Animal Models of Visceral Pain: Pathophysiology, Translational Relevance, and Challenges

Beverley Greenwood-Van Meerveld, Dawn K. Prusator, and Anthony C. Johnson
Veterans Affairs Medical Center (B.G.-V.M.), Department of Physiology (B.G.-V.M.) and
the Oklahoma Center for Neuroscience (A.C.J., D.K.P., B.G.-V.M.), University of
Oklahoma Health Sciences Center, Oklahoma City, Oklahoma, U.S.A.

Corresponding Author:
Beverley Greenwood-Van Meerveld, Ph.D., FACG, AGAF.
V.A. Medical Center, Research Admin. Rm. 151G
921 N.E. 13th St.
Oklahoma City, OK 73104
Tel.: (405) 456-3547
Fax.: (405) 456-1719
E-Mail: Beverley-Greenwood@ouhsc.edu
Abstract

Visceral pain describes pain emanating from the thoracic, pelvic or abdominal organs. In contrast to somatic pain, visceral pain is generally vague, poorly localized and characterized by hypersensitivity to a stimulus such as organ distension. Animal models have played a pivotal role in our understanding of the mechanisms underlying the pathophysiology of visceral pain. This review focuses on animal models of visceral pain and their translational relevance. In addition, the challenges of using animal models to develop novel therapeutic approaches to treat visceral pain will be discussed.
INTRODUCTION

Chronic abdominal pain is the hallmark feature of multiple disorders, some with distinct organ pathology, including inflammatory bowel disease (IBD), pancreatitis, and interstitial cystitis (IC)/painful bladder syndrome (PBS), whereas in other disorders such as irritable bowel syndrome (IBS) and functional dyspepsia (FD) there is no evidence of any structural or histological abnormalities to explain the pain. However, abdominal pain represents one of the main reasons patients seek medical attention. Currently, despite large numbers of patients with chronic, and often debilitating visceral pain, the clinical management of these patients is largely inadequate. There are few effective therapies to treat patients with chronic visceral pain and those that are available are limited by poor side-effect profiles such as addiction, fatigue, constipation, nausea and liver damage. Notwithstanding the large number of patients complaining of chronic visceral pain, the mechanisms leading to this debilitating symptom require further investigation.

Pain pathways innervating the gastrointestinal (GI) tract are shown in Figure 1. Upon stimulation at the peripheral organ, extrinsic nociceptors (Aδ− and C−fibers) synapse in the dorsal horn of the spinal cord. Second order neurons then ascend to the brain through anterolateral pathways such as the spinothalamic and the spinoreticular tracts. Tertiary neurons in the thalamus distribute the pain signal to the primary somatosensory cortex to provide localization of the signal. Other regions, such as the insula and the anterior and midcingulate cortex (ACC/MCC) are activated to provide the ‘feel’ of the stimulus (sharp, dull, aching, burning) and the perception of unpleasantness. Ascending brainstem connections also activate the amygdala to integrate the pain signal with outflow to the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA)
axis. Descending projections from the ACC modulate signaling within the periaqueductal gray (PAG) to activate antinociceptive systems within the brainstem that lead to inhibition of the ascending pain signal within the dorsal horn of the spinal cord. Additionally, there is evidence from both the upper (266) and lower (256) GI tract for vagal modulation of nociception (13, 80). Sensitization of these central and/or peripheral pathways leads to visceral hypersensitivity, which can be affected by multiple mechanisms. For example, stressors can alter the physiology of nuclei that process pain signals, leading to central sensitization. Allelic variations can alter the function of neurotransmitter receptors or re-uptake mechanisms, predisposing an individual to enhanced pain signaling in the central pain matrix. Inflammation can lead to long-term changes in the physiology of the affected organ due to immune mediators released at the site of injury that can sensitize peripheral afferents. Increased epithelial permeability permits luminal contents to directly activate afferent nerve endings, which can lead to sensitization. Shifts in commensal microbiota can lead to changes in fermentation products and/or low-grade immune activation either of which produces mediators that can sensitize afferent nerves. Overlap between these mechanisms adds to the complex, multifactorial nature of visceral hypersensitivity, i.e. genetic variation may amplify stress responses, which increases permeability and changes immune responses to an acute infection, leading to a shift in microbial populations within the GI tract (Figure 2).

This review summarizes the recent literature on the role that animal models have played in enhancing our understanding of the physiology and pathophysiology of visceral pain with a particular focus on abdominal pain of GI origin. We will discuss the translational relevance of the animal models and their importance in studying the i)
mechanisms of visceral pain and ii) development of novel therapeutic approaches to 
treat visceral pain. As each animal model is described we have discussed how well the 
model mimics clinical characteristics of visceral pain (face validity), as well as the 
relevance to human situations (construct validity). We will also describe whether the 
drug studies performed in animal models of visceral pain can predict the efficacy of a 
new drug in patients with visceral pain (predictive validity). In addition, this review 
examines the challenges of using animal models with an emphasis on the specific 
strengths and weaknesses of each model. Together, this review provides an important 
summary of animal models to increase our understanding of the basic mechanisms 
involved in the pathophysiology of visceral pain, their translational relevance for the 
treatment of visceral pain disorders and the important limitations that must be 
considered when interpreting the results from studies that have utilized animal models 
of visceral pain.

ANIMAL MODELS OF COLONIC HYPERSENSITIVITY RELEVANT TO IRRITABLE 
BOWEL SYNDROME (IBS)

Rats and mice are the most commonly used animal models used to assess 
colonic physiology, pathophysiology and new treatment approaches for visceral pain. In 
general, there are multiple, well established methodologies to universally quantify 
visceral nociception in rats and mice. The most commonly used technique involves 
recording devices such as electromyographic (EMG) electrodes or strain gauges 
implanted on the external oblique abdominal musculature, to quantify the number of 
reflex abdominal muscle contractions in response to graded colonic balloon distension.
Despite having numerous strengths such as an objective assessment of pseudoaffective nociceptive reflexes, isobaric distension pressures with a barostat allow for assessment of colonic compliance, as well as mimicking the approach used in clinical studies where sensitivity is assessed in response to rectosigmoid distension, there are weaknesses that must be considered in the data interpretation. For example, the animals may be exposed to stress during the procedure as a result of the novel laboratory environment, or may be restrained to reduce movement artifact during EMG recording. An acclimatization period to the experimental environment has been shown to significantly reduce animal stress and allows for the assessment of visceral sensitivity in a freely moving animal. However, anesthesia will always be necessary to insert the colorectal balloon, which should be kept as brief as possible to avoid interference with the nociceptive reflex. Additionally, the balloon distension paradigm should include randomization of the pressures (0-60 mmHg). An additional approach to assess colonic sensitivity is by the visual assessment of the abdominal withdrawal reflex (AWR) in response to colorectal distension. A benefit of this technique is that the stereotyped nociceptive behavior is induced by a brief distension; however, the AWR is a subjective behavioral measure, requiring larger samples sizes to demonstrate significant differences between treatment groups. An alternative approach to the use of a colonic balloon distension paradigm is to directly stimulate visceral nociceptors through colonic infusion of algesic chemicals, such as capsaicin or mustard oil. Such substances produce spontaneous nocifensive behaviors (perianal licking, abdominal retraction or compression, and hind limb stretching) and take advantage of spontaneous evoked nocifensive behaviors. A note of caution in the data interpretation is that the
inflammatory component of the stimulus suggests that the model may more relevant to inflammatory pain rather than functional visceral pain. In summary, reflex abdominal contractions in response to colonic distension and quantification of nocifensive behaviors in response to colonic infusion of algesic chemicals represent the most widely used approaches for quantifying visceral pain in the specific models that will be described in this section of the review and summarized in Figure 3 or Table 1.

**Genetic/Spontaneous Models of Colonic Hypersensitivity**

*Knockout Animals*

Knockout models provide the opportunity to investigate the role of a specific gene in the regulation of colonic sensitivity. We have previously shown that there is a significant decrease in colonic sensitivity to colorectal distension in corticotropin-releasing factor (CRF)-1 receptor knockout mice, suggesting the importance of CRF in colonic sensitivity (226). Further studies in knockout mice have demonstrated the importance of other signaling molecules and transporters in the regulation of colonic sensitivity, including brain-derived neurotrophic factor (BDNF), guanylate cyclase C (GC-C), serotonin (5HT), and interleukin 10 (IL-10), human excitatory amino acid transporter 2 (EAAT2) and the serotonin reuptake transporter (SERT). These models have also been used to identify key receptors and ion channels involved in visceral sensitivity such as the glial cell line-derived neurotrophic factor family receptor α-3 (GFRα3), G-protein coupled receptor kinase 6 (GRK6), the protease-activated receptor-2 (PAR2), PAR4, purinergic receptor-3 (P2X3), sigma-1 (σ1) receptor, toll-like receptor 4 (TLR4), the short transient receptor potential channel 4 (TRPC4), the transient
receptor potential cation channel, subfamily A, member 1 (TRPA1), and the transient receptor potential cation channel, subfamily V, member 1 (TRPV1).

Wistar-Kyoto (WKY) Rat

Multiple studies have confirmed the original observation that the WKY rat displays a hypersensitive response to colonic distension (81). No single mechanism has been demonstrated to be responsible for the hypersensitive response in WKY rats. Recent studies have shown that both central (21) and peripheral (165) CRF receptors are differentially expressed and that selective CRF antagonists can inhibit colonic hypersensitivity (23, 101). Additionally, treatment with immune modulators (26, 243), probiotics (141), calcium channel inhibition (162), and serotonin receptor inhibition (160) reduced colonic hypersensitivity in WKY rats.

Challenges: Interpretation of data in genetic models is complicated and represents a significant challenge. For example gene deletion may affect the overall health of the animal along with endogenous compensatory and/or redundant mechanisms that mask the true effect of the loss of the gene. An issue with the WKY rats is that, to our knowledge, they are the only rat strain exhibiting spontaneous colonic hypersensitivity. However, the inbred nature of this strain may limit the translational relevance as they may only model the pain experienced by a subgroup of patients with functional visceral pain.

Early Life Stress Models of Colonic Hypersensitivity

Early life stress (ELS), which includes childhood neglect, physical abuse, and sexual abuse, is extremely common in the United States affecting approximately forty
percent of the population before adolescence (48, 60, 68). Increasing evidence from clinical studies suggests that a history of ELS serves as a risk factor for the development of adult pathologies including but not limited to GI disorders such as IBS, with affected patients being two to four times more likely to report an adverse experience during childhood (20). In conjunction with these reports, a history of childhood abuse has been correlated with abnormal bi-directional communication between the brain and the gut, providing a potential explanation for the linkage between ELS and the symptoms of adult GI disorders (25, 184). Despite the strong correlation between ELS and decreased health related quality of life in adults due to GI related abnormalities, the mechanism by which ELS underlies these changes is still unknown. Although the complex nature of the human ELS experience cannot be completely simulated in animal models, we consider that animal models of ELS are important tools to develop our understanding of how adverse neonatal experiences alter brain gut communication that may lead to the development of abnormal visceral perception.

**Maternal Separation**

The most well studied model of ELS is maternal separation (MS), which involves removal of pups from mother and nest, most commonly for 3-hour/day on postnatal (PN) day 2-14 (PN2-14) (173). The purpose of this paradigm is to mimic childhood neglect and abuse through separation and subsequent alterations in maternal care, including altered licking and grooming behaviors and arched back nursing (173). MS pups exhibit decreased weaning body weight at PN22 compared to controls that are left undisturbed in their home cage on PN2, potentially introducing the effects of malnutrition as a side effect of neglect in this ELS model. In adulthood, there are
contradictory results on visceral sensitivity depending on the duration of separation. However, a 3-hour/day separation has been shown to result in visceral hypersensitivity, as evidenced by an increased visceromotor response (VMR) to colorectal distension (CRD) in male Long-Evans rats (49). This model presents an opportunity to investigate the relationship between ELS and subsequent development of visceral hypersensitivity, in conjunction with hyper-reactivity of the HPA axis, two commonly comorbid symptoms in disorders such as IBS (57, 240).

*Odor-Attachment Learning*

The odor-attachment learning (OAL) model of ELS is a classical conditioning model which utilizes predictable or unpredictable odor shock pairings to mirror an attachment to an abusive caregiver (207). Conditioning occurs from PN8-12 wherein rat pups are experiencing both a sensitive and a hyporesponsive period. These are evolutionary advantages that allow the pups to be more sensitive to maternal odors in order to find the dam in the cage for care and nursing. This behavior coincides with an inability to initiate a stress response ensuring that pups do not learn an aversion to the dam when she is stepping on or moving them around the cage by their scruff (208). Therefore, by utilizing an odor and modest shock, this model is able to mimic patterned interactions between pup and dam thus creating an odor attachment to the conditioned odor in response to predictable or paired odor/shock presentations. This paradigm also utilizes an unpaired or unpredictable odor/shock presentation, and an odor only presentation as a control, neither of which learn an attachment or aversion to the conditioned odor. In adulthood, this is the first model to our knowledge that induces female specific visceral hypersensitivity in adult Long Evans rats. Following OAL,
female rats with a history of unpredictable ELS exhibit an increased VMR to CRD, an
effect that has been shown to be estrogen dependent (31). This model of ELS has far
reaching relevance to translational research as it parallels the female predominance
found in patients who experience visceral pain, and thus far is the only rat model
capable of linking ELS to adult visceral hypersensitivity in women (20).

Limited Nesting

A third model of ELS, limited nesting (LN), aims to mirror the neglect and abuse
found in areas of poverty or lower socioeconomic standing (72). From PN2-9, all
bedding material is removed and the dam and pups are placed on a wire cage bottom
with only a single paper towel for nesting material. This limitation of bedding material
causes disruptions in normal maternal care similar to those exhibited by dams during
the MS protocol, however, the LN model does not require removal of pups from the dam
at any time during the experimental paradigm. This advantage also eliminates
differences in weaning weights seen in the MS model, as male and female animals who
experience neonatal LN weigh the same at weaning as their control counterparts who
are left undisturbed until PN22 (175). Furthermore, this particular paradigm produces
increased visceral sensitivity in adult male rats, as quantified by increased VMR to CRD
(175). The LN model is relevant to investigation of the human situation, because this
mirrors neglect and abuse that occurs in the presence and not as a result of absence of
the mother (97).

Neonatal Colonic Irritation

Another model of ELS utilizes neonatal colonic irritation (nCI) using colonic
infusion of mustard oil or repeated CRD in neonates (4). This model relies strictly on
manipulation of the colon; however there are no long lasting observed changes in the colonic mucosa of adult animals. nCI results in altered neuronal excitability and permeability within the colon, as well as visceral hypersensitivity of adult animals (4, 32, 122, 123). This model of ELS may be relevant in instances of repeated physical or sexual abuse, and potentially patients who experience some type of colonic inflammation during childhood.

**Challenges:** Rodent models of ELS aim to mimic specific facets of childhood adverse experiences reported by patients, and are pivotal tools in the investigation of the mechanisms that underlie visceral pain following early life trauma. However, ELS studies as a whole, as well as each paradigm, are not without challenges. Overall, the initial consideration of protocol, strain, and sex differences present major considerations. Within the MS model alone there are many variations of separation duration in terms of hours/day and length of protocol across the neonatal period (163), and this is further complicated by the use of varying strains of rats including Long-Evans, Sprague Dawley (SD), and Wistar (114, 173, 186). The other models, LN, OAL, and nCI have not yet been so widely studied, however these same modifications to the original model developed may become an important factor. A second overarching challenge is the decision to study males, females, or both. As some models have been shown to affect strictly males or strictly females, it is important to decide the merits of studying only an affected group versus investigating potential protective mechanisms within the seemingly unaffected portion of a study. Also, it is important to recognize how each model may be different and include that in the interpretation of results. While MS and LN produce abnormalities in maternal care, the MS model potentially includes the
effects of malnutrition not observed in LN or OAL models. Challenges of a more concrete nature in regards to animal models of ELS include time and resources. While the LN protocol requires little interaction with the observer, the MS, nCl, and OAL models require significant time spent by personnel for separation of pups, manipulation, and conditioning respectively. This does not include further time to allow pups to mature into adult animals, which ranges from PN60-90 depending on the study. All in all, from arrival to first experiment this can be four months before data can be obtained. In conjunction with time, development of ELS models can be costly in terms of animal housing for the duration of the study, which is a major consideration for budgetary resources. Despite these challenges, the importance and relevance of ELS models to investigate the relationship between childhood adverse experiences and adult visceral pain remain.

Stress-Induced Models of Colonic Hypersensitivity in Adulthood

Central Nucleus of the Amygdala (CeA)-Implants

As a model of stress targeting only the amygdala, implantation of corticosterone (CORT) micropellets on the dorsal surface of the central nucleus of the amygdala (CeA) were initially shown to increase colonic sensitivity to innocuous distension (76). An interesting feature of this model is its relevance to patients with IBS based upon imaging studies showing heightened activation of the amygdala in IBS patients in response to colonic distension (154, 242). Careful stereotaxic targeting of the CeA is necessary, as placement of the CORT micropellet in adjacent nuclei does not reproduce the colonic hypersensitivity (148). Follow-up studies demonstrated that the CORT-induced colonic
sensitivity was mediated by non-redundant mechanisms involving both glucocorticoid (GR) and mineralocorticoid receptors (MR) (148, 149), that the effect on colonic sensitivity persisted in the absence of the CORT stimulus (150), and was concurrent with increased CRF expression (222). Further investigation of the persistent effects of the CORT-implant has recently demonstrated that an epigenetic mechanism involving deacetylation of the GR promoter induces the increase in CRF expression, leading to the colonic hypersensitivity (225). Thus, central dysfunction of stress-integrative neurocircuitry is sufficient to induce colonic hypersensitivity, in the absence of peripheral manipulation of the colon.

Restraint Stress-Induced Colonic Hypersensitivity

The most typical protocol is a one- or two-hour acute stress protocol where the animal has either its forelimbs wrapped with tape to restrict movement and grooming behavior or is placed in a restraint apparatus (cage or tube) that prevents turning and grooming. A strength of the model is the robust and reproducible nature of the colonic hypersensitivity induced by restraint stress. However, a notable weakness of all stress models is the translational relevance to clinical stressors. This acute restraint stress induces an increase in colonic sensitivity to distension as measured by EMG or AWR quantification. Restraint stress-induced hypersensitivity to colonic distention can be modulated by serotonergic receptors (167), GC-C receptors (62, 200), PARs (270), or peripheral nociception/orphanin FQ receptors (1). Additionally, while an endothelial cell adhesion molecule inhibitor failed to inhibit the stress-induced hypersensitivity (243), a combination treatment of simethicone and alverine citrate (24) or a probiotic product (2) significantly inhibited post-stress hypersensitivity. Using a repeated restraint stress
protocol, four or seven days of daily two-hour restraint, increased the EMG response to
colic distension, and the hypersensitivity was inhibited by either a cannabinoid
receptor 1 (CB1) agonist (196) or by antibiotic administration (250).

*Water Avoidance Stress (WAS)-Induced Colonic Hypersensitivity*

In this experimental model of stress, the rat is placed on a dry platform
surrounded by water in attempt to mimic a psychological stressor. However, this model
may also engage the fear neurocircuitry, which could evoke freezing behaviors in the
colic distension paradigm and affect interpretation of data using this model. With the
exception of two studies (111, 112), WAS protocols in rats induce colonic
hypersensitivity to distension. A single exposure to WAS has been demonstrated to
induce colonic hypersensitivity either immediately, within 60-min of the acute stressor
(151, 155), or after recovery from the stressor, 24-hour post-stress (62, 237), which
could be modulated by GC-C (62), TRPV1, and 5HT3 receptors (155). A single WAS
exposure can also induce a prolonged colonic hypersensitivity in rats previously
exposed to maternal separation (204, 229-231, 248), mediated in part by mast cells
(229, 230), peripheral histamine receptors (204), and TRPV1 (231). An extension of this
model has been to perform daily 1-hour exposure to the WAS for multiple days in an
attempt to mirror a chronic stressor. A note of caution when using this model is to
measure fecal pellet output or systemic CORT daily to ensure that the rats do not
habituate to the WAS. We have shown in this model that stress-responsive limbic areas
of the brain regulate colonic hypersensitivity induced by 7-days of WAS through an
interaction of GR/MR (151) and CRF receptors (224) and an epigenetic modulation their
expression (221). Epigenetic mechanisms have also been implicated in regulating
reciprocal changes in CB1 and TRPV1 in the dorsal root ganglion (DRG), further modulated by GR, that induce colonic hypersensitivity following 10-days of WAS (86-88, 271). Ten-day WAS-induced hypersensitivity can also be inhibited by treatment with CRF-1 antagonists (19, 71) or antibiotics (250). In recent literature, the effect of WAS on colonic sensitivity in mice has been variable. Two reports have demonstrated that a four-day exposure to WAS induced colonic hypersensitivity to distension, which could be inhibited by pretreatment with probiotics (156) or with a PAR4 agonist (5). A 7-day WAS protocol also increased total pain behaviors induced by intracolonic capsaicin, which were reduced by pre-dosing of broad spectrum antibiotics (3). In contrast, measuring colonic sensitivity non-invasively through colonic manometry, 10-days of WAS produced either hyper- or hyposensitivity, depending on the housing and the surgical status of the mouse (110). Using a similar non-invasive measurement technique, 10-days of WAS only produced a transient increase in colonic hypersensitivity in mice also exposed to an acute dextran sodium sulfate (DSS)-induced colitis, with no apparent direct effect of WAS on colonic sensitivity (113).

**Variable Stress-Induced Colonic Hypersensitivity**

To eliminate the possibility of rats habituating to a single repetitive stressor, several variable stress protocols have been developed that expose animals to a set of randomly presented stressors. The unpredictable nature of the stressors prevents acclimatization to the procedures. One protocol used in rats, termed heterotypic intermittent stress (HIS) or heterotypic chronic stress (HeCS), randomizes presentation of three stressors: 45-min of cold (4 °C) restraint, 60-min of water avoidance stress, or 20-min of forced swim. Each stressor is presented once or twice daily for 9-days. The
duration of colonic hypersensitivity to distension, measured through EMG or AWR, was
strain dependent with a duration of action of up to 48-hour post-stress. Stress-induced
colic hypersensitivity was inhibited by administration of a cystathionine β-synthetase
antagonist (an enzyme responsible for H₂S production) (236), a selective β₂ adrenergic
antagonist (265), antibodies targeting nerve growth factor (NGF) (246), antisense
oligodeoxynucleotides targeting nerve growth factor receptor (246), or through
electroacupuncture activation of endorphin mediated antinociception (277). Colonic
hypersensitivity was further increased when HeCS was combined with DSS colitis,
which was also inhibited with anti-NGF antibodies (36). Colonic hypersensitivity was
also demonstrated in adult male and female rats whose dams were exposed to HeCS
during the last 9-11 days of gestation (244). A further exposure of the adult rats to the
HeCS protocol produced a colonic hypersensitivity that persisted for one week in the
male rats, and for two weeks in the female rats, and could be inhibited by administration
of a histone acetyltransferase antagonist, or through inhibition of BDNF or BDNF
receptor (244). An alternative protocol of chronic unpredictable stress applied over 21-
days induced colonic hypersensitivity to distension that was prevented by neonatal
denervation with capsaicin and inhibited by administration of a mast cell stabilizer (37).
A third protocol has been developed in mice that randomly expose the animals to either
social defeat or overcrowding stress for 19-days to induce colonic hyperalgesia (219).
The colonic hyperalgesia in the mice was decreased in mice deficient for TLR4 or
following peripheral or central administration of a TLR4 antagonist (220).

Challenges: Rodent models of acute stress produce increases in plasma CORT
that are similar in magnitude to the CORT response in stressed humans. However, an
issue with the stress models is their construct validity, i.e. the experimental stressors do
not mirror the types of stress that humans experience. In rat models of stress-related
visceral pain the nature of the stressors, including whether they are physical versus
psychological, acute versus chronic, or predictable versus unpredictable, can have a
profound influence not only on the outcome of the investigation but also on the
biological processes involved. Many rat strains, particularly the commonly used
Sprague-Dawley, will habituate to the repeated presentation of a homotypic stressor
(56, 73), which may affect the development of visceral pain responses; therefore, it is
recommended that strains be employed that do not readily habituate (56, 232) or the
models used are unpredictable in order to better mimic the human pathophysiological
condition.

**Colonic Irritation Models of Colonic Hypersensitivity**

*Non-inflammatory irritation: Acetic Acid & Butyrate*

Acetic acid has been used to induce colonic hypersensitivity to distension
through two different mechanisms. Low concentration (<1.0%) acetic acid produces a
transient sensitization of colonic afferents (109, 174). Higher concentrations of acetic
acid produce a mild damage to the colon, producing inflammation-associated
hypersensitivity. While there are multiple receptors and transmitters that can participate
in acute afferent sensitization, compounds targeting TRPV1 (247), neurokinin-1 receptor
(NK1) (77), 5HT₃ (77), and 5HT₄ (85, 115) have demonstrated efficacy in inhibiting
acetic acid induced colonic hypersensitivity.
In an attempt to produce a non-inflammatory model of colonic hypersensitivity with potential translational relevance to IBS, Bourdu et al. (18) characterized the effect of repeated butyrate enemas on both colonic hypersensitivity to distension and colonic histology. Six enemas of butyrate induced colonic hypersensitivity, reversible with morphine and requiring C-fibers, without evidence of inflammation or histologic damage (18). Recent studies have also demonstrated that antagonists of acid-sensing ion channels (ASICs) (138), anti-NGF antibody (138), serotonin-norepinephrine reuptake inhibitors (SNRIs) (54), or mitogen-activated protein kinase (MAPK) kinase inhibitors (251) can also prevent butyrate-induced colonic hypersensitivity.

Acute inflammatory irritation: Capsaicin, Mustard Oil

Colonic installation of a low volume of capsaicin or mustard oil, which induces pain through direct activation of receptors on afferents as well as establishing an acute inflammation through tissue damage, is typically used to evaluate the analgesic properties of novel therapeutics. Stereotypic pain behaviors, such as abdominal licking, stretching, retraction, or compressing on the cage floor, are counted for 20-30 min after irritant administration. Studies of potential mechanisms for capsaicin-induced visceral pain found that pain behaviors were enhanced in a mouse with a transgenic mutation of a potassium channel in the forebrain (14) and decreased in σ1 receptor knockout mice (74) or in mice with altered sensory nerve development (259). Mustard oil-induced spontaneous nocifensive behaviors can be inhibited by morphine (66), melatonin receptor agonists (35), α-bisabolol (116, 117), and an extract from red algae (34). Behaviors were also decreased in TRPC4 knockout rats (239) and increased in PAR4 knockout mice (9).
**DSS, TNBS, and Zymosan-induced Colitis**

DSS produces a mucosal colitis throughout the colon when administered as a 1-5% solution in the drinking water of rats or mice for 5-7 days. Daily monitoring of a disease activity index (DAI), composed of changes in body weight, stool consistency, and blood in the stool, is used to determine the progression of the colitis. If animals are then returned to normal tap water, recovery from the colitis (measured as decreases in the DAI) will begin within one-two days. Colonic sensitivity can be assessed in response to either distension or acute infusion of an algesic compound in the inflamed colon. Mechanistic studies have demonstrated a role for transient receptor potential cation channel, subfamily M, receptor 8 (TRPM8) (89), glutamate transporters (GLT-1) (124), and endorphins produced by CD4⁺ T cells (16) in the regulation of the colitis-induced hypersensitivity.

In another model, 2,4,6-trinitrobenzenesulfonic acid (TNBS) is a hapten that produces an acute colonic inflammation when administered as an enema in combination with 25-50% ethanol (EtOH) to disrupt the mucosal barrier, in rats and mice. While inflammatory changes in the colonic tissue can be measured within hours of the enema, most protocols investigate colonic sensitivity to distension, with EMG or AWR quantification, 3-7 days post-enema, which will represent different amounts of inflammatory damage depending on the concentration of TNBS and EtOH in the enema. In mice, PAR4 (5), TRPA1 (30), and GC-C receptors (62), cathepsin S (29) and BDNF (260), and GLT-1 (124) have been found to modulate TNBS-induced colonic hypersensitivity. In a similar manner, GC-C (62, 200), estrogen (146), nociception/orphanin FQ (1), prokineticin (237), or 5HT₄ receptors (191), as well as
spinal L- and R-type calcium channels (179) or microglia (103), and tumor necrosis factor alpha (TNFα) (203) all modify TNBS-induced colonic hypersensitivity in rats.

Colonic sensitivity in response to intracolonic zymosan-induced colitis is measured in response to distension either acutely (2-3 hour post-infusion) or following once daily infusion for three days. Recent studies have demonstrated roles for GC-C (64), 5HT₄ (115), P2X3 (197), and TRPV1 (107) in the development of zymosan-induced colonic hypersensitivity.

**Challenges:** While non-inflammatory afferent sensitization models pain experienced in functional bowel disorders, the mechanisms responsible for the acute sensitization may not be directly translatable to the chronic pain experienced by the patient. However, tegaserod, which had efficacy in patients was able to inhibit acetic-acid induced hypersensitivity (78), providing evidence that non-inflammatory afferent sensitization is a model of colonic hypersensitivity with good face validity. While DSS typically affects the whole of the colon, TNBS can produce focal damage depending on the volume of the administered enema. Assessing colonic sensitivity in rats and mice with active colitis likely increases the risk for perforation by the balloon catheter due to the potential presence of ulcerated and/or necrotic tissue. Additionally, selection of mouse strain is an importation factor with the TNBS model of colitis, as the C57BL/6 mouse strain, used as the genetic background for many knockout models, is not as susceptible to TNBS-induced colitis (215).
Post-Inflammatory Models of Colonic Hypersensitivity

Irritant-Induced Inflammation: Acetic Acid, DSS and TNBS

Following recovery from an acute colitis, determined by gross morphology, histology, and/or tissue immune activation markers (cytokines), some animals might develop a hypersensitive response to colonic distension. These models attempt to mirror the post-inflammatory/infec tive hypersensitivity that develops in some IBS patients. The standard protocol for acetic acid-induced post-inflammatory colonic hypersensitivity is to administer and enema of 4% acetic acid, followed by a buffered saline enema, with colonic sensitivity testing 7-days post-enema. Interestingly, while multiple therapeutic targets have been shown to inhibit acetic acid-induced post-inflammatory colonic hypersensitivity (51, 169, 194, 214), common to each study was the ability of nitric oxide synthase inhibitors to reverse the effect of the investigational therapy.

Strong evidence for post-inflammatory colonic hypersensitivity following DSS colitis is lacking in recent literature. While hypersensitivity to distension was demonstrated at 10-days post-DSS administration (36), colonic sensitivity was similar to control animals at 32- (113) or 49-days (61) post-colitis. In rats, post-inflammatory colonic hypersensitivity has been investigated 14-112 days post-TNBS enema. Post-TNBS colonic hypersensitivity is associated with changes in glutamate receptor expression (206, 275), and can be inhibited by treatment with probiotics (100) or with a compound that activates both opioid and nitric oxide signaling (59). In mice, the response to TNBS-colitis had been studied at 14-28 days post-enema, where activation
of GC-C (27) or GFRα3 (213) was able to reduce post-inflammatory colonic hypersensitivity.

*Pathogen-Induced Inflammation: C. rodentium, C. jejuni, & T. spiralis*

In an attempt to model post-infective IBS, colonic sensitivity has been assessed in rat and mouse models following an acute bacterial gastroenteritis. In rats, *Citrobacter rodentium* induced a significant increase in AWR score, which was inhibited by treatment with a traditional herbal medicine or alosetron (92). In mice, *Campylobacter jejuni* infection caused long-term hyperexcitability of colonic DRG neurons (95); however, a clear increase in the EMG response to balloon distension was only found if the mouse was exposed to an additional stressor (96).

*Trichinella spiralis* infection has also been used to produce a long-term colonic hypersensitivity to distension in rats and mice. At 8-weeks post infection, the EMG response to distension was significantly increased along with increases in glutamatergic receptor expression (261) in rats, and the AWR score was increased in mice (130, 131), which could be inhibited by treatment with multiple probiotic strains (235). Similarly, a decrease in withdrawal threshold and an increase in total glutamatergic positive cells in colonic DRGs (257) were found at 100 days post-infection.

**Challenges:** In each of the irritant-induced inflammations, recovery from the colitis does not guarantee the existence for colonic hypersensitivity to balloon distension (275). Thus, the acute effects of the initial inflammatory insult (loose stool/diarrhea, weight loss, occult or explicit bleeding) needs to be monitored during the recovery period to aid in predicting which animals may develop post-inflammatory colonic hypersensitivity. Optimally, colonic sensitivity to distension should be assessed before
testing a therapeutic intervention with only those animals with verified colonic
hypersensitivity being used for subsequent testing. Furthermore, additional precautions
should be exercised when using the post-infective models to protect the experimenters
from the pathogens. Both tegaserod (78) and linaclotide (27) have been shown to inhibit
TNBS-induced post-inflammatory colonic hypersensitivity, providing evidence for the
translational relevance of the model.

ANIMAL MODELS OF VISCERAL HYPERSENSITIVITY RELEVANT TO
FUNCTIONAL DYSPEPSIA (FD) (Table 2)

There are at least three rat models of functional dyspepsia (FD) that have been
investigated in the literature. The first uses oral gavages of 0.1% iodoacetamide in 2%
sucrose administered to male, neonatal SD pups on PN10-16 to induce in adulthood
FD-like responses to gastric balloon distension (128). In the second model, an enema of
TNBS (130 mg/kg in 10% EtOH) is administered to PN10 male SD pups resulting in a
hypersensitive response to stomach distension in adulthood (245). The third model
manipulates the stomach of adult, male SD rats with 15-20 injections of 20% acetic acid
(10 µl/site) into the submucosal layer of the glandular portion of the stomach (52). For
all three models, the change in sensitivity in the stomach is measured as skeletal
muscle contractions in response to isobaric balloon distension. In the adult rat, a balloon
catheter is surgically implanted through the fundus and exteriorized through the base of
the neck. Additionally, EMG electrodes are implanted in the acromiotrapezius and
exteriorized through the base of the neck. The distension paradigm for each study is 20-sec per distension in conscious rats, with pressures ranging from 0-120 mmHg. In
addition to the EMG quantification, AWR score can also be determined (128). Studies investigating possible mechanisms and therapeutic interventions in the FD models have shown that acute pre-dosing of baclofen (γ-aminobutyric acid receptor B agonist, s.c.) or desvenlafaxine succinate (SNRI, p.o.) in adulthood dose dependently inhibited the EMG responses (52, 128). In the neonatal TNBS-induced model of FD, neonatal treatment (PN9-17) with mifepristone (GR antagonist, s.c.) prevented the development of adult gastric hypersensitivity (245). Increased BDNF and decreased voltage-gated potassium channel 1.1 (Kv1.1) within the DRG as well as increased NGF in the fundus in adulthood were also demonstrated to be part of the potential mechanism in the TNBS-induced model of FD (245).

Challenges: The first challenge in these models is the manipulation of the neonatal pups with either p.o. dosing of iodoacetamide or the TNBS enema. None of the papers reported any measure of maternal care or whether the manipulations changed weaning weight of the pups, which can be factors on overall adult health. Additionally, the studies did not report if female pups developed gastric hypersensitivity in adulthood. Another significant challenge is the surgeries needed to implant a chronic balloon catheter within the fundus of the stomach and the EMG electrodes necessary for the assessment of gastric sensitivity. While the chronic nature of the balloon catheter and the EMG electrodes allows for repeated studies within the same animal, care has to be taken to ensure the rat does not damage the instrumentation, and that the balloon does not interfere with eating and gastric emptying. Additionally, while the iodoacetamide and acetic acid models reported complete penetrance, the TNBS-enema model only produced a chronic gastric hypersensitivity in 50% of the treated animals.
Finally, while the use of mice would allow access to the multiple knockout models that exist, adapting the FD models to the mouse scale would be technically difficult.

**ANIMAL MODELS OF VISCERAL PAIN RELEVANT TO PANCREATITIS (Table 2)**

In the rat, while there are multiple, well-characterized models of pancreatitis, there are two models that have been used for investigation of pancreatitis-induced nociceptive behaviors – dibutyltin dichloride (DBTC) and TNBS. DBTC is administered i.v. in an EtOH vehicle at a dose of 8 mg/kg in adult, male SD, Wistar or Lewis rats. DBTC produces a chronic pancreatic inflammation that can be enhanced with 10% EtOH in the drinking water. Visceral hypersensitivity is assessed as mechanical or thermal somatic thresholds with either von Frey filaments or hot plate withdrawal, respectively, or as the number of nocifensive behavioral responses to electrical stimulation of the inflamed pancreas. Studies investigating potential mechanisms for DBTC-induced pancreatitis have demonstrated the decrease in withdrawal response was associated with increased spinal dynorphin content, which could be blocked with anti-dynorphin antiserum or lidocaine in the nodose ganglion (234). Sumatriptan, a mixed 5HT<sub>1B/D</sub> agonist, dose dependently inhibited the decrease in withdrawal threshold, and the effect was reversed by selective 5HT<sub>1B</sub> or 5HT<sub>1D</sub> antagonists (233). Another study demonstrated an increase in calcium channel expression within the spinal cord following induction of pancreatitis along an inhibition of the somatic withdrawal response following administration of gabapentin (calcium channel antagonist, i.p.) (121). Selective endothelin receptor A or B antagonists (i.p.) have also been shown to increase mechanical and thermal withdrawal thresholds in the same
model (168). In the TNBS-induced pancreatitis model, decreased abdominal withdrawal thresholds for both mechanical and thermal stimuli were demonstrated to be modulated by TRPV1 (253), PAR2 (267), and activated microglia.

One study adapted the TNBS-induced pancreatitis model to mice and tested the nociceptive behavioral responses in wild type and TRPA1 knockout mice (28). Aside from increased mortality following the induction of the pancreatitis, TRPA1 knockout mice demonstrated increased withdrawal thresholds, increased voluntary wheel running, and more activity in the open field compared to the wild-type controls (28). The other model of pancreatitis developed in mice is induced by 6-12 injections (i.p.) of caerulein. The caerulein induced pancreatitis model has been used to explore the role of TRPA1 and TRPV1 through the use of selective antagonists (190), and cyclooxygenase receptor 2 (COX-2) with selective knockout animals (211).

**Challenges:** In each of the models, histology of the pancreas verifies that the induced inflammation appears clinically relevant based on the extent of damage and types of inflammatory infiltrate present in the tissue. However, each model has particular challenges associated with the development of the inflammation. For DBTC, pancreatitis develops following an i.v. injection, typically tail vein, and the inflammation can be enhanced with ethanol in the drinking water. For the TNBS-induced pancreatitis models in the rat and mouse, the TNBS is administered slowly through the pancreatic ducts, via the duodenum, which necessarily requires a surgery. When performed by experienced researcher, rats tolerate the TNBS instillation, whereas mortality in the mouse is approximately 20% with TNBS and 10% in the sham surgery group (28). The caerulein model of pancreatitis in the mouse requires multiple i.p. injections that can be
stressful for the mouse if not properly acclimated. All of the pancreatitis models also rely on measuring referred somatic pain responses as an indirect indication of the visceral pain, although stimulation electrodes can be attached to the pancreas after TNBS infusion to directly induce visceral nociceptive responses.

ANIMAL MODELS OF VISCERAL PAIN RELEVANT TO CYSTITIS/PAINFUL BLADDER SYNDROME (PBS) (Table 2)

In rats and mice, intraperitoneal cyclophosphamide (CYP) had been used to induce an acute or chronic model of cystitis. The acute model uses a single bolus injection of 150 mg/kg CYP to produce bladder inflammation and edema, with sensitivity measured within 48-hour of the injection. The chronic model uses repeated injections of 75 mg/kg CYP, typically 3-4 total injections over 8-12 days, with testing 24-48 hour following the final injection. Commonly, referred visceral hyperalgesia is tested in conscious animals as withdrawal to von Frey filament probing on the lower abdomen, although some studies have also measured EMG responses to bladder distension in anesthetized animals. In mice, the cystitis induced increased number of withdrawals to von Frey filaments or increased the EMG response to bladder distension can be inhibited by TRPA1 antagonists (53), a MAPK kinase inhibitor (108), or thrombomdulin (212). An elegant study in rats demonstrated that nociceptive behaviors induced by acute CYP cystitis could be inhibited by, aspirin, ibuprofen, or morphine, without affecting the CYP-induced histologic damage to the bladder (10). Intrathecal botulinum toxin A also had an analgesic effect on CYP-induced decreases in withdrawal threshold (45). Similar to the effect in mice, CYP cystitis induces increases in the MAPK signaling
in DRG (47), and changes in glutamatergic signaling within the spinal cord of rats (33, 105).

In adult rats a 30-min intrabladder infusion of a 1% zymosan solution is used to produce an acute cystitis. A similar concentration is used in neonatal rats, however three total infusions are performed, once each on PN14-16. In the adult rat, zymosan sensitizes lumbosacral spinal neurons to distension (158), and produces an estrous cycle dependent increases the EMG response to bladder distension (11). In the absence of persistent histological damage, adult hypersensitivity to bladder distension was demonstrated in the animals exposed to neonatal zymosan, with differences in sensitivity demonstrated following adult challenge with zymosan (183, 193). The neonatal model has also been used to investigate visceral-organ cross-talk by measuring the EMG response to colonic distension in adult rats (143, 192).

**Challenges:** Histological damage to the bladder in these models of cystitis suggests that the inflammation is relevant to human pathology, with the caveat that these induced-models do not model clinical etiologies. While intraperitoneal injections are easily performed in rodents, CYP has not been studied outside of the effect on the bladder, and thus its effect on other organs is unknown. Intravesical administration of irritants is technically more difficult than intraperitoneal injections, and generally requires study of only female animals to permit easier access to the bladder.

**ANIMAL MODELS OF NON-SPECIFIC ABDOMINAL PAIN (Table 2)**

While still highly reported in recent literature, abdominal writhing can be induced with multiple intraperitoneal irritants, usually acetic acid. However, the excessive
intensity, inescapable nature, and the inability to target a specific visceral organ reduce the translational relevance of this approach.

SUMMARY

Animal models have provided multiple lines of pivotal evidence to enhance our understanding of the physiology and pathophysiology of visceral pain. However, visceral pain is a complex, multifactorial disorder involving higher cognitive and cortical function as well as complicated interactions between biological, psychological, and sociological variables that no animal model is able to mimic completely. Together there are multiple challenges and limitations that must be considered when interpreting the results of studies using animal models to address questions of disease pathology and translational relevance. For example many of the models used to study visceral pain tend to induce exaggerated pain responses or hyperalgesia, however many patients also exhibit allodynia to innocuous stimuli. This matter has relevance for any translational work focused on the development of novel therapeutics to treat visceral pain as different neurobiological mechanisms are involved in hyperalgesia and allodynia. Additionally, mechanisms responsible for acute sensitization in the various animal models may only have low-moderate predictive validity for chronic pain conditions; however, the acute models are excellent screening tools, as efficacy against acute nociceptive behaviors will provide an indication of potential benefit in chronic pain models. To address the somewhat poor face validity of individual models, and due to the multifactorial nature of the clinical condition, efficacy of novel therapeutics should be
assessed in multiple models with different etiologies (e.g., stress-induced, acute sensitization, & post-inflammation) whenever possible and scientifically justified.


23. Buckley MM, O'Halloran KD, Rae MG, Dinan TG, and O'Malley D. Modulation of enteric neurons by interleukin-6 and corticotropin-releasing factor contributes to


41. **Chen ZY, Zhang XW, Yu L, Hua R, Zhao XP, Qin X, and Zhang YM.** Spinal toll-like receptor 4-mediated signalling pathway contributes to visceral hypersensitivity induced by neonatal colonic irritation in rats. *European journal of pain* 2014.


44. **Chung EK, Bian ZX, Xu HX, and Sung JJ.** Neonatal maternal separation increases brain-derived neurotrophic factor and tyrosine kinase receptor B expression in the descending pain modulatory system. *Neuro-Signals* 17: 213-221, 2009.


74. **Gonzalez-Cano R, Merlos M, Baeyens JM, and Cendan CM.** Sigma1 receptors are involved in the visceral pain induced by intracolonic administration of capsaicin in mice. *Anesthesiology* 118: 691-700, 2013.


101. Johnson AC, Tran L, Schulkin J, and Greenwood-Van Meerveld B. Importance of stress receptor-mediated mechanisms in the amygdala on visceral pain


128. Liu LS, Shenoy M, and Pasricha PJ. The analgesic effects of the GABAB receptor agonist, baclofen, in a rodent model of functional dyspepsia.


McKernan DP, Fitzgerald P, Dinan TG, and Cryan JF. The probiotic Bifidobacterium infantis 35624 displays visceral antinociceptive effects in the rat.


167. Ohashi-Doi K, Himaki D, Nagao K, Kawai M, Gale JD, Furness JB, and Kurebayashi Y. A selective, high affinity 5-HT 2B receptor antagonist inhibits visceral...


218. Tjong YW, Ip SP, Lao L, Wu J, Cong HH, Sung JJ, Berman B, and Che CT. Role of neuronal nitric oxide synthase in colonic distension-induced hyperalgesia in


242. Wilder-Smith CH, Schindler D, Lovblad K, Redmond SM, and Nirkko A. Brain functional magnetic resonance imaging of rectal pain and activation of endogenous
inhibitory mechanisms in irritable bowel syndrome patient subgroups and healthy

243. **Winchester WJ, Johnson A, Hicks GA, Gebhart GF, Greenwood-van
Meerveld B, and McLean PG.** Inhibition of endothelial cell adhesion molecule
expression improves colonic hyperalgesia. *Neurogastroenterology and motility :

244. **Winston JH, Li Q, and Sarna SK.** Chronic prenatal stress epigenetically
modifies spinal cord BDNF expression to induce sex-specific visceral hypersensitivity in
offspring. *Neurogastroenterology and motility : the official journal of the European

245. **Winston JH, and Sarna SK.** Developmental origins of functional dyspepsia-like

246. **Winston JH, Xu GY, and Sarna SK.** Adrenergic stimulation mediates visceral
hypersensitivity to colorectal distention following heterotypic chronic stress.

247. **Wiskur BJ, Tyler K, Campbell-Dittmeyer K, Chaplan SR, Wickenden AD, and
Greenwood-Van Meerveld B.** A novel TRPV1 receptor antagonist JNJ-17203212
attenuates colonic hypersensitivity in rats. *Methods and findings in experimental and

Oudenhove L, Van den Wijngaard RM, Van Laere K, and Boeckxstaens G.** Altered
brain activation to colorectal distention in visceral hypersensitive maternal-separated
rats. *Neurogastroenterology and motility : the official journal of the European

249. **Wu JC, Ziea ET, Lao L, Lam EF, Chan CS, Liang AY, Chu SL, Yew DT,
Berman BM, and Sung JJ.** Effect of electroacupuncture on visceral hyperalgesia,
serotonin and fos expression in an animal model of irritable bowel syndrome. *Journal of

250. **Xu D, Gao J, Gillilland M, 3rd, Wu X, Song I, Kao JY, and Owyang C.**
Rifaximin alters intestinal bacteria and prevents stress-induced gut inflammation and

251. **Xu GY, Winston JH, Shenoy M, Yin H, Pendyala S, and Pasricha PJ.** Butyrate-induced colonic
hypersensitivity is mediated by mitogen-activated protein kinase activation in rat dorsal

252. **Xu GY, Winston JH, and Chen JD.** Electroacupuncture attenuates visceral
hyperalgesia and inhibits the enhanced excitability of colon specific sensory neurons in
a rat model of irritable bowel syndrome. *Neurogastroenterology and motility : the official

253. **Xu GY, Winston JH, Shenoy M, Yin H, Pendyala S, and Pasricha PJ.**
Transient receptor potential vanilloid 1 mediates hyperalgesia and is up-regulated in

254. **Xu GY, Winston JH, Shenoy M, Zhou S, Chen JD, and Pasricha PJ.** The
endogenous hydrogen sulfide producing enzyme cystathionine-beta synthase
contributes to visceral hypersensitivity in a rat model of irritable bowel syndrome.


Figure 1. Visceral pain signaling pathways. A) Noxious visceral stimulation activates nociceptors that synapse in the superficial lamina of the dorsal horn of the spinal cord. Ascending pain signals are transmitted through the anterolateral pathways in the dorsal column. Tertiary neurons in the thalamus distribute the pain signal to cortical areas for localization while other regions provide intensity and emotional components of the pain stimulus. The amygdala is also activated to integrate the responses of the autonomic nervous system and the hypothalamic-pituitary-adrenal (HPA) axis. B) Pain inhibition is modulated by descending projections from the anterior cingulate that activate the periaqueductal gray (PAG) to further activate serotonergic (caudal raphe) and opioidergic (rostral ventral medulla) antinociceptive systems. Pain facilitatory systems can be engaged by amygdala modulation of the paraventricular nucleus of the hypothalamus for HPA axis activity and of the locus coeruleus for the sympathomedullary axis. The amygdala can also modulate descending antinociception through connections to the PAG. ACC = anterior cingulate cortex. MCC = midcingulate cortex. PAG = periaqueductal gray. The figure is adapted from Grover and Drossman 2010 (79).

Figure 2. Mechanisms of visceral hypersensitivity. Combined evidence from animal models and clinical studies supports the hypothesis that visceral hypersensitivity is due to multiple central and peripheral mechanisms. Interactions between each mechanism (i.e. stress changes permeability, which can alter microbiota, leading to immune
activation) make the development of adequate therapies a continuing challenge for future research.

**Figure 3: Rodent models and mediators of colonic hypersensitivity.** A) Models of visceral hypersensitivity. Hypersensitivity can be induced through global or tissue specific knockout of neurotransmitters or their receptors or via overexpression of human proteins. Additionally, variation between strains has identified the Wistar-Kyoto rat as displaying spontaneous colonic hypersensitivity. Chronic hypersensitivity can be established by manipulation of neonatal pups, including: maternal separation protocols, direct irritation of the colon, or through the use of conditioning protocols. Acute stress protocols produce colonic hypersensitivity of varying duration depending on the type of stressor and the strain of rodent. The central nucleus of the amygdala (CeA) and be directly targeted with stress hormones to produce a colonic hypersensitivity that persists beyond the exposure to the hormone. Restraint stress typically produces an acute hypersensitivity (< 1 day), whereas exposing rats to variable stress or repeated water avoidance stress protocols can produce colonic hypersensitivity that persists for several weeks. Acute sensitization of colonic afferents produces hypersensitivity that is either transient (several hours) or short-lived (days), depending on whether the irritant induced an inflammatory response. Acetic acid and butyrate can be used to sensitize afferents without inducing inflammation. Capsaicin and mustard oil induce spontaneous noxious behaviors. Dextran sodium sulfate (DSS), 2,4,6-trinitrobenzenesulfonic acid (TNBS), and zymosan all produce colonic inflammation of varying severity. Post-inflammatory colonic hypersensitivity can occur following recovery from colitis induced by acetic acid,
DSS or TNBS. Additionally, recovery from colitis induced by pathogenic bacteria (Citrobacter rodentium, Campylobacter jejuni) or nematodes (Trichinella spiralis) can also lead to chronic colonic hypersensitivity. See Table 1 for recent references for each model. B) Mediators of visceral hypersensitivity. While not a comprehensive list, mediators of visceral hypersensitivity identified in the literature from Table 1 have been summarized based on the model system used and the potential site of action. For the purposes of this figure, mediators (either ligands, signaling pathways, processes, or receptors) were classified as ‘central’ if a therapeutic was administered centrally (brain or spinal cord) and/or if only changes within the brain and spinal cord were analyzed; mediators were considered ‘peripheral’ if a therapeutic was administered peripherally and/or if only tissues outside of the spinal cord (including the dorsal root ganglion) were investigated; mediators were listed as ‘both’ central and peripheral if a therapeutic was administered both centrally and peripherally, if the therapeutic was known to cross the blood-brain barrier with targets in both sites, and/or if the study analyzed both central and peripheral tissues. ASICs = acid-sensing ion channels; ATP = adenosine triphosphate; BDNF = brain-derived neurotrophic factor; Ca_v = voltage gated calcium; CRF = corticotropin releasign-factor; EAAT2 = excitatory amino-acid transporter type 2; ERKs = extracellular-signal-regulated kinases; GABA = γ-aminobutyric acid; GC-C = guanylate cyclase C; GDNF = glia cell line-derived neurotropic factor; GLT-1 = glutamate transporter-1; GRK6 = G protein-coupled receptor kinase 6; HCN = hyperpolarization-activated cyclic nucleotide-gated; K_v = voltage gated potassium; IL-1β = interleukin 1β; IL-6 = interleukin 6; IL-10 = interleukin 10; Na_v = voltage gated sodium; NE = norepinephrine; NGF = nerve growth factor; NO = nitric oxide; N/OFQ =...
nociception/orphanin FQ; SERT = serotonin re-uptake transporter; SP = substance P;

TLR4 = toll-like receptor 4; TNFα = tumor necrosis factor α; TRPA1 = transient receptor
potential cation channel, subfamily A, member 1; TRPC4 = short transient receptor
potential channel 4; TRPM8 = transient receptor potential cation channel subfamily M
member 8; TRPV1 = transient receptor potential cation channel, subfamily V, member
1; VGLUT3 = vesicular glutamate transporter 3.
Table 1: Rodent Models of Colonic Hypersensitivity (2009-2014)

**Genetic/Spontaneous Models**

<table>
<thead>
<tr>
<th>Model</th>
<th>Mice</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDNF HET</td>
<td>260, 264</td>
<td></td>
</tr>
<tr>
<td>GC-C KO</td>
<td>62</td>
<td></td>
</tr>
<tr>
<td>GFRα3 KO</td>
<td>213</td>
<td></td>
</tr>
<tr>
<td>GRK6 KO</td>
<td>61</td>
<td></td>
</tr>
<tr>
<td>IL-10 KO</td>
<td>276</td>
<td></td>
</tr>
<tr>
<td>Mast Cell (Ws/Ws) KO</td>
<td></td>
<td>258</td>
</tr>
<tr>
<td>PAR2 KO</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>PAR4 KO</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>P2X2/P2X3 KO</td>
<td>185</td>
<td></td>
</tr>
<tr>
<td>P2X3 KO</td>
<td>197</td>
<td></td>
</tr>
<tr>
<td>P2X3/TRPV1 KO</td>
<td>107</td>
<td></td>
</tr>
<tr>
<td>P2X7 KO</td>
<td>106</td>
<td></td>
</tr>
<tr>
<td>SERT KO</td>
<td></td>
<td>69</td>
</tr>
<tr>
<td>σ1 Receptor KO</td>
<td>74</td>
<td></td>
</tr>
<tr>
<td>TLR4 KO</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>TRPA1 KO</td>
<td>22, 30</td>
<td></td>
</tr>
<tr>
<td>TRPC4 KO</td>
<td></td>
<td>239</td>
</tr>
<tr>
<td>TRPV1 KO</td>
<td>155</td>
<td></td>
</tr>
<tr>
<td>EAAT2 OE</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>Wistar-Kyoto</td>
<td>21, 23, 26, 70, 101, 141, 160-162, 164, 165, 243</td>
<td></td>
</tr>
</tbody>
</table>

**Early Life Stress Models**

<table>
<thead>
<tr>
<th>Model</th>
<th>Mice</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Limited Nesting  175

Maternal Separation  144  12, 15, 40, 44, 58, 63, 75, 90, 91, 93, 119, 134, 162, 164, 202, 216-218, 227-231, 248, 249, 268, 269


Odor Attachment Learning  31

**Stress-Induced Models**

<table>
<thead>
<tr>
<th>Model</th>
<th>Mice</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>CeA-Implants</td>
<td>82, 149-152, 222, 223, 225</td>
<td></td>
</tr>
<tr>
<td>Restraint Stress</td>
<td>1, 2, 24, 62, 167, 196, 200, 209, 243, 250, 270</td>
<td></td>
</tr>
<tr>
<td>Variable Stress</td>
<td>219, 220</td>
<td></td>
</tr>
</tbody>
</table>

**Colonic Irritation Models**

<table>
<thead>
<tr>
<th>Model</th>
<th>Mice</th>
<th>Rats</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetic Acid</td>
<td>77, 85, 115, 247</td>
<td></td>
</tr>
<tr>
<td>Butyrate</td>
<td>8, 54, 138, 139, 251</td>
<td></td>
</tr>
<tr>
<td>Capsaicin</td>
<td>3, 14, 35, 61, 66, 74, 83, 116, 140, 259</td>
<td>181, 188, 231</td>
</tr>
<tr>
<td>DSS</td>
<td>16, 89, 94, 124</td>
<td></td>
</tr>
<tr>
<td>Mustard Oil</td>
<td>9, 30, 34, 35, 66, 116, 117, 155, 172, 237</td>
<td>98, 170, 171, 198, 199, 239</td>
</tr>
<tr>
<td>TNBS</td>
<td>5, 22, 29, 30, 62, 124, 237, 260</td>
<td>1, 62, 103, 146, 179, 191, 200, 203</td>
</tr>
<tr>
<td>Zymosan</td>
<td>64, 107, 197</td>
<td>115</td>
</tr>
<tr>
<td>Model</td>
<td>Mice</td>
<td>Rats</td>
</tr>
<tr>
<td>---------------</td>
<td>------------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td></td>
<td>51, 169, 194, 209, 214</td>
</tr>
<tr>
<td><em>C. rodentium</em></td>
<td>95, 96</td>
<td></td>
</tr>
<tr>
<td><em>C. jejuni</em></td>
<td></td>
<td>92</td>
</tr>
<tr>
<td>DSS</td>
<td>61, 113</td>
<td>36</td>
</tr>
<tr>
<td>TNBS</td>
<td>27, 59, 65, 100, 142, 155, 166, 167, 206, 213, 275</td>
<td>59, 100, 142, 155, 166, 167, 206, 275</td>
</tr>
<tr>
<td><em>T. spiralis</em></td>
<td>46, 106, 130, 131, 185, 235</td>
<td>257, 258, 261</td>
</tr>
</tbody>
</table>

BDNF = brain-derived neurotrophic factor; CeA = central nucleus of the amygdala; DSS = dextran sodium sulfate; EAAT2 = excitatory amino-acid transporter type 2; GC-C = guanylate cyclase C; GFRα3 = GDNF family receptor alpha-3; GRK6 = G protein-coupled receptor kinase 6; HET = heterozygous; IL-10 = interleukin 10; KO = knockout; OE = overexpression; PAR2 = protease activated receptor 2; PAR4 = protease activated receptor 4; P2X2 = P2X purinoceptor 2; P2X3 = P2X purinoceptor 3; P2X7 = P2X purinoceptor 7; SERT = serotonin re-uptake transporter; TLR4 = toll-like receptor 4; TNBS = 2,4,6-trinitrobenzenesulfonic acid; TRPA1 = transient receptor potential cation channel, subfamily A, member 1; TRPC4 = short transient receptor potential channel 4; TRPV1 = transient receptor potential cation channel, subfamily V, member 1
<table>
<thead>
<tr>
<th>Disease</th>
<th>Method</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Functional Dyspepsia</td>
<td>Oral iodoacetamide in neonatal rats</td>
<td>120, 128</td>
</tr>
<tr>
<td></td>
<td>TNBS enema in neonatal rats</td>
<td>245</td>
</tr>
<tr>
<td></td>
<td>Acetic acid in the submucosal layer of the stomach in rats</td>
<td>52, 210</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>Intravenous DBTC in rats</td>
<td>121, 168</td>
</tr>
<tr>
<td></td>
<td>Intraductal TNBS in mice</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>Intraductal TNBS in rats</td>
<td>129, 267</td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal caerulein in mice</td>
<td>190, 201</td>
</tr>
<tr>
<td>Cystitis/Painful Bladder Syndrome</td>
<td>Intraperitoneal injections of CYP in mice</td>
<td>53, 108, 212</td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal injections of CYP in rats</td>
<td>7, 10, 33, 42, 45, 47, 105, 133</td>
</tr>
<tr>
<td></td>
<td>Intravesical PS infusion in mice</td>
<td>205</td>
</tr>
<tr>
<td></td>
<td>Intravesical PS infusion in rats</td>
<td>136, 145</td>
</tr>
<tr>
<td></td>
<td>Intravesical zymosan infusion in rats</td>
<td>11, 158</td>
</tr>
<tr>
<td></td>
<td>Intravesical zymosan infusion in neonatal rats</td>
<td>143, 183, 192, 193</td>
</tr>
<tr>
<td>Non-specific Abdominal Pain</td>
<td>Intraperitoneal acetic acid in mice</td>
<td>14, 54, 67, 84, 132, 137, 153, 187, 189, 238</td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal acetic acid in rats</td>
<td>17, 55, 102, 118, 147</td>
</tr>
<tr>
<td></td>
<td>Intraperitoneal lactic acid in rats</td>
<td>157</td>
</tr>
<tr>
<td></td>
<td>Other intraperitoneal irritants in mice – CYP, misoprostol, neostigmine, PPQ</td>
<td>66, 117, 172, 241</td>
</tr>
</tbody>
</table>

CYP = cyclophosphamide; DBTC = dibutyltin dichloride; PPQ = para-phenylquinone; PS = protamine sulfate; TNBS = 2,4,6-trinitrobenzenesulfonic acid
Stress

- HPA Axis Alteration
- Central Sensitization

Altered Microbiome
- Fermentation Products
- Host Immune Response

Genetic Vulnerability
- Allelic Variations in Neurotransmitter Function

Increased Permeability
- Afferent Nerve Stimulation
- Immune System Stimulation

Post-Infective
- Immune System Activation
- Peripheral Sensitization

Visceral Hypersensitivity