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Visceral Pain

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Abstract

Most of us live blissfully unaware of the orchestrated function that our internal organs conduct. When this peace is interrupted, it is often by routine sensations of hunger and urge. However, for >20% of the global population, chronic visceral pain is an unpleasant and often excruciating reminder of the existence of our internal organs. In many cases, there is no obvious underlying pathological cause of the pain. Accordingly, chronic visceral pain is debilitating, reduces the quality of life of sufferers, and has large concomitant socioeconomic costs. In this review, we highlight key mechanisms underlying chronic abdominal and pelvic pain associated with functional and inflammatory disorders of the gastrointestinal and urinary tracts. This includes how the colon and bladder are innervated by specialized subclasses of spinal afferents, how these afferents become sensitized in highly dynamic signaling environments, and the subsequent development of neuroplasticity within visceral pain pathways. We also highlight key contributing factors, including alterations in commensal bacteria, altered mucosal permeability, epithelial interactions with afferent nerves, alterations in immune or stress responses, and cross talk between these two adjacent organs.

INTRODUCTION

Our internal or visceral organs undertake a variety of physiological functions that are essential for sustaining life. We are generally unaware of these complicated processes, but when awareness is triggered, it is usually by sensations of hunger, fullness, or urge. Akin to pain arising from other regions of the body, visceral pain alerts us of potential or actual tissue damage. Visceral pain encompasses a plethora of common acute and chronic clinical conditions that are experienced by millions of people around the globe. This includes sudden onset pain associated with serious clinical conditions, such as peptic/intestinal ulcers, cholecystitis, and appendicitis. However, visceral pain also encompasses wide-ranging chronic syndromes, including noncardiac chest pain, functional abdominal pain, endometriosis, pancreatitis, as well as chronic pain from the bladder and bowel. While some of these clinical entities have a clear underlying pathology, in many cases there is no obvious reason for the pain, and they are therefore often referred to as idiopathic or functional disorders. This is particularly troubling for the afflicted individual and makes accurate diagnosis difficult, particularly when the diagnosis is based on exclusion of other serious diseases. To further complicate diagnosis, visceral pain is poorly localized and diffuse, often affecting multiple organs at once, with visceral differentiation often relying on the determination of associated pathology and changes in organ function.

In this review, for brevity, we discuss key mechanisms contributing to chronic abdominal and pelvic pain. In particular, it covers pain originating from the colon and bladder and the highly prevalent clinical conditions they are associated with. These include interstitial cystitis/bladder pain syndrome (IC/BPS), irritable bowel syndrome (IBS), and inflammatory bowel disease (IBD), which are major and debilitating forms of chronic visceral pain. A lack of suitable treatments for these disorders is a major contributing factor to their debilitating nature and the large socioeconomic cost accrued by patients, their families, and society (1, 2). Conventional analgesics, such as nonsteroidal anti-inflammatory drugs and opioids, are unsuitable for therapy, as they are associated with severe side effects. This includes tolerance, a lack of efficacy and, importantly for some inflammatory disorders, the potential to exacerbate the disease (3, 4). Chronic use of opioids for pain management causes dependence and reduced analgesic efficacy, resulting in the current opioid epidemic (5). Importantly, chronic opioid use also causes severe constipation, which is already a common symptom of many patients with IBS, making opioid use even more problematic in this patient cohort. Consequently, understanding the underlying etiology of these disorders is the first step in identifying new effective analgesic treatments for visceral pain syndromes.

CLINICAL RELEVANCE

IBD, which includes ulcerative colitis and Crohn's disease, is a chronic, relapsing inflammatory disorder of the gastrointestinal tract that affects 0.5% of the Western population, including 2.5 million in Europe and 1 million people in the United States (6). Although the exact etiology of IBD is unclear, development likely occurs in genetically susceptible individuals through an inadequately suppressed or exaggerated immune response to luminal antigens, probably derived from gut microbiota (7). Abdominal pain, diarrhea, and gastrointestinal bleeding are the major clinical symptoms of IBD, with medical and surgical therapies aimed at resolving mucosal inflammation and correspondingly reducing symptoms (7). Accordingly, IBD has considerable economic costs totaling >$6 billion per annum in the United States alone (1, 6).

In contrast, IBS is a chronic functional gastrointestinal disorder characterized by abdominal pain or discomfort associated with altered bowel habits. IBS is subclassified as constipation-predominant IBS (IBS-C), diarrhea-predominant IBS (IBS-D), alternating or mixed IBS (A/M-IBS), and postinfectious IBS (PI-IBS). IBS is distinct from IBD in that it presents

without overt inflammation-induced pathology to the intestine and is diagnosed based on the Rome IV criteria (1). IBS affects more than 11% of the global population, with ~65% of patients being female (1, 8). The etiology of IBS is multifactorial, and additional risk factors may be required for development, including altered stress and immune responses in afflicted individuals, with a strong correlation between a prior exposure of the patient to gastrointestinal infection and symptom occurrence (8, 9). This includes a preceding bout of gastroenteritis induced by pathogens such as *Escherichia coli*, *Campylobacter*, *Giardia lamblia*, and *Salmonella*, with symptoms potentially lasting for more than eight years after the initial infection (9, 10). IBS patients report a reduced quality of life and have additional clinical symptoms, including stool irregularities, as well as somatic and visceral comorbidities, with higher levels of anxiety and depression than healthy people (1, 8, 11). In the United States, the total direct and indirect cost of IBS is ~$30 billion per annum (12).

IC/BPS is a prevalent, chronic bladder disorder affecting >5% (11% of women and 5% of men) of the Western population (13–15). Patients with IC/BPS exhibit sensations of bladder fullness, urge to void, and allodynia and hyperalgesia to cystometric bladder filling compared to healthy controls; they also report sensations of urgency and pain at lower bladder distension volumes (16). Although the pathophysiology of IC/BPS is not completely understood, prior bladder *E. coli* infection, stress, and changes to neural pathways likely play key roles in the associated urgency, frequency, and pain (17). In the United States alone, IC/BPS costs ~$20–40 billion per annum to treat (15).

VISCERAL COMORBIDITIES

Although these conditions exist as distinct clinical entities, it is becoming clear that they do not occur in isolation, with considerable overlap in symptom profiles across patient cohorts. For example, ~40% of IBD patients in remission from inflammation meet the diagnostic symptom criteria for a functional gastrointestinal disorder (18), with incidence of IBS 2–3 times higher in IBD patients in remission from inflammation than in the general population (19). IC/BPS patients are 100 times more likely to have concurrent IBD than healthy controls (20), whereas bladder dysfunction is significantly more common among IBS patients than in healthy subjects (21). Correspondingly, patients with IBS are more likely to report bladder symptoms, including nocturia, urgency, and in some cases, urge incontinence (22). Conversely, 20–30% of men and women with IC/BPS have IBS among their most common comorbidity (20, 23). Recent evidence suggests that these clinical comorbidities are likely underscored by the overlap of colonic and bladder sensory networks, which ensure homeostatic coordination of these organs (11). Sensory signals emanating from these visceral organs project to the dorsal horn of the spinal cord where they activate postsynaptic pathways responsible for spinal and supraspinal autonomic reflexes. If the signal is of sufficient intensity, brain stem, limbic, and cortical regions provide emotional affective and conscious modulation of visceral sensation. As such, visceral sensations are susceptible to modulation at each stage along this pathway, and the induction and maintenance of visceral pain have been investigated as a consequence of peripheral and central sensitization of the afferent pathways. Supraspinal modifications such as emotional state and stress can have significant effects on the perception of visceral pain.

EXTRINSIC SENSORY AFFERENT INNERVATION OF THE COLON AND BLADDER

The colon/rectum and bladder are innervated by specialized spinal sensory afferents traveling via two distinct anatomical spinal pathways: the lumbar splanchnic and sacral pelvic nerves (7, 24, 25) (**Figure 1**). Colon-innervating splanchnic and pelvic afferents have their cell bodies

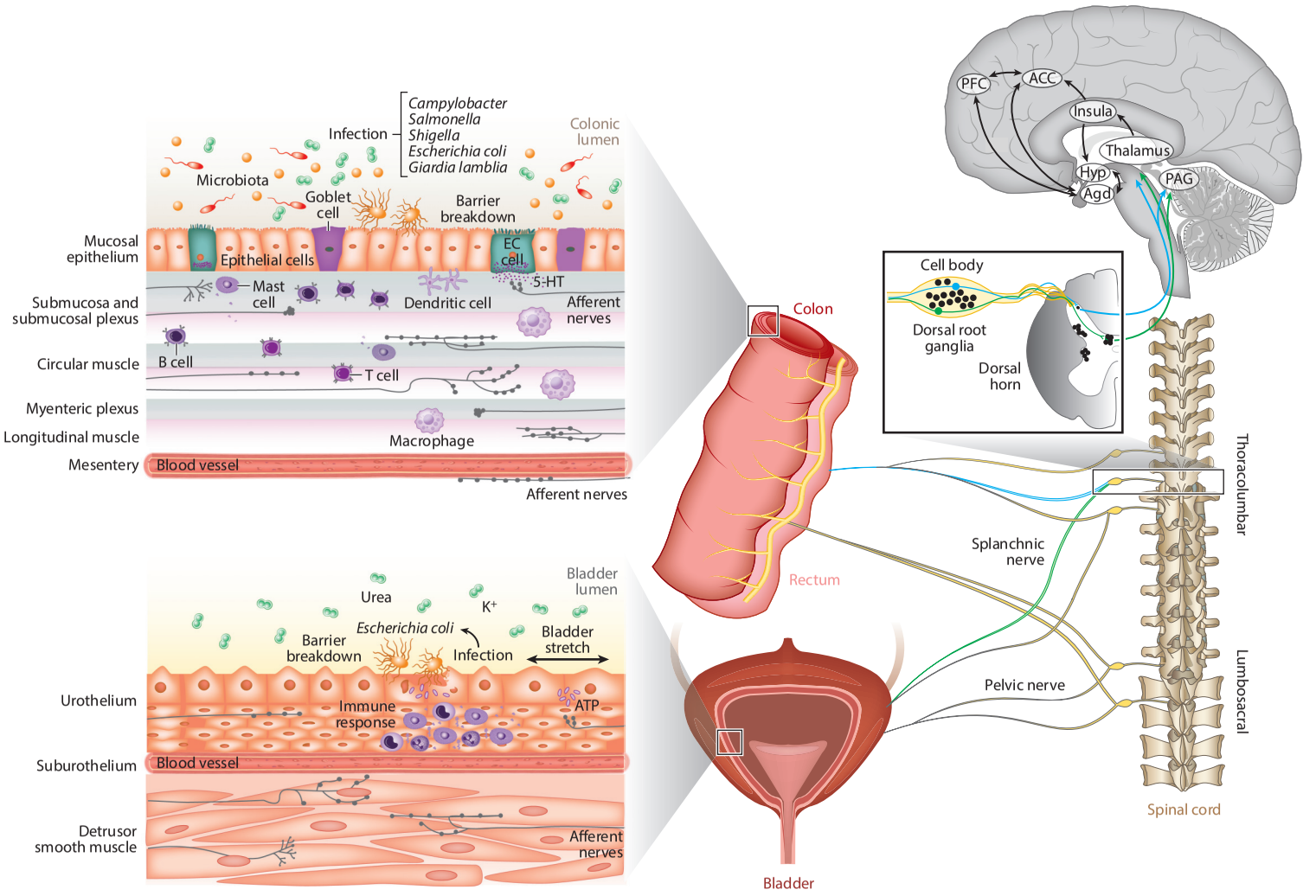

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Figure 1 (*Figure appears on preceding page*)

Schematic overview of the extrinsic sensory innervation of the colon/rectum and bladder. The colon and rectum are innervated by spinal afferents that follow the splanchnic and pelvic nerves. These afferents have cell bodies within the thoracolumbar and lumbosacral dorsal root ganglia, respectively. Their central axons terminate within the respective thoracolumbar and lumbosacral dorsal horn of the spinal cord, where they synapse onto second-order neurons. The peripheral projections of these afferents innervate the mucosa, myenteric/submucosal ganglia, and muscle; they also wrap around blood vessels within the submucosa and on the mesenteric attachment. This gives rise to distinct functional classes of afferents: muscular, mucosal, and vascular (splanchnic and pelvic pathway); and mesenteric (splanchnic only) and muscular/mucosal (pelvic only). Several populations of mechanically insensitive silent afferents also exist. Combined, these afferents allow detection of the full range of mechanical and chemical stimuli occurring within the colon and rectum. The bladder is also innervated by afferents from the pelvic and splanchnic pathways, with central projections to the thoracolumbar and lumbosacral dorsal horn of the spinal cord. Bladder afferents have specialized peripheral endings located within the urothelium, but predominantly within the detrusor smooth muscle. These afferents have both low- and high-mechanical activation thresholds to distension, allowing the full range of bladder distension and contraction to be detected. Populations of mechanically insensitive afferents also exist. Abbreviations: ACc, anterior cingulate cortex; Agd, amygdala; EC, enterochromaffin; Hyp, hypothalamus; PAG, periaqueductal gray; PFC, prefrontal cortex.

located within the thoracolumbar (TL; T10–L2) and lumbosacral (LS; L5–S1) dorsal root ganglia (DRG), respectively. These afferents synapse in the dorsal horn of the spinal cord with excitatory and inhibitory interneurons and second-order neurons of the dorsal column, spinothalamic tract, and spinoparabrachial pathway. The spinoparabrachial pathway is made predominantly of superficial dorsal horn projections associated with autonomic and affective responses to painful stimuli, whereas the spinoparabrachial projections feed into limbic and cognitive centers, including the amygdala, hypothalamus, and periaqueductal gray (PAG). The spinothalamic tract signal is relayed via the thalamus to cortical areas for sensory discrimination and localization via somatosensory inputs, while also feeding into limbic areas for the emotional component of the pain response (3, 26). The thalamus influences prefrontal cortex signaling associated with visceral pain responses, including those in the anterior cingulate cortex (ACc). Output from the cortical and limbic regions in response to pain activates descending inhibitory circuitry within the brainstem that causes release of inhibitory neurotransmitters within the dorsal horn of the spinal cord to regulate autonomic output responses (3, 26).

Neuronal cell bodies of bladder-innervating afferents are distributed within DRG at TL (T10–L2) and LS (L5–S1) spinal levels, with a predominance within LS DRG and the LS spinal cord (27, 28) (**Figure 1**). The spinal cord terminals of bladder afferents are found predominantly in the lateral spinal nucleus, superficial dorsal horn, and the dorsal commissure (DCN) (27, 28). Interestingly, these regions do not appear to differentiate between non-nociceptive and nociceptive mechanical stimuli and chemical stimuli. The major destination for bladder signals entering the spinal cord is the PAG (28), such that the degree of excitation within the PAG is closely linked to bladder volume, and thus the degree of afferent firing from the bladder. The PAG coordinates ascending excitatory input from the spinal cord with excitatory and inhibitory signals from the ACC, insula, and hypothalamus to provide an overview of the appropriateness to urinate. This is ultimately determined by the prefrontal cortex, allowing conscious control of urination. When a consensus is reached, subsequent activation of the pontine micturition center initiates the switch from a storage to voiding phase, and urination commences (28, 29). A number of studies have also shown that an important pelvic pain pathway may exist in the DCN, involving postsynaptic DCN neurons that project directly to the nucleus gracilis, before continuing on to activate regions of the thalamus and cortex contributing to pain sensation (30).

AFFERENT SUBTYPES INNERVATING THE COLON AND BLADDER

Although the colon/rectum and bladder are innervated by spinal sensory afferents, their physiological properties and anatomical structures are distinct from those innervating the skin (**Figure 1**).

Hence, the traditional nomenclature for subclassifying cutaneous afferents is not appropriate or relevant for visceral organs. Generally speaking, visceral afferents are predominantly peptidergic [calcitonin gene-related peptide (CGRP), NF200, and TRPV1 expressing] C-fibers that display polymodal characteristics to mechanical and chemical stimuli. These afferents express a wide variety of pro- and antinociceptive ion channels and receptors that dictate visceral afferent sensitivity and peripheral drive to the spinal cord (**Figure 2**). This balance can be dramatically shifted during inflammation, or in chronic visceral hypersensitivity (CVH) states, as discussed later in this review.

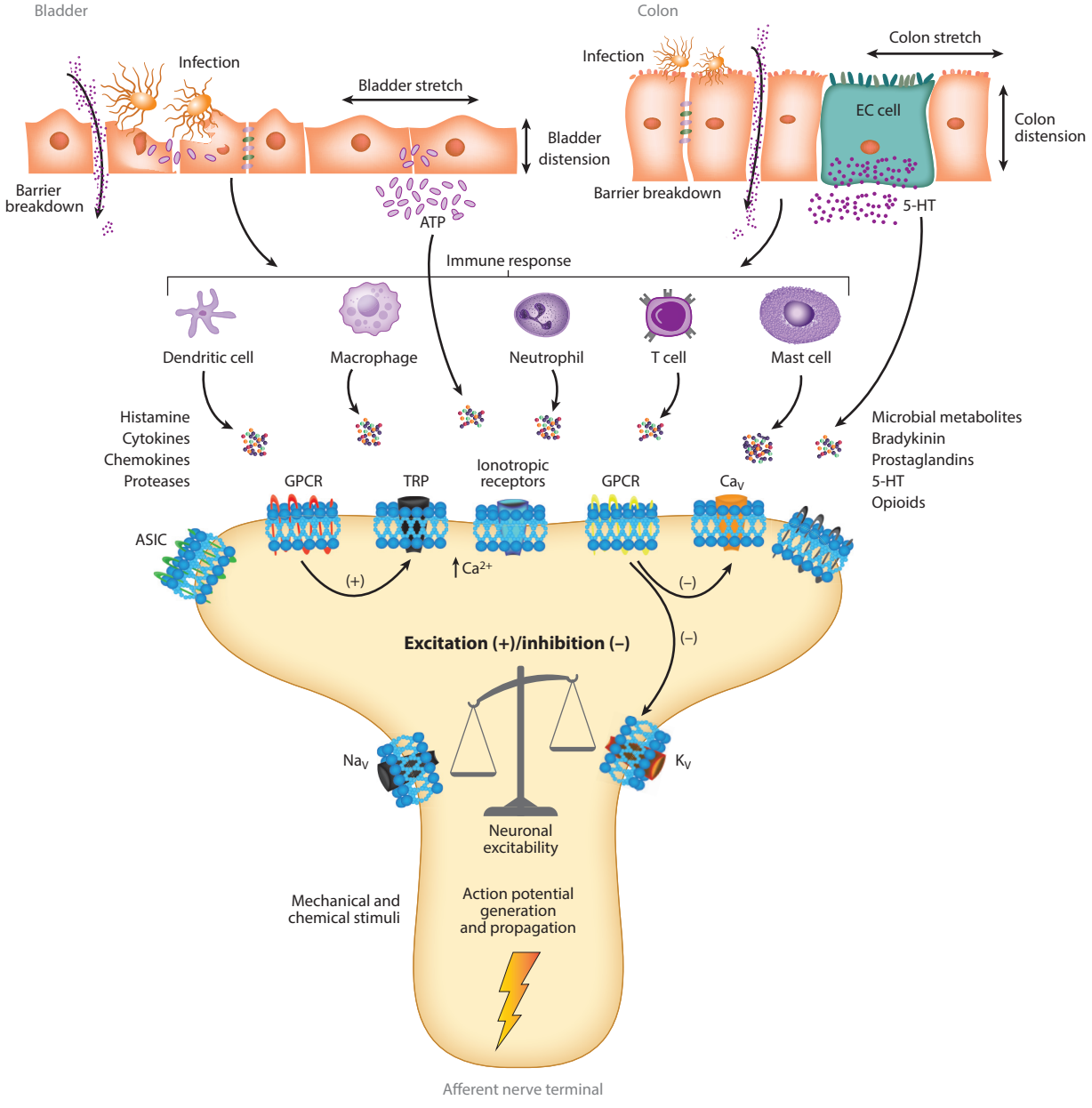

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Figure 2 (*Figure appears on preceding page*)

Schematic overview of the signaling mechanisms that affect visceral afferent sensitivity. The colon and bladder are highly dynamic signaling environments. Both undergo contraction and relaxation, providing direct mechanical activation of sensory afferents. Furthermore, specialized colonic EC cells can be activated by a range of endogenous mediators, including microbial metabolites, resulting in the release of 5-HT, which activates mucosal afferents via synaptic connections. In the bladder, release of ATP from urothelial cells results in subsequent activation of bladder afferents to modulate afferent sensitivity. Both the colon and bladder are susceptible to infection and inflammation resulting in barrier breakdown, allowing intraluminal mediators to access afferent nerve endings. Infection and inflammation also recruit distinct immune processes. These immune cells release a plethora of mediators that can activate and sensitize afferents via an arsenal of ion channels and receptors expressed by afferent nerve terminals. Some of these processes are excitatory (pronociceptive), while some are inhibitory (antinociceptive). The respective balance between the two processes regulates overall neuronal excitability and peripheral afferent drive to the central nervous system. Abbreviations: ATP, adenosine 5′-triphosphate; Ca_V, voltage-gated calcium channel; EC, enterochromaffin; GPCR, G protein–coupled receptor; Kv, voltage-gated potassium channel; Na_V, voltage-gated sodium channel; TRP, transient receptor potential channel.

The generalized nomenclature of visceral afferents therefore reflects their overall function, their mechanical responsiveness to a variety of stimuli, their mechanical activation thresholds, and the layer of gut or bladder they reside in.

Colon

The peripheral projections of spinal afferents innervate all layers of the colon/rectum wall, with 13 morphologically distinct endings identified to date (31). They include various types of mucosal endings, submucosal endings, intraganglionic laminar endings, intramuscular arrays, and afferents that wrap around blood vessels (32–34) (**Figure 1**). These endings correlate with eight different functional classes of afferents found within the splanchnic and pelvic innervations of the mouse colon. Importantly, the first five afferent classes discussed below have been confirmed in recordings from human colon (35–38).

Mucosal afferents. These have very low activation thresholds, are highly sensitive to distortion of the colonic mucosal epithelium, and likely play a crucial role in detecting the particle size of luminal contents (24, 39). These afferents are incredibly rare in the splanchnic nerve (1% of all afferents) but common in the pelvic nerve (15–25% of all afferents) (24, 39, 40). Pelvic mucosal afferents also communicate directly with enterochromaffin (EC) cells via 5-HT acting as an intermediary, whereby 5-HT is released from EC cells in response to norepinephrine acting on α2A-adrenoceptors (Adrα2A) or microbial metabolites, such as isovalerate, acting on Olfr558 receptors expressed on EC cells (41). Mucosal afferents also express TRPA1 and become mechanically hypersensitive during CVH states (39, 42).

Muscular afferents. Muscular afferents respond at low distension thresholds with a wide dynamic range. They represent 6–10% of the afferents in the splanchnic pathway and 16–25% of afferents in the pelvic pathway (24, 39, 40). Muscular afferents signal distension (and contraction) caused by fecal matter in the distal colon and rectum that provides information necessary for coordinating reflex loops and stimulating defecatory pathways (24, 39). These afferents also signal into the noxious range and likely contribute to nociception at high stimulus intensities. Antagonism of the voltage-gated sodium (Na_V) channel subtype $Na_V1.6$ reduces their action potential firing to stretch (43), whilst deletion of TRPV1 or ASIC3 also reduces their functional responsiveness (44).

Muscular/mucosal afferents. Muscular/mucosal afferents respond to both mucosal distortion and circular stretch, and contribute to 16–25% of the afferents within the pelvic pathway (24, 39, 40). Interestingly, these afferents have not been observed in the splanchnic innervation of

the distal colon, suggesting they may play a key role in spinal defecatory circuits and conscious sensation of urge (24, 39). ASIC3 and TRPV1 contribute to their responsiveness to mechanosensory stimuli, and they can undergo long-term sensitization following zymosan treatment (44, 45). Muscular/mucosal afferents can be inhibited by $GABA_B$ receptor agonists or by μ-opioid receptor agonists (46, 47).

Vascular endings. These afferents wrap around blood vessels in the mesentery and submucosa and respond to high-threshold stimuli and a variety of inflammatory and immune mediators (24, 33, 39, 48). Vascular afferents also respond to noxious levels of distension and serve a role in signaling mechanically induced pain (24, 39, 42, 49). Afferents within the submucosa represent ~30% of afferents within both the splanchnic and pelvic pathways (24, 39, 40). Interestingly, mesenteric vascular afferents are exclusive to the splanchnic pathway (28–50% of all afferents) and respond to noxious distension of the colon and to changes in intramesenteric arterial pressure (49, 50). Overall, vascular afferents show the greatest similarity to traditional cutaneous nociceptors. Vascular afferents also display reduced activation thresholds and enhanced responsiveness during colitis and in models of CVH (39, 46, 51–55). Key targets include the Na_V channel isoforms $Na_V1.1$ (55, 56), $Na_V1.8$ (57), and $Na_V1.9$ (58); the voltage-gated calcium (Ca_V) channels $Ca_V2.2$ and $Ca_V2.3$ (46); the voltage-gated potassium (K_V) channel K_V7 (59); and the transient receptor potential channels TRPA1, TRPV1, and TRPV4 (42, 49, 60). In particular, $Na_V1.1$, $Ca_V2.2$, $Ca_V2.3$, TRPA1, and TRPV1 play key roles in contributing to CVH (48). Importantly, numerous inhibitory mechanisms have also been identified on subpopulations of these afferents, including TRPM8 (61) and the oxytocin (OTR) (53), $GABA_B$ (46, 51), κ-opioid (KOR) (54, 62), δ-opioid (63), and μ-opioid receptors (63). For both OTR and KOR, these inhibitory effects only become apparent during inflammation or in CVH states (54, 62), perhaps as a compensatory mechanism in response to reduced abundance of their respective endogenous agonists.

Mechanically insensitive or silent afferents. These constitute ~25% of the afferents innervating the splanchnic and pelvic pathways (40). There are three different types of mechanically insensitive/silent afferents: those chemically activated by inflammatory or immune mediators [including histamine, 5-HT, bradykinin, capsaicin and adenosine 5′-triphosphate (ATP)] that do not subsequently develop mechanical sensitivity; those not chemically activated but mechanically sensitized; and those chemically activated and mechanically sensitized (40, 45, 60, 64). Interestingly, the silent afferents that develop mechanical sensitivity following inflammatory mediator application display the properties of high-threshold vascular afferents. In sensitized states, there is a decrease in the proportion of mechanically insensitive afferents but a corresponding increase in the proportion of mechanically sensitive high-threshold vascular afferents (40). These changes likely contribute to an increased afferent barrage from the periphery in response to distension and contraction, resulting in persistent pain states. Recent studies suggest that silent nociceptors are characterized by the expression of the nicotinic acetylcholine receptor subunit alpha 3 (CHRNA3) and that the mechanically gated ion channel PIEZO2 mediates nerve growth factor (NGF)-induced mechanosensitivity in these neurons (65).

Overall, these different afferent structures, combined with a differing arsenal of ion channels and receptors to regulate their neuronal excitability, underlie the vastly different sensory functions of these afferent classes (7, 48, 66) (**Figure 2**). Molecular expression profiles identified via RNAseq of colon-innervating DRG neurons suggest seven broad classes of colon-innervating afferents (67), which broadly accounts for the functional classes described above. This reflects the different stimuli transduced by these nerves and their overall function, including transmission of nociceptive information by the splanchnic nerves (but also the pelvic nerves) and encoding

of defecatory reflexes by pelvic nerves. Therefore, these subclasses of afferents allow detection of non-noxious physiological stimuli, including luminal events, muscle stretch during organ distension and contraction, as well as noxious mechanical (bloating, intense distension/contraction) and chemical stimuli (7). These afferents are also activated and sensitized by inflammatory and immune mediators, including those acting via P2X (60), P2Y (35), 5-HT_3 (67), histamine 1 (68), PAR_1 (69), PAR_2 (70), bradykinin 1 (64), TNF-α (47), interleukin (IL)-β (47), IL-6 (47), and IL-2 (71) receptors. For further information regarding specific interactions between these ion channels and receptors, please see recent comprehensive reviews (48, 66, 72, 73).

Bladder

In the bladder, spinal afferents have specialized peripheral endings within the urothelium and three distinct types of ending, with branching, simple, or complex morphology in the detrusor smooth muscle (74). These endings broadly correlate with four broad functional classes of afferents found within the bladder.

Muscle mechanoreceptors. These respond to both contraction and distension, acting as tension receptors to accurately determine the degree of bladder stretch during urine accommodation. In the mouse, these afferents represent 30% of the splanchnic pathway and 63% of the pelvic pathway (75). In keeping with the role of these afferents in sensing bladder volume in the physiological range, most mechanosensitive afferents are active at low levels (3–15 mm Hg) of intravesical pressure, when the first sensations of bladder fullness in humans occur. A smaller population of afferents become active only when bladder pressures extend into the noxious range, including those that would induce sensations of urgency, discomfort, and eventually pain (28). Both low- and high-threshold muscle mechanoreceptors can be sensitized by inflammatory mediators (75). Electrophysiology recordings measuring conduction velocity reveal bladder afferents to be either myelinated (Aδ) or unmyelinated (C) fibers. However, in another break with somatic afferent properties, conduction velocity does not correlate with response threshold, with both fiber types demonstrating low or high thresholds to graded distension, as well as the majority of afferents expressing CGRP, NF200, and TRPV1 (17, 76). Interestingly, more low-threshold afferents are capsaicin sensitive than the high-threshold afferents (77, 78). However, the high-threshold capsaicin-sensitive bladder afferents also coexpress TRPA1 (79), whereas TRPV4 is expressed by a population of TRPV1-negative, bladder-innervating neurons (80), suggesting subtype specialization. Therefore, although TRPV4 inhibition alone improves bladder function in cyclophosphamide-induced cystitis (81), coadministration of TRPV4 and TRPV1 antagonists has greater combined effects (80). $P2X_3$ receptor–mediated mechanisms also contribute to both non-nociceptive and nociceptive mechanosensory transduction, with populations of both low- and high-threshold muscular afferents responding to $P2X_3$ agonists (82). Correspondingly, their muscular bladder afferent responsiveness is reduced by $P2X_3$ antagonists (82). Similarly, onabotulinumtoxinA reduces both low- and high-threshold bladder afferent nerve firing (83), which may be a consequence of reduced ATP release from the urothelium.

Urothelial afferents. These are distension insensitive, but their position embedded within or in close proximity to the urothelium indicates a role as sentinels to rapidly detect urothelial breakdown, bladder infection, and inflammation through bidirectional communication with the urothelium and chemical environment. These urothelial afferents are activated by urothelial stroking and various chemical stimuli (75, 78, 84, 85). They have not been found in the splanchnic innervation of the bladder, but they represent ~10% of the pelvic innervation (75).

Muscular/urothelial afferents. These are similar to muscular/mucosal afferents in the colon. They represent 3% of the splanchnic and 14% of the pelvic innervation of the bladder (75). They respond to lower threshold stimuli than bladder muscle mechanoreceptors and respond to both mechanical stretch and light stroking of the urothelium. These afferents can be activated by ATP (78), and thus their location close to the urothelium suggests that they might also respond to ATP released by urothelial distension (86).

Serosal afferents/mechanically insensitive silent afferents. Serosal afferents are distension-insensitive afferents that can be activated by punctate stimulation of their receptive fields. They represent 67% of the splanchnic innervation but only 14% of the pelvic innervation of the bladder (75). They are called serosal afferents, as their physiological properties resembled afferents recorded within the colon. However, in the colon at least, serosal afferents have been subsequently associated with blood vessels innervating the submucosa and termed vascular afferents (see above). Meanwhile, the anatomical structure of serosal afferents within the bladder remains unclear. Some serosal afferents have likely been termed mechanically insensitive silent afferents in a number of previous studies, because like those found in the colon, they are unresponsive to distension under normal conditions but become activated following irritation or inflammation (82). This includes activation and sensitization via P2X receptors (82).

Persistent Changes in Visceral Sensory Afferent Pathways

Overall, most colonic and bladder afferents show exquisite sensitivity for mechanical distension or luminal distortion, enabling them to detect physiologically relevant stimuli and provide the afferent component of spinal and extraspinal reflexes. High-threshold afferents provide further resolution between normal and nociceptive stimuli, which result in visceral sensations ranging from discomfort, urgency, and ultimately to pain. Sensitization of these afferents, particularly high-threshold, distension-sensitive and vascular afferents, plus the recruitment of mechanically insensitive afferents, leads to an increase in sensory signaling from the periphery to the spinal cord. In many situations this sensitization resolves following resolution of the initial pathology (inflammation or physical challenge) (7). However, in CVH, this sensitization does not resolve and can be exacerbated for months after the initial infectious or inflammatory insult. This leads to persistent neuroplasticity (synaptic or intrinsic changes of afferent endings) that may drive subsequent changes in downstream sensory pathways (7). For example, in the colon during CVH there is evidence of chronic afferent ending sensitization (39, 46, 51–55), hyperexcitability of colon-innervating DRG neurons (51), and increased activation of dorsal horn neurons within the spinal cord in response to noxious distension (25). The latter is also accompanied by sprouting of the central terminals of colonic afferents, which normally reside predominantly within laminae I (LI) and V (LV) and project down the middle and lateral dorsal horn collateral pathways (25). However, during postinflammatory CVH, there is an increased density and more widespread distribution of terminals within LI, with terminals now also present within deeper laminae. Such changes are also accompanied by activation of dorsal horn neurons within deeper laminae of the spinal cord in response to noxious stimuli (25). A key driver of persistent changes following colitis is granulocyte colony–stimulating factor (G-CSF) signaling in spinal microglia, as ablating microglia or blocking the G-CSF receptor prevents colitis-induced sensitization (87).

In relation to the bladder, cyclophosphamide-induced cystitis causes hyperexcitability of bladder-innervating DRG neurons (88) and evokes bladder overactivity in addition to allodynia and hyperalgesia to bladder distension (89–91), thereby mimicking the symptoms of IC/BPS. Consequently, a key concept in visceral pain research is the switch from a healthy environment to

a sensitized acute pain state and progression toward a recurrent sensitization and CVH state. We discuss the key contributing factors below (**Figure 3**).

Neuroepithelial Interactions

The colon and bladder epithelia provide a key interface between luminal contents and the underlying sensory afferents. In the colon, a particular type of epithelial cell, called EC cells, are electrically excitable and act as sentinels of noxious chemical stimuli or other insults (41). EC cells are polymodal chemosensors that respond to specific mediators, including TRPA1 agonists; the microbial metabolites isovalerate, isobutyrate, and butyrate; and dopamine, epinephrine, and norepinephrine (41). This results in the basolateral release of 5-HT, via a TRPC4-dependent mechanism. 5-HT then acts on 5-HT_3 receptors expressed on pelvic mucosal afferents, resulting in mechanical sensitization (41). Most 5-HT in the body is synthesized, stored, and released by these EC cells. Alterations in the fundamental properties of 5-HT signaling, including synthesis, release, or reuptake of 5-HT, may play a significant role in the development of visceral pain in IBD and IBS (92). Although effective, 5-HT_3 receptor antagonists, such as alosetron, are only approved for treating very specific cohorts of IBS patients, due to incidences of adverse effects, including ischemic colitis (93). Voltage-gated sodium currents generated by $Na_V1.3$ likely allow EC cells to respond to the detection of mechanical and chemical stimuli within the lumen of the colon (41, 94) and may provide a novel therapeutic target for treating pain symptoms.

The bladder urothelium expresses numerous receptors and ion channels, including TRPV4, TRPV2, and TRPM8 (95), and secretes a range of mediators that are capable of modulating sensory afferents and inflammatory cells. Under physiological conditions, the urothelium is not thought to come into direct contact with urine, as it is protected by the hydrophobic glycosaminoglycan (GAG) layer. However, under pathological conditions, such as bacterial infection, the urothelium is exposed and can provide input to autonomic reflexes regulating micturition. ATP has been the most extensively studied urothelial release factor, and there are several reports providing evidence of dysfunction in the urothelial/sensory afferent purinergic signaling complex. Knockout of $P2X_3$ and $P2X_{2/3}$ receptors in mice reduces bladder mechanosensitivity by limiting P2X activation on afferents in response to urothelial ATP release (96). Urothelial release of ATP from patients with IC/BPS is enhanced compared to controls, which may be a result of reduced ATP metabolism. As such, there has been significant interest in this pathway for the development of novel analgesics for IC/BPS patients (97); however, pharmacological exploitation of this pathway has not been forthcoming in clinical trials. An alternative therapeutic intervention is the intramural injection of botulinum toxin type A (BOTOX), which does show efficacy in treating visceral hyperalgesia and is the fourth-line treatment for IC/BPS (98, 99). BOTOX acts by preventing vesicular release of neurotransmitters and was originally proposed to act by blocking parasympathetic-mediated acetylcholine release at the neuromuscular junction. However, a recent study showed that BOTOX, instilled into the bladder during graded distension, significantly reduced bladder afferent mechanosensitivity and luminal ATP release without influencing bladder compliance (83). BOTOX administration also increased luminal release of nitric oxide, which acts as an inhibitory transmitter on bladder afferents (100).

Epithelial Breakdown

As the colon and bladder are responsible for storing and eliminating toxic waste metabolites from the body, the epithelial lining of these organs provides an essential barrier between luminal contents and the underlying interstitium. A leaky gut refers to increased permeability of the luminal

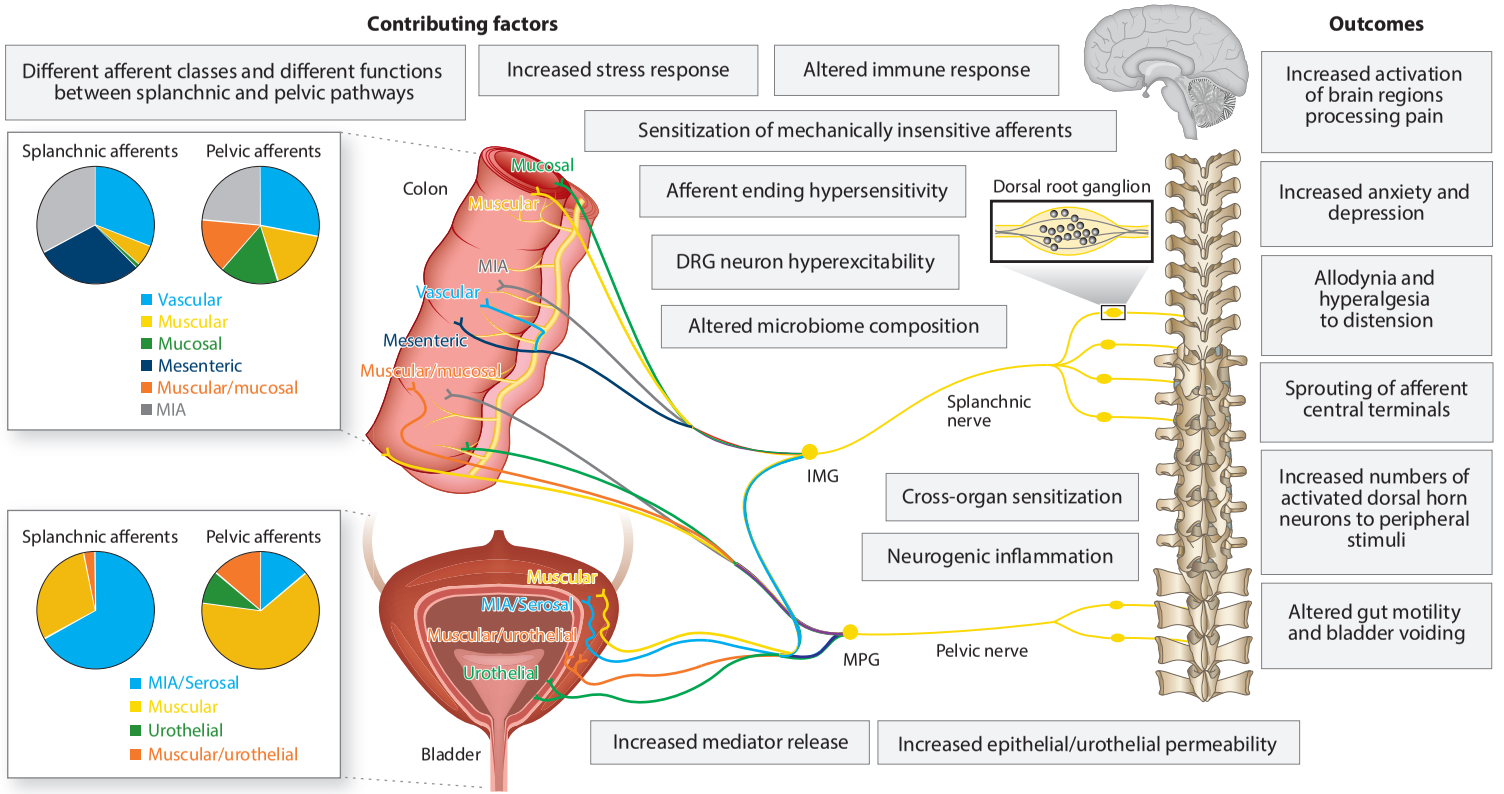

Figure 3

Schematic overview of the major factors contributing to chronic visceral pain from the colon and bladder. Many visceral pain disorders, such as irritable bowel syndrome or painful bladder syndrome, present without an obvious underlying cause, such as a pronounced epithelial/urothelial inflammation at the time of referral to generalist or specialist practitioners. The schematic highlights numerous contributing factors and their resultant outcomes that produce chronic abdominal and pelvic pain. For example, in some cases, inflammation or infection is the underlying trigger; however, following healing of the tissue, neuroplasticity in pain pathways exists for several years following the initial insult, resulting in chronic pain. In other cases, there is evidence for altered immune responses, while stress can trigger and also exacerbate symptoms. Cross-organ sensitization can allow alterations in neuronal signaling in one organ to subsequently alter the adjacent organ, due to their common neural innervations. Abbreviations: DRG, dorsal root ganglia; IMG, inferior mesenteric ganglion; MIA, mechanically insensitive afferent; MPG, major pelvic ganglion.

epithelial layers of the intestinal mucosa, such that luminal content gains unregulated access to underlying tissues. Increased gut permeability can be caused by numerous factors, including high-fat or high-fructose diets, alcohol ingestion, vitamin A deficiency, and changes in the intestinal microbiome. A leaky gut can cause pain due to access of sensitizing agents from food consumption and substances released from the microbiome to afferents that innervate the tissue. Patients with IBD, IBS, and celiac disease have increased gut permeability, which may contribute to increased pain signaling (1, 7, 8). Although pharmacologically improved cell–cell adhesion of the intestinal epithelial layer can decrease permeability, further research on whether it can improve visceral pain is needed (101).

A key component of the urothelial barrier is the GAG mucus layer, composed of sulfated polysaccharides, that has hydrorepellent properties, which block the movement of small molecules and urine access to the underlying tissue. Upon histological examination, many, but not all, IC/BPS patients show a diminished urothelium or loss of umbrella cells and GAG layer. It remains to be determined if bladder permeability is part of the underlying pathology of visceral hypersensitivity or a downstream consequence of an inflammatory process. However, a positive response to the potassium sensitivity test, in which high potassium is infused into the bladder, is indicated by allodynia and hyperalgesia to bladder distention and is reported by >80% of IC/BPS patients, as well as patients diagnosed with chronic pelvic pain (102). The use of oral pentosane polysulphate therapy to repair the GAG layer is the only treatment for IC/BPS approved by the US Food and Drug Administration. Pentosane polysulphate induces broad anti-inflammatory actions, including the inhibition of mast cell histamine release in the bladder (103, 104), which may provide an additional mechanism of action to alleviate IC/BPS symptoms.

Infection and Inflammation

The importance of visceral afferents in pain sensation and homeostatic regulation is exemplified during acute bacterial infections, whereby physiological responses enhance gastrointestinal motility and urinary frequency to rapidly eliminate the pathogen from the body. Inflammation manifests as a local influx of immune cells, release of proinflammatory mediators, edema, and pain sensation via sensitization of sensory neurons innervating the affected tissue. Inflammatory mediators, such as NGF, TNF-α, bradykinin, substance P, histamine, prostaglandins, and ATP, activate specific receptors on sensory afferents, which leads to a localized membrane depolarization (7, 17, 48). The change in membrane potential can be sufficient to activate voltage-gated ion channels, leading to action potential generation and transmission to the central nervous system (48, 66, 105). Recurring episodes of visceral inflammation can lead to long-term neuroplasticity within the peripheral and central nervous systems, which can contribute to development and maintenance of CVH (7, 17, 48).

Altered Immune Responses

Recent studies utilizing clinical samples show that subgroups of IBS patients display altered immunological function (47, 106–108). For example, colonic biopsies of IBS patients have increased numbers of $CD3^+$ T cells and mast cells versus control biopsies (106, 109, 110). Colonic biopsies from IBS patients also display increased release of key mediators, including histamine, tryptase, trypsin-3, and the proinflammatory cytokine IL-1β, which can correlate with the severity and frequency of abdominal pain (109–111). Histamine, proteases, and IL-1β can all act on receptors expressed by colonic afferents to cause sensitization via TRPV1-, TRPV4-, and $Na_V1.7$-dependent mechanisms (47, 48, 68, 69). Correspondingly, supernatants from IBS patient biopsies, but not

healthy controls, cause activation of colonic afferents (110). Immune changes in IBS patients are also apparent in peripheral blood mononuclear cells (PBMCs) (47, 107, 112). The proinflammatory cytokines, TNF-α, IL-1β, and IL-6, are all increased in PBMC supernatants from IBS-D patients, which correlates with symptoms of abdominal pain (47, 112). Conversely, immune-derived opioidergic inhibition is decreased in IBS patients, with monocyte-derived β-endorphin levels and colonic macrophage numbers lower in IBS patients than controls (107). The latter represents the loss of a key antinociceptive mechanism, as preclinical studies show that immune-derived endogenous opioids play a key role in modulating visceral hypersensitivity (113–115).

Neurogenic Inflammation

In addition to their sensory role, visceral afferents also exhibit an efferent function, as the vast majority of afferents innervating the colon and bladder are peptidergic. Accordingly, they produce and release neuropeptides such as substance P, CGRP, neurokinin A, and neurokinin B to enhance sensory fidelity. However, under the correct conditions, they promote local inflammation, termed neurogenic inflammation, which promotes vasodilatation and plasma extravasation, leading to immune cell infiltration. Although neurogenic inflammation can be a beneficial process during naïve states, neuronal hypersensitivity in CVH states may result in persistent neurogenic inflammation.

Stress and the Gut–Brain and Bladder–Brain Axes

As described above, the processing of sensory stimuli from visceral organs requires neural pathways linking autonomic afferent excitability with emotional and cognitive centers in the brain. These core neural circuits are also dependent on neuroendocrine, immune, and sensory afferent integration. Together, these processes undergo bidirectional communication to regulate visceral homeostasis via a brain–gut or brain–bladder axis. The evolutionary development of the fight or flight response relies on activation of the sympathomedullary axis and the hypothalamic–pituitary–adrenal (HPA) axis during stressful situations. Corticotrophin-releasing factor (CRF) initiates the HPA axis by binding to CRF_1 receptors (CRF_1Rs) in the anterior pituitary and leads to subsequent release of cortisol from the adrenal cortex in preparation for a physical or psychological challenge. However, CRF and CRF_1R are also widely distributed in various brain regions, including those linked with anxiogenic and digestive behaviors (116). As such, a common human reflection on the physiological perception of acute stress or anxiety is the presence of butterflies in the stomach and the need to urinate (117).

However, maladaptive responses to physical or psychosocial stress represent a common risk factor for visceral pain disorders. In preclinical rodent studies employing acute and chronic stress, early life stress, and high anxiety, Wistar-Kyoto rats exhibit hyperalgesia to colorectal distension and altered micturition (117–119). Similarly, CRF injections induce allodynia and hyperalgesia to colorectal distension and bladder overactivity (120–122), while CRF_1R antagonism prevents visceral hyperalgesia induced by acute and chronic stress (123, 124). From a clinical point of view, symptoms of IBS, IBD, and IC/BPS patients are enhanced during periods of stress and exhibit significant overlap with psychiatric disorders such as anxiety and depression and posttraumatic stress disorder, as well as a history of early life stress/trauma (118, 125–128). In support of centrally mediated visceral pain mechanisms, translational studies identify altered brain activation patterns in response to nociceptive stimuli in IBS, IBD, and IC/BPS patients suggestive of central sensitization (129–132). These findings provide strong evidence that stress can cause or exacerbate visceral sensation. However, despite these obvious connections, there has been considerable

translational failure of CRF_1R antagonists owing to persistent side effect profiles that are likely the result of ubiquitous expression of the CRF_1R throughout the body and brain (133).

Cross-Organ Sensitization Between the Colon and Bladder

Coordination of afferent signals between the bladder and colon is essential for efficient and synchronized bladder and bowel evacuation. However, it is now apparent that disease of one visceral organ can induce the development of pathology in an adjacent, otherwise unaffected organ (11). As such, this pathological occurrence is thought to underlie the comorbidity of a number of visceral pain disorders, including but not limited to IBS, IBD, and IC/PBS (134). Preclinical research has consistently implicated cross-organ sensitization of common afferent pathways as a key player in this phenomenon (11). This is because both bladder and colonic afferents innervate the same levels of the DRG and spinal cord. Several studies have shown that colitis can also induce hypersensitivity of bladder afferent pathways (135, 136), with up-regulation of brain-derived neurotrophic factor in the DRG being a key contributing factor (137). This phenomenon has classically been explained by viscero-visceral convergence of afferents within the DRG and spinal cord, such that sensitization of one axon induces sensitization of all convergent axons. However, the proportion of dichotomizing afferents within the DRG and convergent neurons within the spinal cord remains relatively small, approximately 10–20% of the total population of bladder or colonic afferents (11). Furthermore, lumbosacral DRG and spinal neurons with axons innervating only the bladder also display neuronal hypersensitivity following chemically induced colitis (135). These findings therefore suggest a more widespread sensitization of the entire visceral afferent network as a result of neuroplasticity. Because the central terminals of colonic afferents exhibit sprouting within the dorsal horn following recovery from colitis (25), an intriguing possibility exists that additional input onto convergent visceral axon terminals occurs in CVH states.

Gut Microbiome

One key factor that can potentially integrate the mechanisms described above is the gut microbiome, which comprises trillions of microbes from a wide diversity of species. Their potential influence in visceral pain is profound, as they and their metabolic products influence intestinal permeability, immune function, the gut–brain axis, and visceral pain responses (138, 139). Consequently, dysbiosis (alterations in intestinal microbial composition) has been linked to IBS and IBD, and more recently, to conditions outside of the gut, including, stress, anxiety, and cognition (140, 141).

Early indicators of the role the microbiome plays in colonic hypersensitivity arose from studies utilizing antibiotic and probiotic treatments. In these studies, mice treated with antibiotics had altered gut microbiome composition, increased inflammatory markers, and visceral hypersensitivity to colorectal distension (142). Treatment of these mice with the probiotic *Lactobacillus paracasei* normalized colonic hypersensitivity (142), while other studies have shown that *Lactobacillus acidophilus* reduces colonic hypersensitivity via opioid and cannabinoid receptor mechanisms (143). Furthermore, decreasing *Lactobacillus* abundance and butyrate production also exacerbates colitis in mice (144). In terms of possible direct interactions between bacteria and afferent nerves, recent studies of cutaneous afferents demonstrate that *Staphylococcus aureus* can directly activate DRG neurons via the pore-forming toxin α-haemolysin (145). Other studies show that different bacterial species can suppress DRG neuron excitability through activation of PAR-4 (146). Similarly, a lipopeptide produced by the probiotic *E. coli* strain Nissle 1917 directly inhibits DRG neurons and reduces visceral pain by crossing the epithelial barrier and activating $GABA_B$ receptors

expressed on afferents (147). Overall, these findings suggest that the microbiome can alter colonic afferent sensitivity via their roles in barrier function, immune responses, as well as metabolite-epithelial-neuronal, metabolite-neuronal, or bacterial-neuronal interactions. Microbial dysbiosis likely changes the balance of these mechanisms, resulting in persistent sensitization via a variety of mechanisms.

Numerous studies have associated symptoms in IBS patients to microbial dysbiosis, in particular, the decreased abundance of *Lactobacillus* and *Bifidobacterium* species and increases in Firmicutes and Bacteroidetes (139, 148, 149). Interestingly, changes in gut microbial composition correlate with differential activation of brain regions in IBS patients (149), which likely reflects altered emotional processing of sensory input. The relevance of the microbiome to these clinical conditions is also highlighted by recent studies transferring abnormal human phenotypes to mice, whereby transplantation of fecal microbiota from IBS-D patients into germ-free mice results in altered gastrointestinal transit, intestinal barrier dysfunction, innate immune activation, and anxiety-like behavior in the recipient mice (150). Hence, pre- and probiotics are of therapeutic interest in the treatment of IBS (141). However, recent studies also suggest that dysbiosis in IBS and IBD is not only limited to bacterial species but extends to both viral and fungal species (151, 152). Indeed, the correct composition of these respective organisms is deemed so important for gastrointestinal function that fecal microbiota transplantation to IBD patients with recurrent *Clostridium difficile* infection is offered as a genuine therapeutic option (153).

Bladder Microbiome

The recent identification of a bladder-specific microbiome has triggered links between the balance of bacteria in the bladder and the symptoms of IC/BPS (154, 155). Patients with bladder symptoms may have genuine infections but are misdiagnosed, as large numbers of bacteria are undetected by routine midstream urine cultures (156). Changes in bacterial species that make up the bladder microbiome have been associated with the presence and severity of IC/BPS (154, 157, 158). Recent studies have identified women with IC/BPS as having a less diverse microbiota than normal subjects (159, 160). Despite significant interpatient variability in bladder microbiomes, a decrease in *Lactobacillus* in IC/BPS patients compared to controls is a common finding (160), with an absence of *L. acidophilus* associated with higher pain scores and higher scores on the interstitial cystitis symptom index (159).

CONCLUSIONS

As discussed within this review, chronic visceral pain is an extremely common symptom experienced by >20% of the global population. Chronic visceral pain is debilitating for those afflicted individuals and has considerable economic burden, costing >$65 billion per annum in the United States for IBS, IBD, and IC/BPS. The mechanisms contributing to chronic visceral pain are incredibly complex and involve a range of processes from the level of the microbiome to the brain. The mechanisms and therefore the etiologies of these clinical conditions are further complicated by cross talk between adjacent visceral organs. Therefore, future studies will need to study underlying mechanisms and potential treatment strategies at a holistic level.

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