, to our knowledge we were the first to demonstrate that decreasing GR within the CeA was sufficient to induce visceral hypersensitivity in a stress-naive animal. Taken together, our data shows that stress is associated with a loss of GR signaling and thus an impaired ability of the CeA to respond appropriately to stress. Key to building upon these observations and to further understand the underlying mechanisms of chronic stress-induced CeA dysfunction, we used the approach of examining whether a stressor itself drives alterations in amygdala function in a rodent model. We confirmed earlier work that daily exposure to water avoidance stress for 7-10 days increases visceral sensitivity to CRD. We then advanced these observations by demonstrating that the water avoidance stressor increased CRH and decreased CRH expression in the CeA closely resembling that seen in rats with CORT micropellets on the CeA. In the water avoidance stress model, we found that CRH knockdown in the CeA inhibits the stress-induced visceral hypersensitivity.

Sex Linked Differences in Visceral Sensitivity

From epidemiological studies, evidence has shown that IBS-patients are more commonly female. In developed countries, the estimated female bias for IBS is between 2 to 5 for every male IBS patient, which is similar to other chronic pain and affective disorders. For example visceral pain disorders such as functional dyspepsia or chronic pelvic pain, somatic pain disorders such as fibromyalgia or migraines, and affective disorders such as chronic fatigue syndrome, anxiety or depression, all have female-predominate sex ratios. These sex-related differences in symptom expression begin around puberty, with the surge of gonadal hormones, but continues to increase through the mid-forties. Interestingly, the rate of newly diagnosed IBS patients decreases after the age of 50 and the sex ratio for IBS patients is similar in the elderly. Clinical evidence suggests that there may be differences in IBS symptom expression between men and women; men with IBS are more likely to experience loose and more frequent stools, whereas women with IBS experience more bloating, abdominal distension, and infrequent, hard stools, although some women with IBS report increased episodes of diarrhea during menses. While peripheral mediators likely affect GI motility, gender-associated differences in visceral pain perception in IBS patients have been demonstrated using brain imaging techniques such as positron emission tomography. A study by Naliboff et al has shown that compared to men with IBS, women with IBS utilized different areas of their brain, such as the amygdala, while experiencing a rectal distension they perceived as aversive. Moreover, other clinical studies have provided evidence that women's perception of visceral pain can be influenced by their cyclical changes in gonadal hormones.

Characteristic features of IBS can be recapitulated in experimental models. For example, sex differences in pain responsiveness and differences in regional brain activity resembling that observed in IBS have been seen in male and female animals. In rodents experimental methodology that includes CRD to assess visceral sensitivity or a mechanical stimulus applied to the hindpaw or tail to assess somatic sensitivity, have uncovered sex differences in nociceptive behaviors following ELS, with responses in females being greater than males. Studies performed in adult rodent models have demonstrated that estrogen and progesterone modulate pain reporting, and that variations in the response to colonic distension occurred during the estrus cycle. Specifically, in rodent models, colonic sensitivity was increased during proestrus or estrus, with the
highest plasma levels of estrogen and progesterone, compared to diestrus or metestrus with significantly lower circulating hormonal levels.88,99 Other studies support the concept that female hormones modulate visceral pain; we showed that CeA-CORT micropellet implantation visceral hypersensitivity in females only during proestrus/estrus.96 Thus, clinical and preclinical data support a significant and complicated role for ovarian hormones to modulate not only pain perception, but also the underlying pain circuitry, which may promote the sexual dimorphism in IBS. Estrogen has been shown to be an important modulator of brain development, specifically influencing the plasticity of nociceptive circuits.97,98 In one study, direct administration of female hormones into the brain induced visceral hypersensitivity in adult female rats.99 Visceral hyperalgesia can also be induced in male rats by estrogen administration,100 further demonstrating that estrogen can be a pivotal modulator of visceral pain processing. Evidence suggests that, within the central nervous system, estrogen alters the expression of specific genes related to pain signaling to increase perception of peripheral nociceptive stimuli.101-103 These estrogen-dependent alterations in gene expression increase neuronal excitability by promoting synaptic plasticity leading to increased visceral sensitivity in females, especially during periods of high cycling estrogen.104 Another estrogen-mediated mechanism that could drive visceral hypersensitivity is via the induction of µ-opioid receptor internalization within the medial preoptic nucleus and the posterodorsal medial amygdala.105 At the level of the spinal cord, multiple studies have characterized estrogen as an important modulator of visceral nociceptive signaling, specifically through an effect on the N-methyl-D-aspartate receptor,106 metabotropic glutamate receptor 2 (mGluR2),107 and ionotropic glutamate receptor subunit 2B activity108 within the spinal cord.

Models of Stress-induced Visceral Hypersensitivity in Rodents

To model stress in preclinical models, researchers have developed numerous animal models. We recently reviewed the most relevant rodent models, along with potential mediators of visceral pain and the reader is referred to this review, Greenwood-Van Meerveld et al.109

Rodent Models of Adult Stress

To understand the central pathways and cellular mechanisms underlying changes observed in the human brain and to design novel therapeutics for stress-induced visceral pain, there are multiple rodent models of stress. The Wistar Kyoto rat is a spontaneous or genetically-induced model in which a high anxiety trait is associated with elevated colonic sensitivity as demonstrated by an increased visceromotor response (VMR) induced by low levels of CRD.110-114 In other models, adult animals are exposed to stressors such as restraint stress, water avoidance stress, and a chronic variable stress.89,94,115,116 In these models, there is an increase in plasma CORT resembling that seen in IBS patients and an increase in visceral sensitivity.

Rodent Models of Early Life Stress

Evidence suggests that stressful events during development can have long-lasting effects. For example, neonatal adversity increases the likelihood of developing a functional gastrointestinal disorder in adulthood.117 To enhance our understanding of the mechanisms by which adversity in early life contributes to IBS, animal models permit longitudinal studies of the life-long effects of early life adversity on visceral pain perception. Adverse early childhood experiences, such as neglect, poverty, or an abusive caregiver, have been modeled by specific ELS models. Preclinical studies in these rodent models have provided important experimental evidence to suggest that brain circuits are primed by exposure to stress or pain during early life, predisposing individuals to chronic pain disorders as adults.118 Furthermore, in adulthood, these models of ELS have been shown to induce chronic, sexually dimorphic visceral hypersensitivity.119 As a model for neglect, maternal separation induces visceral hyperalgesia and enhanced HPA-activity in adulthood compared to non-separated controls. Visceral hypersensitivity has also been observed in adult rats following ELS induced by limited nesting, a model for poverty-associated neglect or abuse.120 Although valid for some types of ELS, maternal separation and limited nesting do not directly model abusive relationships in early life in order to assess visceral pain behaviors in adulthood. By relying on conditioned responses to an odor to model an abusive relationship, the odor attachment learning (OAL) model of ELS exploits key developmental time points by using different pairings of an odor and a shock to control for trauma predictability and to teach a preference for the conditioned odor. In this model, we have shown that neonatal female rats exposed to an unpredictable odor-shock pairing group showed a colonic hypersensitivity in adulthood compared to females in the predictable ELS group or odor only controls. In contrast, colonic sensitivity was unaffected in adult males exposed to any of the OAL treatments.94,121 Building upon our observation in female rats, we found that removal of all circulating ovarian hormones via ovarioectomy after puberty reversed unpredictable ELS-induced colonic hypersensitivity, whilst estradiol replacement restored colonic hyper-
sensitivity in those same animals. These data indicate an essential role for circulating ovarian hormones in females in the maintenance of ELS-induced chronic visceral pain. Ours was the first study to show sex-related differences in visceral sensitivity following unpredictable ELS and suggests that the activational effects of estradiol may maintain IBS-like symptomatology.

Any childhood abuse has the potential to alter an individual’s ability to cope with stress in adulthood by changing the development of the nervous system. This observation can be recapitulated in experimental models in which adult rats exposed to an ELS paradigm as neonates are subjected to an additional stressor such as a repetitive water avoidance stress. Under this multiple “hit” experimental paradigm there was enhanced colonic hypersensitivity to distension when compared to non-manipulated control groups. Although adult stress induced visceral hypersensitivity in all animals exposed to ELS, the effect of a second stress in adult animals showed a sex dependency, with female rats being more vulnerable to the chronic adult stressor. Particularly, adult female rats exposed to the predictable ELS developed an exaggerated visceral hypersensitivity that resembled the effect apparent in females who experienced unpredictable ELS as neonates. Our findings suggested to us that the resilience factor that previously allowed female animals in the predictable ELS group to be normosensitive was disrupted by repeated exposure to a stressful experience in adulthood. Taken together, the neonatal OAL model in conjunction with an adult stress, provide a solid foundation for exploring the mechanisms by which sex, early life adverse experiences, and adult stress act together to induce chronic pain in adulthood representing an opportunity for future investigations to enhance our understanding of the etiology of functional pain disorders that may lead to promising novel therapeutic targets.

**Epigenetic Mechanisms That Contribute to Irritable Bowel Syndrome Symptomatology**

Having shown that long term exposure of the amygdala elevated levels of CORT or repetitive stress decreases GR and increases CRH expression in the CeA that persist despite depletion of the source of CORT or removal of the stressor, an intriguing question is what are the underlying mechanism(s) leading to the prolonged disruption in GR and CRH-regulated gene transcription? There is the potential that these changes in gene expression may have a significant and long-term impact on the brain-gut axis, suggesting the involvement of epigenetic mechanisms within the brain to mediate stress-induced visceral hypersensitivity and provide a foundation for exploring epigenetic mechanisms that contribute to IBS symptomatology. Epigenetics does not describe alterations to the genomic DNA sequence itself but refers to phenotypic trait variations that result from environmental cues. The specific mechanisms through which these epigenetic variations develop encompass modifications that regulate nucleosome assembly (the histone octamer and associated DNA segments), which forms the basic unit of chromatin. Dynamic remodeling of chromatin structure by histone modifications, such as acetylation, changes accessibility of the associated DNA to RNA polymerase to result in differential gene expression. In addition to the histone modifications, epigenetic mechanisms include modification of the DNA structure through methylation that alters binding of the transcriptional machinery leading to a further level of gene regulation, a concept referred to as the epigenome. Epigenetic mechanisms also involve small RNAs, termed microRNA (miRNA), that are not transcribed and do not participate in translation, but instead regulate gene expression by causing degradation of specific mRNAs. Thus, miRNAs are indirect modulators of the epigenome by altering mRNA translation in response to environmental cues without affecting transcription through chromatin modification as with the other epigenetic mechanisms. We explored the hypothesis that chronic stress-induced visceral hypersensitivity could be regulated by epigenetic mechanisms within the CNS (Fig. 2). We found that central administration of trichostatin A (TSA), a histone deacetylase inhibitor, significantly inhibited visceral hypersensitivity in adult rats exposed to repeated WAS. Ours was the first study to demonstrate that stress-induced visceral hypersensitivity involved a central epigenetic mechanism, providing a rationale for future studies investigating whether IBS-like symptomatology involved additional epigenetic mechanisms. Subsequently, visceral hypersensitivity induced by a repeated stressor or by CORT was shown to modify histone acetylation in the brain and spinal cord, leading to specific changes in pro- and anti-nociceptive gene expression. DNA methylation patterns within the brain and an increase in the expression of pronociceptive neurotransmitters have also been seen in a model of stress-induced visceral pain. Through the use of currently available histone deacetylase inhibitors such as TSA and suberoylanilide hydroxamic acid (SAHA), in our laboratory, we showed that subjecting male rats to water avoidance stress-induced visceral hypersensitivity could be inhibited by intracerebroventricular infusions of TSA preceding the stress exposure. In a second study, we examined histone deacetylation in the brain using another model of visceral hypersensitivity induced by stereotaxic application of CORT to the CeA. Our study showed that prolonged...
exposure of the CeA to CORT produced not only visceral hypersensitivity but also decreased expression in lifetime 3 at lysine 9 (H3K9) at the GR promoter. Loss of promoter region acetylation lead to decreased GR expression within the CeA and a subsequent increase in the expression of CRH due to a loss of GR-mediated repression.\(^{107}\) Bilateral CeA-infusions of TSA or SAHA inhibited visceral hypersensitivity by preventing the change in histone acetylation.\(^{62}\) Concurrently, another laboratory employed the water avoidance stress model to investigate epigenetic mechanisms of visceral hypersensitivity within the dorsal root ganglion (DRG). They found that stress increased GR promoter methylation, causing a DRG-specific decrease in GR expression. The authors also found that exposing a male rat to the water avoidance stress had multiple additional effects on gene expression within the DRG including increased methylation of the cannabinoid receptor-1 promoter, causing decreased receptor expression and increased histone acetylation at the transient receptor potential cation channel subfamily V member 1 promoter, causing increased receptor expression.\(^{126}\) The stress-induced visceral hypersensitivity could be reversed through targeted knockdown of the DNA methyltransferase or the histone acetyltransferase that regulated the change in receptor expression.\(^{126}\) In a rodent model of chronic visceral hypersensitivity induced by neonatal maternal separation, a decrease in acetylation of histone 4 at lysine 12 in the lumbosacral spinal cord correlated with visceral hypersensitivity in adulthood.\(^{127}\) Furthermore, in a model of visceral hypersensitivity induced by 17β-estradiol in female rats, SAHA infused on to the lumbosacral spinal cord lead to hyperacetylation of H3K9 at the promoter for the metabotropic glutamate receptor-2 (mGluR2).\(^{107}\) Counter intuitively, the SAHA-induced increase in mGluR2 expression inhibited the estradiol-induced visceral hypersensitivity.\(^{107}\)

Evidence from models of chronic cystitis or esophageal reflux disease identified several miRNAs associated with chronic visceral pain.\(^{128,129}\) In a series of pivotal studies, Zhou and coworkers determined the importance of miR-29 on visceral hypersensitivity via a mechanism involving increased intestinal permeability.\(^{130,131}\) Increased expression of miR-29a and miR29b were associated with increased intestinal permeability in mice following stress exposure. In support of a role miR-29 they employed Mirt29\(^{-/-}\) mice and discovered that stress-induced intestinal permeability was significantly attenuated via a mechanism involving regulation of Claudin-1 mRNA expression.\(^{132}\)

Through the use of animal models, the epigenetic modulation of gene expression in the early postnatal environment causes life-long alterations in pain responsiveness. Following exposure to ELS, epigenetically induced alterations in gene promoter accessibility regulate vulnerability or resilience to additional stressor in adulthood. Having previously shown that chronic psychological stress induces visceral hypersensitivity in adulthood via alterations in GR and CRH signaling within the CeA,\(^{61,72}\) whether the same mechanism(s) are involved following ELS remained to be explored. We therefore hypothesized that within the CeA, female-specific visceral hypersensitivity induced by exposure to unpredictable ELS, resulted in altered GR and CRH expression. Our results revealed marked differences in GR and CRH expression in adult female rats following both predictable and unpredictable ELS that were dependent on the context of the neonatal experience.\(^{113}\) We showed that GR expression in the CeA was increased in adult rats following predictable and unpredictable ELS, suggesting that in the CeA different mechanisms underlie ELS-induced and adult stress induced visceral hypersensitivity in the OAL model. GR has been shown to be a negative regulator of CRH via binding to the promoter region of the CRH gene to inhibit transcription.\(^{114}\) Increases in GR expression observed together with an increase in CRH following unpredictable ELS, suggest a dysfunction in the regulatory relationship between GR and CRH within the CeA. This mechanistic difference is likely a product of the complex interaction between the type of stress experienced and sex-related vulnerabilities. In support, we found that adult male rats exposed to an identical neonatal adverse early life experience showed no alterations in GR and CRH expression in the CeA, which supports our earlier observation that the colonic sensitivity was not significantly different between groups of adult males in the OAL model.\(^{113}\) These data suggest different mechanisms in the CeA in from adult rats exposed to ELS as compared to adult rats experiencing only adulthood stress. In an attempt to delineate the molecular mechanism within the CeA in adult rats exposed to ELS, we examined the role of CRH in modulating visceral hypersensitivity following ELS using targeted knockdown of CRH in the CeA via ASO sequences. Having shown that only unpredictable ELS increased CRH expression within the CeA of adult female rats, we found that following targeted knockdown of CRH in the CeA, there was an attenuation in the VMR to CRD following unpredictable ELS establishing that enhanced CRH expression underlies increased pain behaviors as a result of unpredictable ELS.\(^{113}\) In support, a selective CRH type-1 receptor antagonist administered directly into the CeA significantly decreased visceral hypersensitivity in the rats exposed to neonatal unpredictable ELS. These data provide compelling evidence that the predictability of an ELS experience plays a pivotal role in the reprogramming of CRH expression in adulthood and the regulation of visceral hypersensitivity in a top-down manner through the
CeA. Using a targeted knockdown approach, we also uncovered an inhibitory role of GR up-regulation on visceral hypersensitivity, as targeted knockdown of GR in the CeA resulted in an exaggerated visceral hypersensitivity in both predictable and unpredictable ELS groups compared to controls. Although not directly investigated in this study, these data suggest that up-regulation of GR serves as a compensatory mechanism within the CeA attempting to prevent the development of visceral hypersensitivity. To our knowledge, this is the first study to identify a potential protective role for GR up-regulation in the CeA following ELS. We speculate that disruption of this compensatory mechanism by GR knockdown could explain the exaggerated visceral hypersensitivity in animals exposed to ELS as compared to odor only controls.

Summary and Conclusion

Chronic physical and emotional stress can lead to potentially life-long visceral pain disorders such as IBS. Due to the complex etiology of functional bowel disorders, preclinical models are an essential tool for identifying and testing the efficacy of novel therapeutics. Researchers must strive to develop new animal models with multiple readouts that better mirror IBS and strengthen their translational relevance and predictive value for patients with IBS. Despite this lack of a perfect animal model that recapitulates all the symptoms of IBS, investigating the basic mechanisms of IBS-associated visceral pain can be done in experimental models. To date animal models have proven important for defining the central mechanisms of sensitization and nociceptive signaling, as well as discovering that chronic stress appears to be pivotal to all aspects of IBS in perpetuating and exacerbating abnormalities in GI function that resemble IBS including visceral hypersensitivity. We have provided evidence for a mechanism by which deregulation of corticosteroid receptors and CRH within the central amygdala induces stress-mediated chronic visceral pain. Given this link between altered amygdala function and stress-related IBS, we believe that understanding the central mechanisms of stress-induced visceral pain will be of significance for the future development of novel treatment options.

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