Chapter 9
Altered Ion Channel/Receptor Expression and Function in Extrinsic Sensory Neurons: The Cause of and Solution to Chronic Visceral Pain?

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Introduction

Neural control of gastrointestinal function is a highly integrated system, comprised of distinct populations of neurons, whose cell bodies are either intrinsic or extrinsic to the gut wall. Neural control involves interactions between; (1) local enteric reflexes within the gut wall; (2) reflexes that pass through prevertebral sympathetic ganglia and (3) reflexes that pass to and from the gut via the central nervous system (CNS) (Furness 2012). To add further intricacy the gastrointestinal tract is an incredibly complex signalling environment. Neurons are subjected mechanical events such as distension and contraction, whilst being inundated with a constantly changing milieu of endogenous mediators (Brierley and Linden 2014). Inflammation of the gut, either through abnormal immune responses or via gut infection has been consistently demonstrated to cause neuroplasticity and abnormal neuronal function. These profound effects result in disregulated neuronal signalling, abnormal secretion, motility and sensory signalling resulting in the development of diarrhea, constipation, discomfort and pain. The importance of this neuroplasticity is highlighted in a number of highly prevalent organic and functional gastrointestinal disorders. In organic disorders such as Inflammatory Bowel Disease (IBD), which includes Crohn's disease and Ulcerative Colitis, chronic uncontrolled inflammation of the

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intestinal mucosa is recognised as the pathogenesis of neuronal dysfunction and correspondingly the presentation of symptoms (Kaser et al. 2010; Nell et al. 2010). However, for functional bowel disorders such as Irritable Bowel Syndrome (IBS), where macroscopic mucosal damage is not evident, but symptoms of persistent abdominal pain, discomfort and abnormal bowel function are evident (Brierley and Linden 2014). The underlying source of this neuronal deregulation remained unclear, until the recent association with infectious gastroenteritis. Numerous clinical studies have attributed IBS symptom development to a preceding bout of gastroenteritis induced by pathogens such as *Campylobacter* (Spiller et al. 2000; Thabane et al. 2010), *Escherichia coli* (Thabane et al. 2010), *Salmonella* (Dunlop et al. 2003), *Giardia lamblia* (Hanevik et al. 2009). Whilst IBS is multi-factorial and several additional risk factors may also be required for development (Dinan et al. 2010; Ohman and Simren 2010), acute gastroenteritis can trigger IBS symptoms that persist for at least 8 years (Marshall et al. 2010). In the relatively short term setting of tissue damage, inflammation is a protective process which facilitates wound healing, however these clinical findings suggests that in these individuals the neuroplasticity induced by infection and inflammation, fails to reset back to normal long after healing of the intestinal tissue. As increasing effort has been directed towards determining the extent of neuroplasticity that is associated with gut disorders then the number of different models used to investigate it has also increased. Experimental models of gut inflammation have included administration of dextran sodium sulphate (DSS), chemical irritants such as mustard oil and acetic acid, infection with nematodes (e.g. *Trichinella spiralis*) or bacteria (e.g. *Citrobacter rodentium*), and haptens such as trinitrobenzene sulphonic acid (TNBS). However, the time course and nature of the resultant inflammation is different between these models and is defined by the different categories of immune cells involved in the response (Antalis et al. 2007; Fasano and Shea-Donohue 2005). For example TNBS combines with endogenous proteins and antigens to evoke a transmural Th1-mediated inflammation, whilst DSS is more dependent on innate immunity and is restricted to the mucosa. Furthermore, zymosan which unlike the models described above, does not induce an increase in myeloperoxidase (MPO) activity at any time after intracolonic treatment, does result in a brief monocyte-based inflammation (Feng et al. 2012a). However, despite these differences an increasing amount of data suggests neuroplasticity can occur inextrinsic sensory afferent neurons, their peripheral and central projections and their resultant communication with the CNS. These changes are likely to further alter communication along the brain-gut axis, and have a profound effect on resultant neuronal plasticity. Given this complexity and that understanding of many of these interactions remain in their infancy; this review will focus on the resultant effects of gut inflammation on neuronal function in the gut-brain pathway and the persistent long term neuroplasticity than remains following resolution of inflammation (Fig. 9.1). Where apparent this review will also highlight the channels, receptors and mediators involved in this process.

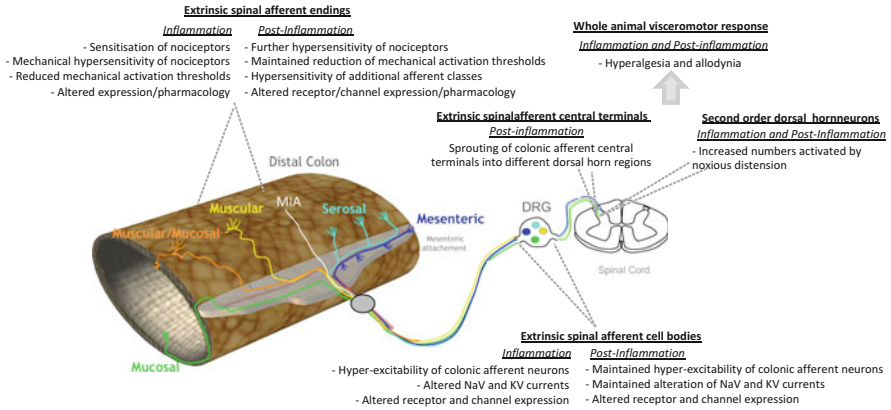

Fig. 9.1 Neuroplasticity in extrinsic sensory afferent pathways during and following resolution of gut inflammation. During inflammation nociceptive sensory afferent endings are hypersensitive, are activated at lower stimulus intensities and displayed enhanced mechanical responsiveness, whilst their cell bodies in the DRG also display hyperexcitability. This translates to increased activation of dorsal horn neurons in the spinal cord and in whole animal studies enhanced pain responses to colorectal distension. Many of these changes are still present or are even enhanced following resolution of inflammation. Nociceptive sensory afferent endings now display even greater mechanical hypersensitivity, and their cell bodies in the DRG remain hyper-excitability. An increased density of colonic afferent central afferent terminals is now evident, as is sprouting of these terminals into different regions of the dorsal horn of the spinal cord. This plasticity results in greater numbers of second order dorsal horn neurons in the spinal cord being activated in response to noxious colorectal distension. There is evidence of enhanced pain responses to colorectal distension, which can be dependent upon the experimental model used and influenced by the severity of the initial insult

Extrinsic Sensory Afferent Pathways Innervating the Gastrointestinal Tract

Distinct from the enteric and sympathetic nervous systems are the extrinsic sensory innervations of the gastrointestinal tract. These pathways have become one of the most intensely studied areas of neuro-gastroenterology, as they are the first step in generating sensations. In particular they are responsible for signaling nociceptive stimuli from the gut, and ultimately the conscious perception of pain. Therefore, identifying the afferents, mediators and mechanisms involved in this process is crucial in understanding the mechanisms of neuroplasticity underlying inflammatory and chronic visceral pain. Most studies have focused on the innervations of the small intestine, colon and rectum, as these regions are associated with the symptoms of IBD and IBS. The complexity of this intact system means that many of its individual components; afferent endings in the gut wall, cell bodies in the DRG, activation of pathways in the spinal cord and the overall pain-related behavior to gut distension (visceromotor response to colorectal distension) have been studied

independently, either in vitro or in vivo. These studies indicate that mechanisms underlying inflammatory and chronic post-inflammatory visceral pain are varied, but originate from changes in the periphery (Barbara et al. 2002, 2004, 2007; Liebregts et al. 2007; Spiller and Garsed 2009). The peripheral endings of particular afferent subtypes feed into nociceptive pathways within the spinal cord and pain sensing regions in the brain. Whilst pain is an emotive process, the threshold of nociceptors has to be high enough not to interfere with normal physiology, but low enough that it can be evoked before marked tissue damage occurs (Costigan et al. 2009). In order to achieve this function nociceptive nerve endings express a variety of ion channels and receptors, which regulate neuronal excitability and transduce mechanical or chemical stimuli (Beyak 2010; Beyak and Vanner 2005; Blackshaw et al. 2010; Brierley 2010; Brierley and Kelber 2011). To add further complexity there are several schools of thought regarding the types of afferent that contribute to nociceptive signaling and therefore inflammatory and chronic pain. These subtypes include low- and high-threshold afferents, and mechanically insensitive 'silent' afferents. However, by definition nociceptors selectively respond to noxious or potentially tissue damaging stimuli and can be sensitized, or increase their excitability in response to tissue insult or inflammation.

Neuroplasticity in Extrinsic Sensory Afferent Pathways Innervating the Gut

It is clear that experimentally induced inflammation or infection causes afferent hypersensitivity, neuronal hyper-excitability and correspondingly hyperalgesia and allodynia in whole animal models. Consistent findings of neuroplasticity have been most apparent when studying isolated neuronal cell bodies across different regions of gut and across different experimental models. Most studies utilizing inflammatory (TNBS), nematode (*T. Spiralis, Nippostrongylus brasiliensis*) or bacterial models (*Citrobacter rodentium*) show that neurons innervating the stomach (Dang et al. 2004; Gebhart et al. 2002; Bielefeldt et al. 2002a, b), small intestine (Hillsley et al. 2006; Moore et al. 2002; Stewart et al. 2003; Keating et al. 2008) and the colon (Beyak et al. 2004; Ibeakanma et al. 2009; King et al. 2009) display pronounced hyper-excitability after the initial insult. This hyper-excitability is characterized by a decreased threshold for activation, increased firing rate, increases in TTX-resistant Na_V currents and suppression of K_V, I_A and I_K channels. Recent reports indicate a crucial role for $Na_V1.8$ in colonic innervating DRG neurons, with its expression differentially regulated across different time points during colitis (King et al. 2009). Furthermore, *Nippostrongylus brasiliensis* induced jejunal neuronal hyper-excitability is lost in $Na_V1.8$-/- mice, but not $Na_V1.9$-/- mice (Hillsley et al. 2006). Longer term neuroplasticity is also evident as K_V, I_A and I_K currents are reduced in colonic innervating DRG neurons 10 days post-*Citrobacter rodentium* infection, whilst suppression of K_V and I_A currents contributes to neuronal hyper-excitability 30 days post-infection (Ibeakanma et al. 2009).

Neuroplasticity of peripheral sensory afferent endings is also evident across a range of different experimental models; however different afferent subtypes, different neuronal pathways and time courses are involved in this process. For example, following *T. spiralis* infection both low- and high-threshold jejunal afferents initially display significant *reductions* in mechanosensitivity at 14 days post-infection. However, at 28 and 56 days post-infection pronounced mechanical hypersensitivity is now evident (Keating et al. 2008). The development of this longer term mechanical hypersensitivity is dependent upon a $P2X_7$ receptor-dependent increase in immune cell IL-1β expression and release. Notably these $P2X_7R$ -/- animals display a clear attenuation of the innate inflammatory response and no post-infectious mechanical hypersensitivity at any time point (Keating et al. 2011).

DSS-induced colonic inflammation does not induced afferent mechanical hypersensitivity (Coldwell et al. 2007), or short or long term hyperalgesia in response to colorectal distension (Larsson et al. 2006). However, DSS treated animals display increased visceral sensitivity to capsaicin and 5-HT (Larsson et al. 2006; Eijkelkamp et al. 2007). By contrast, TNBS induced colitis causes high-threshold nociceptors to become mechanically sensitized, have reduced activation thresholds, and display hypersensitive responses in inflammatory and post-inflammatory states (Hughes et al. 2009a, b). This hypersensitivity is particularly apparent in splanchnic afferents with high mechanical activation thresholds, which is partially mediated by TRPA1 (Brierley et al. 2009). A potential contributing factor is also a reduction in the mechanosensitive K2P channels TREK-1 and TREK-2, as these hyperpolarizing K^+ channels are significantly reduced in splanchnic and pelvic colonic DRG neurons during TNBS inflammation (La and Gebhart 2011). The extent of this mechanical hypersensitivity in high threshold afferents is greater following recovery from overt tissue damage (28 days post-TNBS) (Hughes et al. 2009a, b). This hypersensitivity translates to an increased density and sprouting of colonic afferent central terminals in the thoracolumbar spinal cord and an increased number of activated DH neurons in the spinal cord in response to noxious colorectal distension (Harrington et al. 2012). In contrast, the same investigators have shown TNBS induced mechanical hypersensitivity is not evident during inflammation in afferents with low-thresholds (mucosal, muscular and muscular/mucosal). However, pelvic high-threshold and mucosal afferents only become hypersensitive post-inflammation (Hughes et al. 2009a, b). Other studies have shown transient, absent or inconsistent effects of TNBS-induced inflammation on low-threshold distension-sensitive afferents (De Schepper et al. 2008a; Lynn et al. 2008; Sengupta et al. 1999; Feng et al. 2012b) and transient hypersensitivity during in vivo colorectal distension studies (Lamb et al. 2005). The apparent discrepancy of these findings with TNBS may relate to the severity of mucosal inflammation, which is a predictor for alterations of visceral sensory function in rodents (Adam et al. 2006) and in humans. However, acute zymosan treatment, which recruits a different immune response, does lead to low-threshold sensitive afferents displaying short and long term hypersensitivity (Feng et al. 2012a, b), which is partially dependent on TRPV1 (Jones et al. 2007), ASIC3 (Jones et al. 2007) and P2X receptors (Shinoda et al. 2010). Inflammatory mediators, TNBS and zymosan treatment can also activate or sensitize two different types of mechanically insensitive afferents (MIAs), also known as ‘silent afferents’.

One population is silent, responds to chemical stimuli, but doesn't subsequently display mechanosensitivity (Brierley et al. 2005a, b), whilst the other population is sensitized by mediators and develops mechanosensitivity (Feng and Gebhart 2011). The proportion of this second type of MIA is increased in a number of inflammatory and post-inflammatory states (Feng et al. 2012a, b). Another model utilizing intracolonic administration of deoxycholic acid, an unconjugated secondary bile acid, induces a mild, transient colonic inflammation within 3 days, which resolves within 3 weeks. This causes exaggerated visceromotor responses to colorectal distension, referred pain to mechanical stimulation, and increased dorsal horn neuron activity, which persists for at least 4 weeks (Traub et al. 2008).

Various stress models have been shown to increase visceral pain sensitivity (Larauche et al. 2012; Winston et al. 2010). However, stress, combined with prior acute colitis induced by *C. rodentium*, results in exaggerated peripheral nociceptive signaling of colonic afferents, their cell bodies and correspondingly visceromotor reflex thresholds via protease, β-2 adrenergic, glucocorticoid receptor and PAR2 mechanisms (Ibeakanma et al. 2011). However, such an interaction does not occur with stress and DSS treatment (Larsson et al. 2009), which again may suggest specific neuroimmune interactions in the development of neuroplasticity and chronic colonic hyperalgesia.

One of the most consistent and long-term displays of visceral neuroplasticity occurs following neonatal insult. In these cases neonatal animals receive either mechanical or chemical colonic irritation between post-natal days 8 and 21 and are then tested when they are adults (Al-Chaer et al. 2000). Colonic irritation in neonates results in chronic visceral hypersensitivity, allodynia and hyperalgesia, associated with central neuronal sensitization, in the absence of identifiable peripheral abnormalities. Evidence exists for TRPV1 (Jones et al. 2007; Hong et al. 2009; Winston et al. 2007) and TRPA1 (Christianson et al. 2010) initiating colonic hypersensitivity and TRPV1 (Winston et al. 2007), P2X (Xu et al. 2008) and TRPA1 (Christianson et al. 2010) maintaining colonic hypersensitivity induced by neonatal acetic acid or mustard oil colonic irritation. More recent studies indicate similar mechanisms in the upper gut, which may be applicable to Functional Dyspepsia. Gastric irritation in neonates results in chronic gastric hypersensitivity and gastric motor dysfunction in adults, in the absence of detectable gastric pathology (Liu et al. 2008). This gastric hypersensitivity in adults can be attenuated by the $GABA_B$ agonist baclofen, although this analgesic affect appears to occur via central rather than peripheral mechanisms (Liu et al. 2011).

Insights into the Mechanisms of Neuroplasticity Using IBS Patient Biopsies and Samples

In some subgroups of IBS patient's persistent low-grade inflammation within the gut wall (Barbara et al. 2002, 2004) and altered immunological function (Liebregts et al. 2007, 2011; Hughes et al. 2013) are evident and may lead to recurrent

re-sensitisation of nerve function within the gut (Hughes et al. 2009c, 2013). One of the first reports of this interaction demonstrated IBS patients have greater colonic mast cell infiltration and an increased release of key mediators, tryptase and histamine. Crucially these activated mast cells are in closer proximity to nerve fibres in IBS patients, which correlates with the severity and frequency of abdominal pain and discomfort (Barbara et al. 2004). Correspondingly, supernatants from IBS patient biopsies, but not healthy subjects, causes activation of afferent nerve endings and their cell bodies, via histamine H1 receptor and serine protease mechanisms (Barbara et al. 2007). Similar findings have been demonstrated using supernatants from Ulcerative Colitis patients, where application of supernatants enhances the neuronal excitability of colonic sensory DRG neurons. However, in this case the pro-inflammatory cytokine, TNFα is the key mediator, as it is elevated in Ulcerative Colitis biopsies, and acts at neuronal TNFR1 to modulate K_v and Na_v currents. These findings have increased importance as TNFα and the Ulcerative Colitis supernatants both enhance Na_V currents, and suppress K_V (I_A and I_K) currents (Ibeakanma and Vanner 2010), which are the same currents that are altered in inflammatory and post-inflammatory states (Beyak 2010).

Changes in IBS patients are also evident in peripheral blood mononuclear cells (PBMCs) (Liebregts et al. 2007, 2011; Hughes et al. 2009c, 2013). In particular several pro-inflammatory cytokines, TNF-α, IL-1β and IL-6, are all increased in PBMC supernatants from diarrhoea-predominant IBS (IBS-D) patients, which correlate with symptoms of pain frequency and intensity (Liebregts et al. 2007; Hughes et al. 2013). Notably, these supernatants from IBS-D patients evoke pronounced mechanical hypersensitivity in high- and low-threshold splanchnic and pelvic colonic afferents (Hughes et al. 2009c, 2013). As these colonic afferents express the receptors for these cytokines they can individually sensitise splanchnic and pelvic colonic afferents to mechanical stimuli (Hughes et al. 2009c, 2013). Whilst IL-1β causes direct firing of colonic afferents via a $NaV_{1.7}$ mechanism, TNF-α induces mechanical hypersensitivity, via a TRPA1 dependent mechanism (Hughes et al. 2013). This is one of numerous interactions that exist between pro-nociceptive mediators and TRP channels, which play key roles in inducing neuronal hypersensitivity and neuroplasticity.

TRP Channels: Key Roles for Neuroplasticity

In addition to its interaction with TNF-α (Hughes et al. 2013), TRPA1 also mediates the mechanical hypersensitivity induced by bradykinin (Brierley et al. 2009), as well as PAR2-induced hyperalgesia (Cattaruzza et al. 2009) (Fig. 9.2). This is important as TRPA1 plays a major role in visceral nociception, as TRPA1 deletion causes pronounced mechanosensory deficits, predominantly in high-threshold colonic afferents (Brierley et al. 2009, 2011) and correspondingly reduces viscero-motor responses to noxious colorectal distension (Brierley et al. 2009). Furthermore, activation of TRPA1 by numerous agonists, including mustard oil, cinnamaldehyde

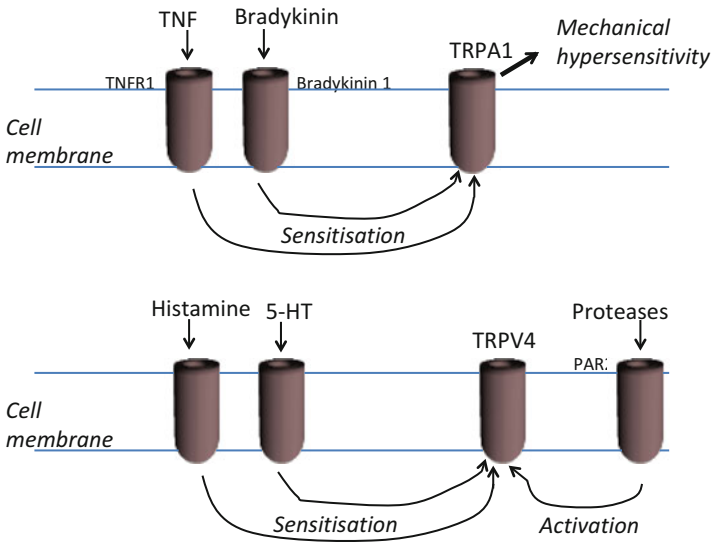

Fig. 9.2 TRP channels are key mediators of visceral afferent hypersensitivity and are downstream targets of receptor activation. (**a**) Whilst TRPA1 can be activated directly by compounds such a 4-Hydroxynonenal, mustard oil and cinnamaldehyde to induce mechanical hypersensitivity, TRPA1 can also be sensitised by interactions with TNFR1 and bradykinin 1 receptors. Binding of TNFα to TNFR1 and bradykinin to bradykinin 1 respectively can both independently evoke mechanical hypersensitivity of nociceptors by a TRPA1 dependent process. (**b**) Similarly, histamine and 5-HT can cause sensitisation of TRPV4, evoking neuronal hypersensitivity. This occurs via mitogen-activated protein kinase kinase (MAPKK) and phospholipase A2 (PLA2)-dependent mechanisms and increased TRPV4 dependent hypersensitivity in response to colorectal distension. By contrast, the interaction between TRPV4 and PAR-2 appears more fundamental, with expression of TRPV4 being required for PAR-2-induced mechanical hyperalgesia and excitation of colonic afferent neurons

and 4-hydroxynonenal, can tune nociceptor responses, inducing pronounced mechanical hypersensitivity (Brierley et al. 2009), and visceral mechanical hyperalgesia (Cattaruzza et al. 2009). Notably, TRPA1 function is increased during TNBS induced inflammation (Brierley et al. 2009) and TRPA1 deletion markedly reduces TNBS-induced colonic mechanical hyperalgesia (Cattaruzza et al. 2009), suggesting TRPA1 is also a key contributor to inflammatory pain. In addition to these effects on neurons, TRPA1 can also contribute to the inflammatory response itself, via neurogenic inflammation, as activation and sensitization of TRPA1 and release of substance P induces and maintains colitis in mice (Engel et al. 2011), which correspondingly re-sensitises nociceptors.

Another member of the TRP channel family, TRPV4, also plays a key role in nociception, neuroplasticity and pain. TRPV4 is predominantly expressed in spinal neurons innervating the colon and in the gut only contributes to the mechanosensory function of high-threshold colonic afferents (Brierley et al. 2008). These changes in colonic neuronal function translate to decreased visceromotor responses to colorectal

distension in TRPV4 -/- mice, or in mice with siRNA induced down-regulation of TRPV4 (Brierley et al. 2008; Cenac et al. 2008; Sipe et al. 2008). TRPV4 also has a crucial interaction with PAR2, whereby TRPV4 is required for PAR2-induced excitation of colonic afferent neurons and colonic mechanical hyperalgesia (Sipe et al. 2008). PAR2 is also a key receptor for inducing neuroplasticity, as PAR2 agonists can evoke sustained hyperexcitability of colonic nociceptive neurons by suppressing I_K currents, via a PKC and ERK(1/2) pathway (Kayssi et al. 2007). More recently another key PAR2-dependent mediator has been identified, cathepsin-S, which is activated in macrophages during TNBS colitis and evokes hyperexcitability of colonic nociceptive neurons and visceral hyperalgesia (Cattaruzza et al. 2011). TRPV4 can also be sensitised by a series of other mediators leading to neuronal hyperexcitability. Pre-exposure of colonic DRG neurons to 5-HT or histamine increases TRPV4 agonist induced responses and increases TRPV4 expression at the plasma membrane via PKC, PLA(2), PLCβ and MAPKK-dependent mechanisms (Cenac et al. 2010). TRPV4 can also contribute to the inflammation response itself, by inducing neurogenic inflammation, via activation of neuronal TRPV4 stimulating neuropeptide release from peripheral afferent terminals (Vergnolle et al. 2010). Secondly, TRPV4 is also expressed on intestinal epithelial cells, where its activation induces chemokine release and induces colitis (D'Aldebert et al. 2011).

TRPV1 is the most identifiable of the TRP channels and has long been implicated in gut nociception and altered neuronal function. Intra-colonic administration of the TRPV1 agonist, capsaicin, causes pronounced visceral pain (Laird et al. 2001, 2002), whilst TRPV1 -/- mice display decreases in visceromotor responses to colorectal balloon distension (Jones et al. 2005). TRPV1 appears to have a transient role in neuroplasticity, with initial increases in TRPV1 expression and function during the height of active colonic inflammation (De Schepper et al. 2008a, b; Miranda et al. 2007; Yang et al. 2008), which may return to normal levels at later post-inflammatory time points (Miranda et al. 2007). Correspondingly, TRPV1 deletion or pharmacological blockade partially reverses inflammation induced mechanical hypersensitivity and hyperalgesia (Jones et al. 2007; Miranda et al. 2007). However, a key interaction in this process appears to be via TRPV1 and the G protein-coupled receptor kinase 6 (GRK6) (Eijkelkamp et al. 2009). The pro-inflammatory cytokine IL-1β sensitizes TRPV1, which can be prevented by over-expressing GRK6. Following colitis, TRPV1-induced behavioural pain responses are more pronounced in GRK6 -/- mice than in wild-type mice, suggesting GRK6 can regulate inflammation-induced sensitization hyperalgesia (Eijkelkamp et al. 2009).

Neuroplasticity Induced by Bacterial Cell Products

Mucosal barrier function is crucial for the overall function of the gastrointestinal tract; however it is disturbed during inflammation associated with IBD (Turner 2009), whilst alterations and increased epithelial permeability are also evident in the small intestine and colon of IBS patients (Bertiaux-Vandaele et al. 2011;

Dunlop et al. 2006). These changes may allow bacteria to access the interstitial compartment of the gut and several recent studies have identified that bacterial cell products can profoundly alter gut neuronal function. In the jejunum lipopolysaccharide (LPS), a component of the cell wall of gram-negative bacteria, activates extrinsic sensory afferents (Wang et al. 2005; Donovan and Grundy 2012), an effect which is reduced by a non-selective cannabinoid agonist, an anandamide transport inhibitor, but not by a fatty acid amide hydrolase (FAAH) inhibitor (Donovan and Grundy 2012). Interestingly, activation of afferents appears to be specific for certain types of LPS, as luminally applied LPS from *Salmonella typhimurium*, but not LPS from *Escherichia coli,* activates these jejunal afferents (Liu et al. 2009). Increased afferent activity and an increased afferent sensitivity to a 5-HT3-receptor agonist following *Salmonella typhimurium* LPS can be blocked via a cyclo-oxygenase or EP1/EP2 mediated mechanism (Liu et al. 2009). Afferents innervating the colon can also be activated by LPS. Standard-grade LPS applied acutely for 3 min or chronically incubated for 24 h induces significant increases colonic DRG neuronal excitability (Ochoa-Cortes et al. 2010). These effects can be mimicked by acute application of bacterial lysate from *Escherichia coli* NLM28, which is exaggerated during DSS induced colitis. However, these effects cannot be blocked in TLR4 -/- mice or be replicated by the use of selected bacterial products activating individual TLRs, suggesting additive or alternate mechanisms may be involved. As ultrapure LPS cannot mimic the hyper-excitability effects of standard-LPS and lysate, but does stimulate TNF-α secretion from acutely dissociated DRG neurons, bacterial cell products may also sensitize colonic afferents via the release of pronociceptive cytokines from both immune cells and the neurons themselves. This appears to be evident by intracolonic administration of a toll-like receptor TLR7 activator, which causes inflammation, and induces short term hyperalgesia which is reduced in $Na_V1.9$ -/- mice. As wild-type and -/- mice display similar acute inflammatory responses and similar increases in pro-inflammatory cytokines, this reduction in hyperalgesia in $Na_V1.9$ -/- mice presumably occurs via the loss of neuronal $Na_V1.9$ (Martinez and Melgar 2008).

Endogenous Factors that Reduce Nociceptor Signalling

In addition to the myriad of nociceptive mediators and mechanisms described above, several key anti-nociceptive mechanisms have also been described that can reduce nociceptor signalling and prevent hyperalgesia and allodynia. Protease activated receptor 4 (PAR4) agonists suppresses the excitability of colonic DRG neurons (Karanjia et al. 2009) and significantly reduce the visceromotor response to colorectal distension in whole animal studies (Auge et al. 2009). PAR4 is actually co-localised in the same neurons as PAR2 and TRPV4 (discussed above) and correspondingly PAR4 activation attenuates both PAR2 agonist and TRPV4 agonist-induced allodynia and hyperalgesia in response to colorectal distension. Interestingly PAR4 agonist exposure inhibits free intracellular calcium mobilization induced by

the pro-nociceptive agonists of PAR2 and TRPV4 (Auge et al. 2009). As such the resultant balance between PAR2, PAR4 and TRPV4 activation is likely to determine the resultant effect on nociceptor responsiveness and therefore visceral pain.

Endogenous opioids are also key regulators of anti-nociceptive function (Karanjia et al. 2009; Verma-Gandhu et al. 2006, 2007). The lack of hyperalgesia and allodynia associated with chronic DSS colitis is actually accompanied by an increase in β-endorphin and μ-opioid receptor expression and CD4 +ve T-cells. This suggests chronic DSS induced-inflammation involves infiltration by lymphocytes, which is accompanied by μ-opioid receptor and β-endorphin up regulation, providing an anti-nociceptive input that restores normal visceral perception (Verma-Gandhu et al. 2007). In addition, colonic supernatants from chronic DSS treated mice have a 14-fold increase in β-endorphin levels, and their incubation suppresses the excitability of nociceptive colonic DRG neurons (Valdez-Morales et al. 2013). However, the timing of these effects may be disease specific as different opioid induced effects are evident in IBS. It has recently been shown that supernatants from PBMCs taken from healthy subjects actually inhibit colonic afferent mechanosensitivity, via a μ-opioid receptor mechanism (Hughes et al. 2013). Moreover, the number of β-endorphin expressing colonic mucosal lamina propria cells actually decreases in constipation predominant-IBS (C-IBS) patients compared with healthy subjects, suggesting that healthy human immune cells actively secrete β-endorphin, which dampens colonic mechanosensation (Hughes et al. 2013). As this inhibitory effect from PBMC supernatants is lost in C-IBS patients, and actually switches to sensitisation in diarrhea predominant (D-IBS) patients, where increases in pro-inflammatory cytokines are evident, these results suggests that resultant neuronal function is a constant balance between pro- and anti-nociceptive mechanisms.

More recently, it was demonstrated that inflammation can induce the function of kappa-opioid receptors, as demonstrated by the inhibitory effects of the agonist asimadoline on colonic nociceptor function (Hughes et al. 2014). Furthermore, the oxytocin receptor is not expressed in healthy colonic DRG neurons, however its expression is induced following inflammation and oxytocin receptor analogues inhibit colonic nociception in vitro and in vivo in post-inflammatory chronic visceral hypersensitivity models (de Araujo et al. 2014).

Conclusions and Future Perspectives

Recent studies have clearly demonstrated the capacity of inflammation or infection to cause long term neuroplasticity and the development of gut symptoms. In the absence of a ‘perfect’ pre-clinical model to replicate the multifactorial nature of many gut disorders, such as IBS, concurrent studies on numerous models have allowed identification of several distinct mechanisms that may potentially underlie neuroplasticity in the clinical setting. Specific immune pathways are recruited in response to different insults, which in turn leads to specific interactions between inflammatory cells, immune cells and neurons. This leads to alterations in neuronal

ion channel and receptor expression and function, leading to neuroplasticity. These studies also suggest some commonality in the mechanisms underlying neuroplasticity and that several mechanisms may have to interact to cause pronounced long term neuroplasticity. Crucially, several different therapeutic strategies may exist for the treatment and prevention of gastrointestinal dysfunction. Selective targeting of the individual neuronal populations displaying neuroplasticity is the ultimate goal for patients currently experiencing chronic pain or alterations in gut motility. However, another therapeutic window of opportunity exists, whereby reducing the initial inflammatory response, for example during the early stages of gastroenteritis, may reduce or prevent subsequent inflammation-induced neuroplasticity. Future research will need to identify how the differing extrinsic and intrinsic neural pathways communicate with one another and the complex interactions that each of them have concurrently with stress mediators, immune responses, enteric/spinal glia and gut microbiota to underlie normal gut physiology. Determining how these interactions are altered during pathophysiology will be crucial in the next phase of understanding the mechanisms of neuroplasticity, which underlie gastrointestinal dysfunction.

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References

Adam B et al (2006) Severity of mucosal inflammation as a predictor for alterations of visceral sensory function in a rat model. Pain 123:179–186

Al-Chaer ED, Kawasaki M, Pasricha PJ (2000) A new model of chronic visceral hypersensitivity in adult rats induced by colon irritation during postnatal development. Gastroenterology 119:1276–1285

Antalis TM, Shea-Donohue T, Vogel SN, Sears C, Fasano A (2007) Mechanisms of disease: protease functions in intestinal mucosal pathobiology. Nat Clin Pract Gastroenterol Hepatol 4:393–402

Auge C, Balz-Hara D, Steinhoff M, Vergnolle N, Cenac N (2009) Protease-activated receptor-4 (PAR 4): a role as inhibitor of visceral pain and hypersensitivity. Neurogastroenterol Motil 21:1189-e107

Barbara G, De Giorgio R, Stanghellini V, Cremon C, Corinaldesi R (2002) A role for inflammation in irritable bowel syndrome? Gut 51:i41–i44

Barbara G et al (2004) Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterology 126:693–702

Barbara G et al (2007) Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. Gastroenterology 132:26–37

Bertiaux-Vandaele N et al (2011) The expression and the cellular distribution of the tight junction proteins are altered in irritable bowel syndrome patients with differences according to the disease subtype. Am J Gastroenterol 106:2165–2173

Beyak MJ (2010) Visceral afferents – determinants and modulation of excitability. Auton Neurosci 153:69–78

Beyak MJ, Vanner S (2005) Inflammation-induced hyperexcitability of nociceptive gastrointestinal DRG neurones: the role of voltage-gated ion channels. Neurogastroenterol Motil 17:175–186

Beyak MJ, Ramji N, Krol KM, Kawaja MD, Vanner SJ (2004) Two TTX-resistant Na+ currents in mouse colonic dorsal root ganglia neurons and their role in colitis-induced hyperexcitability. Am J Physiol Gastrointest Liver Physiol 287:G845–G855

Bielefeldt K, Ozaki N, Gebhart GF (2002a) Mild gastritis alters voltage-sensitive sodium currents in gastric sensory neurons in rats. Gastroenterology 122:752–761

Bielefeldt K, Ozaki N, Gebhart GF (2002b) Experimental ulcers alter voltage-sensitive sodium currents in rat gastric sensory neurons. Gastroenterology 122:394–405

Blackshaw LA, Brierley SM, Hughes PA (2010) TRP channels: new targets for visceral pain. Gut 59:126–135

Brierley SM (2010) Molecular basis of mechanosensitivity. Auton Neurosci 153:58–68

Brierley SM, Kelber O (2011) Use of natural products in gastrointestinal therapies. Curr Opin Pharmacol 11:604–611

Brierley SM, Linden DR (2014) Neuroplasticity and dysfunction after gastrointestinal inflammation. Nat Rev Gastroenterol Hepatol 11:611–627

Brierley SM, Jones RCW III, Xu L, Gebhart GF, Blackshaw LA (2005a) Activation of splanchnic and pelvic colonic afferents by bradykinin in mice. Neurogastroenterol Motil 17:854–862

Brierley SM et al (2005b) Differential chemosensory function and receptor expression of splanchnic and pelvic colonic afferents in mice. J Physiol 567:267–281

Brierley SM et al (2008) Selective role for TRPV4 ion channels in visceral sensory pathways. Gastroenterology 134:2059–2069

Brierley SM et al (2009) The ion channel TRPA1 is required for normal mechanosensation and is modulated by algesic stimuli. Gastroenterology 137:2084–2095.e3

Brierley SM et al (2011) TRPA1 contributes to specific mechanically activated currents and sensory neuron mechanical hypersensitivity. J Physiol 589:3575–3593

Cattaruzza F et al (2009) Transient receptor potential ankyrin-1 has a major role in mediating visceral pain in mice. Am J Physiol Gastrointest Liver Physiol 298:G81–G91

Cattaruzza F et al (2011) Cathepsin S is activated during colitis and causes visceral hyperalgesia by a PAR2-dependent mechanism in mice. Gastroenterology 141:1864–1874, e1–3

Cenac N et al (2008) Transient receptor potential vanilloid-4 has a major role in visceral hypersensitivity symptoms. Gastroenterology 135:937–946, 946.e1–2

Cenac N et al (2010) Potentiation of TRPV4 signalling by histamine and serotonin: an important mechanism for visceral hypersensitivity. Gut 59:481–488

Christianson JA, Bielefeldt K, Malin SA, Davis BM (2010) Neonatal colon insult alters growth factor expression and TRPA1 responses in adult mice. Pain 151:540–549

Coldwell JR, Phillis BD, Sutherland K, Howarth GS, Blackshaw LA (2007) Increased responsiveness of rat colonic splanchnic afferents to 5-HT after inflammation and recovery. J Physiol 579:203–213

Costigan M, Scholz J, Woolf CJ (2009) Neuropathic pain: a maladaptive response of the nervous system to damage. Annu Rev Neurosci 32:1–32

D'Aldebert E et al (2011) Transient receptor potential vanilloid 4 activated inflammatory signals by intestinal epithelial cells and colitis in mice. Gastroenterology 140:275–285

Dang K, Bielefeldt K, Gebhart GF (2004) Gastric ulcers reduce A-type potassium currents in rat gastric sensory ganglion neurons. Am J Physiol Gastrointest Liver Physiol 286:G573–G579

de Araujo AD et al (2014) Selenoether oxytocin analogues have analgesic properties in a mouse model of chronic abdominal pain. Nat Commun 5, 3165

De Schepper HU et al (2008a) TRPV1 receptors on unmyelinated C-fibres mediate colitis-induced sensitization of pelvic afferent nerve fibres in rats. J Physiol 586:5247–5258

De Schepper HU et al (2008b) TRPV1 receptor signaling mediates afferent nerve sensitization during colitis-induced motility disorders in rats. Am J Physiol Gastrointest Liver Physiol 294:G245–G253

Dinan TG, Cryan J, Shanahan F, Keeling PW, Quigley EM (2010) IBS: an epigenetic perspective. Nat Rev Gastroenterol Hepatol 7:465–471

Donovan J, Grundy D (2012) Endocannabinoid modulation of jejunal afferent responses to LPS. Neurogastroenterol Motil 24:956-e465

Dunlop SP, Jenkins D, Neal KR, Spiller RC (2003) Relative importance of enterochromaffin cell hyperplasia, anxiety, and depression in postinfectious IBS. Gastroenterology 125:1651–1659
Dunlop SP et al (2006) Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. Am J Gastroenterol 101:1288–1294
Eijkelkamp N et al (2007) Increased visceral sensitivity to capsaicin after DSS-induced colitis in mice: spinal cord c-Fos expression and behavior. Am J Physiol Gastrointest Liver Physiol 293:G749–G757
Eijkelkamp N et al (2009) G protein-coupled receptor kinase 6 controls post-inflammatory visceral hyperalgesia. Brain Behav Immun 23:18–26
Engel MA et al (2011) TRPA1 and substance P mediate colitis in mice. Gastroenterology 141:1346–1358
Fasano A, Shea-Donohue T (2005) Mechanisms of disease: the role of intestinal barrier function in the pathogenesis of gastrointestinal autoimmune diseases. Nat Clin Pract Gastroenterol Hepatol 2:416–422
Feng B, Gebhart GF (2011) Characterization of silent afferents in the pelvic and splanchnic innervations of the mouse colorectum. Am J Physiol Gastrointest Liver Physiol 300:G170–G180
Feng B et al (2012a) Long-term sensitization of mechanosensitive and -insensitive afferents in mice with persistent colorectal hypersensitivity. Am J Physiol Gastrointest Liver Physiol 302:G676–G683
Feng B et al (2012b) Altered colorectal afferent function associated with TNBS-induced visceral hypersensitivity in mice. Am J Physiol Gastrointest Liver Physiol 303:G817–G824
Furness JB (2012) The enteric nervous system and neurogastroenterology. Nat Rev Gastroenterol Hepatol 9:286–294
Gebhart GF, Bielefeldt K, Ozaki N (2002) Gastric hyperalgesia and changes in voltage gated sodium channel function in the rat. Gut 51(Suppl 1):i15–i18
Hanevik K, Dizdar V, Langeland N, Hausken T (2009) Development of functional gastrointestinal disorders after Giardia lamblia infection. BMC Gastroenterol 9:27
Harrington AM et al (2012) Sprouting of colonic afferent central terminals and increased spinal mitogen-activated protein kinase expression in a mouse model of chronic visceral hypersensitivity. J Comp Neurol 520:2241–2255
Hillsley K et al (2006) Dissecting the role of sodium currents in visceral sensory neurons in a model of chronic hyperexcitability using Nav1.8 and Nav1.9 null mice. J Physiol 576:257–267
Hong S et al (2009) Reciprocal changes in vanilloid (TRPV1) and endocannabinoid (CB1) receptors contribute to visceral hyperalgesia in the water avoidance stressed rat. Gut 58:202–210
Hughes PA, Brierley SM, Blackshaw LA (2009a) Post-inflammatory modification of colonic afferent mechanosensitivity. Clin Exp Pharmacol Physiol 36:1034–1040
Hughes PA et al (2009b) Post-inflammatory colonic afferent sensitisation: different subtypes, different pathways and different time courses. Gut 58:1333–1341
Hughes PA et al (2009c) TRPV1-expressing sensory fibres and IBS: links with immune function. Gut 58:465–466
Hughes PA et al (2013) Sensory neuro-immune interactions differ between Irritable Bowel Syndrome subtypes. Gut 62:1456–1465
Hughes PA et al (2014) Increased kappa-opioid receptor expression and function during chronic visceral hypersensitivity. Gut 63:1199–1200
Ibeakanma C, Vanner S (2010) TNFalpha is a key mediator of the pronociceptive effects of mucosal supernatant from human ulcerative colitis on colonic DRG neurons. Gut 59:612–621
Ibeakanma C et al (2009) Citrobacter rodentium colitis evokes post-infectious hyperexcitability of mouse nociceptive colonic dorsal root ganglion neurons. J Physiol 587:3505–3521
Ibeakanma C et al (2011) Brain-gut interactions increase peripheral nociceptive signaling in mice with postinfectious irritable bowel syndrome. Gastroenterology 141:2098–2108.e5
Jones RC 3rd, Xu L, Gebhart GF (2005) The mechanosensitivity of mouse colon afferent fibers and their sensitization by inflammatory mediators require transient receptor potential vanilloid 1 and acid-sensing ion channel 3. J Neurosci 25:10981–10989

Jones RC 3rd et al (2007) Short-term sensitization of colon mechanoreceptors is associated with long-term hypersensitivity to colon distention in the mouse. Gastroenterology 133:184–194
Karanjia R, Spreadbury I, Bautista-Cruz F, Tsang ME, Vanner S (2009) Activation of protease-activated receptor-4 inhibits the intrinsic excitability of colonic dorsal root ganglia neurons. Neurogastroenterol Motil 21:1218–1221
Kaser A, Zeissig S, Blumberg RS (2010) Inflammatory bowel disease. Annu Rev Immunol 28:573–621
Kayssi A, Amadesi S, Bautista F, Bunnett NW, Vanner S (2007) Mechanisms of protease-activated receptor 2-evoked hyperexcitability of nociceptive neurons innervating the mouse colon. J Physiol 580:977–991
Keating C et al (2008) Afferent hypersensitivity in a mouse model of post-inflammatory gut dysfunction: role of altered serotonin metabolism. J Physiol 586:4517–4530
Keating C, Pelegrin P, Martinez CM, Grundy D (2011) P2X7 receptor-dependent intestinal afferent hypersensitivity in a mouse model of postinfectious irritable bowel syndrome. J Immunol 187:1467–1474
King DE, Macleod RJ, Vanner SJ (2009) Trinitrobenzenesulphonic acid colitis alters Na 1.8 channel expression in mouse dorsal root ganglia neurons. Neurogastroenterol Motil 21:880-e64
La JH, Gebhart GF (2011) Colitis decreases mechanosensitive K2P channel expression and function in mouse colon sensory neurons. Am J Physiol Gastrointest Liver Physiol 301:G165–G174
Laird J, Martinez-Caro L, Garcia-Nicas E, Cervero F (2001) A new model of visceral pain and referred hyperalgesia in the mouse. Pain 92:335–342
Laird JM, Souslova V, Wood JN, Cervero F (2002) Deficits in visceral pain and referred hyperalgesia in Nav1.8 (SNS/PN3)-null mice. J Neurosci 22:8352–8356
Lamb K, Zhong F, Gebhart GF, Bielefeldt K (2005) Experimental colitis in mice and sensitization of converging visceral and somatic afferent pathways. Am J Physiol Gastrointest Liver Physiol 290(3):G451–G457
Larauche M, Mulak A, Tache Y (2012) Stress and visceral pain: from animal models to clinical therapies. Exp Neurol 233:49–67
Larsson MH, Rapp L, Lindstrom E (2006) Effect of DSS-induced colitis on visceral sensitivity to colorectal distension in mice. Neurogastroenterol Motil 18:144–152
Larsson MH, Miketa A, Martinez V (2009) Lack of interaction between psychological stress and DSS-induced colitis affecting colonic sensitivity during colorectal distension in mice. Stress 12:434–444
Liebregts T et al (2007) Immune activation in patients with irritable bowel syndrome. Gastroenterology 132:913–920
Liebregts T et al (2011) Small bowel homing T cells are associated with symptoms and delayed gastric emptying in functional dyspepsia. Am J Gastroenterol 106:1089–1098
Liu LS et al (2008) A rat model of chronic gastric sensorimotor dysfunction resulting from transient neonatal gastric irritation. Gastroenterology 134:2070–2079
Liu CY, Mueller MH, Rogler G, Grundy D, Kreis ME (2009) Differential afferent sensitivity to mucosal lipopolysaccharide from Salmonella typhimurium and Escherichia coli in the rat jejunum. Neurogastroenterol Motil 21:1335-e129
Liu LS, Shenoy M, Pasricha PJ (2011) The analgesic effects of the GABAB receptor agonist, baclofen, in a rodent model of functional dyspepsia. Neurogastroenterol Motil 23:356–361, e160–1
Lynn PA, Chen BN, Zagorodnyuk VP, Costa M, Brookes SJ (2008) TNBS-induced inflammation modulates the function of one class of low-threshold rectal mechanoreceptors in the guinea pig. Am J Physiol Gastrointest Liver Physiol 295:G862–G871
Marshall JK et al (2010) Eight year prognosis of postinfectious irritable bowel syndrome following waterborne bacterial dysentery. Gut 59:605–611
Martinez V, Melgar S (2008) Lack of colonic-inflammation-induced acute visceral hypersensitivity to colorectal distension in Na(v)1.9 knockout mice. Eur J Pain 12:934–944
Miranda A et al (2007) The role of transient receptor potential vanilloid 1 in mechanical and chemical visceral hyperalgesia following experimental colitis. Neuroscience 148:1021–1032

Moore BA, Stewart TM, Hill C, Vanner SJ (2002) TNBS ileitis evokes hyperexcitability and changes in ionic membrane properties of nociceptive DRG neurons. Am J Physiol Gastrointest Liver Physiol 282:G1045–G1051

Nell S, Suerbaum S, Josenhans C (2010) The impact of the microbiota on the pathogenesis of IBD: lessons from mouse infection models. Nat Rev Microbiol 8:564–577

Ochoa-Cortes F et al (2010) Bacterial cell products signal to mouse colonic nociceptive dorsal root ganglia neurons. Am J Physiol Gastrointest Liver Physiol 299:G723–G732

Ohman L, Simren M (2010) Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. Nat Rev Gastroenterol Hepatol 7:163–173

Sengupta JN, Snider A, Su X, Gebhart GF (1999) Effects of kappa opioids in the inflamed rat colon. Pain 79:175–185

Shinoda M, La JH, Bielefeldt K, Gebhart GF (2010) Altered purinergic signaling in colorectal dorsal root ganglion neurons contributes to colorectal hypersensitivity. J Neurophysiol 104:3113–3123

Sipe WEB et al (2008) Transient receptor potential vanilloid 4 mediates protease activated receptor 2-induced sensitization of colonic afferent nerves and visceral hyperalgesia. Am J Physiol Gastrointest Liver Physiol 294:G1288–G1298

Spiller R, Garsed K (2009) Infection, inflammation, and the irritable bowel syndrome. Dig Liver Dis 41:844–849

Spiller RC et al (2000) Increased rectal mucosal enteroendocrine cells, T lymphocytes, and increased gut permeability following acute Campylobacter enteritis and in post-dysenteric irritable bowel syndrome. Gut 47:804–811

Stewart T, Beyak MJ, Vanner S (2003) Ileitis modulates potassium and sodium currents in guinea pig dorsal root ganglia sensory neurons. J Physiol 552:797–807

Thabane M, Simunovic M, Akhtar-Danesh N, Garg AX, Clark WF, Collins SM, Salvadori M, Marshall JK (2010) An outbreak of acute bacterial gastroenteritis is associated with an increased incidence of irritable bowel syndrome in children. Am J Gastroenterol 105(4):933–939

Traub RJ et al (2008) A rat model of chronic postinflammatory visceral pain induced by deoxycholic acid. Gastroenterology 135:2075–2083

Turner JR (2009) Intestinal mucosal barrier function in health and disease. Nat Rev Immunol 9:799–809

Valdez-Morales E et al (2013) Release of endogenous opioids during a chronic IBD model suppresses the excitability of colonic DRG neurons. Neurogastroenterol Motil 25:39–45.e4

Vergnolle N et al (2010) A role for transient receptor potential vanilloid 4 in tonicity-induced neurogenic inflammation. Br J Pharmacol 159:1161–1173

Verma-Gandhu M et al (2006) CD4+ T-cell modulation of visceral nociception in mice. Gastroenterology 130:1721–1728

Verma-Gandhu M et al (2007) Visceral pain perception is determined by the duration of colitis and associated neuropeptide expression in the mouse. Gut 56:358–364

Wang B et al (2005) Lipopolysaccharide-induced changes in mesenteric afferent sensitivity of rat jejunum in vitro: role of prostaglandins. Am J Physiol Gastrointest Liver Physiol 289:G254–G260

Winston J, Shenoy M, Medley D, Naniwadekar A, Pasricha PJ (2007) The vanilloid receptor initiates and maintains colonic hypersensitivity induced by neonatal colon irritation in rats. Gastroenterology 132:615–627

Winston JH, Xu GY, Sarna SK (2010) Adrenergic stimulation mediates visceral hypersensitivity to colorectal distension following heterotypic chronic stress. Gastroenterology 138:294–304.e3

Xu GY, Shenoy M, Winston JH, Mittal S, Pasricha PJ (2008) P2X receptor-mediated visceral hyperalgesia in a rat model of chronic visceral hypersensitivity. Gut 57:1230–1237

Yang J et al (2008) Transient receptor potential ankyrin-1 participates in visceral hyperalgesia following experimental colitis. Neurosci Lett 440:237–241