Contribution of membrane receptor signalling to chronic visceral pain

Running title: Receptor signalling and chronic visceral pain

Mahsa Sadeghi [3], Andelain Erickson [1,2], Joel Castro [1,2], Annemie Deiteren [1,2], Andrea M. Harrington [1,2], Luke Grundy [1,2], David J. Adams [3,] Stuart M. Brierley [1,2]

[1] Visceral Pain Research Group, Human Physiology, Centre for Neuroscience, College of Medicine and Public Health, Flinders University, Bedford Park, South Australia, 5042, AUSTRALIA.

[2] Centre for Nutrition and Gastrointestinal Diseases, Discipline of Medicine, University of Adelaide, South Australian Health and Medical Research Institute (SAHMRI), North Terrace, Adelaide, South Australia 5000, AUSTRALIA.

[3] Illawarra Health & Medical Research Institute (IHMRI), University of Wollongong, Wollongong, NSW 2522, AUSTRALIA.

Corresponding Author:

A/Prof. Stuart M. Brierley: Visceral Pain Research Group, Flinders University, Level 7, South Australian Health and Medical Research Institute (SAHMRI), North Terrace, Adelaide, SA 5000, AUSTRALIA.

Email: stuart.brierley@flinders.edu.au

Phone: +61 8 8128 4848

Acknowledgements: S.M.B is a National Health and Medical Research Council of Australia (NHMRC) R.D Wright Biomedical Research Fellow (APP1126378) and is funded by NHMRC Australia Project Grants #1083480, #1139366 and #1140297. A.M.H received funding via the Australian Research Council (ARC) Discovery Early Career Research Award (DE130100223). A.M.H and S.M.B receive funding via the ARC Discovery Project DP180101395. Dr Ann Turnley is thanked for editorial assistance.

Key Words: Pain; nociception; afferents; ion channels; colon

HIGHLIGHTS:

- Irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD) are major forms of chronic visceral pain (CVP), which affect over 15% of the global population.

- In order to identify new therapies, it is important to understand the underlying causes of CVP.

- Inflammation or infection of the gut triggers changes in the sensory pathways that transmit nociceptive information from the periphery to the CNS.

- Such changes include altered expression and function of a variety of ion channels and receptors, which underlies neuronal hyper-excitability.

- Neuronal hyper-excitability enhances peripheral drive from the viscera, providing an underlying basis for enhanced nociceptive signalling during CVP.

ABBREVIATIONS:

- α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)
- Calcitonin gene-related peptide (CGRP)
- Cannabinoid receptor (CB)
- Central nervous system (CNS)
- Chronic visceral pain (CVP)
- Chronic visceral hypersensitivity (CVH)
- constipation-predominant IBS (IBS-C)
- δ-theraphotoxin-Hm1a (Hm1a)
- diarrhoea-predominant IBS (IBS-D)
- Enteric nervous system (ENS)
- Gamma-aminobutyric acid (GABA)
- Guanylate cyclase-C (GC-C)
- G protein-coupled receptor (GPCR)
- Irritable bowel syndrome (IBS)
- Inflammatory bowel disease (IBD)
- Metabotropic glutamate (mGlu) receptors
- N-methyl-D-aspartate (NMDA)
- Positron emission tomography (PET)
- Protease-activated receptors (PARs)
- Substance P (SP), neurokinin A (NKA), and neurokinin B (NKB)
- Tachykinin receptor 1 (NK_1R)
- Tachykinin receptor 2 (NK_2R)
- Tachykinin receptor 3 (NK_3R)
- Tetrodotoxin (TTX)
- Transient receptor potential (TRP)
- Transient receptor potential ankyrin repeat (TRPA)
- Transient receptor potential (TRP) canonical (TRPC)
- Transient receptor potential (TRP) melastatin (TRPM)
- Transient receptor potential (TRP) mucolipin (TRPML)
- Transient receptor potential (TRP) polycystin (TRPP)
- Transient receptor potential (TRP) vanilloid (TRPV)
- Voltage-gated calcium (Ca_V) channels
- Voltage-gated potassium (K_V) channels
- Voltage-gated sodium (Na_V) channels

ABSTRACT

Irritable bowel syndrome and inflammatory bowel disease are major forms of chronic visceral pain, which affect over 15% of the global population. In order to identify new therapies, it is important to understand the underlying causes of chronic visceral pain. This review provides recent evidence demonstrating that inflammation or infection of the gastrointestinal tract triggers specific changes in the neuronal excitability of sensory pathways responsible for the transmission of nociceptive information from the periphery to the central nervous system. Specific changes in the expression and function of a variety of ion channels and receptors have been documented in inflammatory and chronic visceral pain conditions relevant to Irritable bowel syndrome and inflammatory bowel disease. An increase in pro-nociceptive mechanisms enhances peripheral drive from the viscera and provides an underlying basis for enhanced nociceptive signalling during chronic visceral pain states. Recent evidence also highlights increases in anti-nociceptive mechanisms in models of chronic visceral pain, which present novel targets for pharmacological treatment of this condition.

1: CLINICAL RELEVANCE OF CHRONIC VISCERAL PAIN

Pain is an unpleasant sensory and emotional experience that normally serves as an alarm mechanism. This allows the body to protect itself against actual or potential tissue damage, by allowing withdrawal from and avoidance of noxious stimuli. In chronic pain, this alarm signal fails to reset following subsidence of the threat or healing of the damaged tissue. Accordingly, chronic pain is a maladaptive, relapsing and remitting condition characterised by nociceptor sensitisation, allodynia (pain response to stimuli that do not normally cause pain) and hyperalgesia (an enhanced pain response) in the absence of overt tissue damage (Brierley and Linden, 2014; Costigan et al., 2009). Chronic visceral pain (CVP) derives from our internal organs and is a common and debilitating symptom for patients with gastrointestinal disorders, such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD).

IBS and IBD are major clinical problems affecting up to 15% of the global population (Chey et al., 2015; Enck et al., 2016). Consequently, CVP is an important public health issue, which places a large economic burden on healthcare resources (Camilleri and Williams, 2000; Chey et al., 2015; Sandler et al., 2000). In the early 2000's, the economic impact of IBS and IBD

were estimated in the USA alone to exceed $25 billion and $6 billion per annum respectively. Since then the incidence rates of IBS and IBD, and their associated costs have steadily increased over time (Enck et al., 2016; Kaplan, 2015). Despite such a burden, treatments for IBS and IBD are generally lacking. IBS and IBD patients report reduced quality of life and have additional clinical symptoms, including stool irregularities, as well as somatic, visceral and psychiatric co-morbidities, including referred pain and higher levels of anxiety and depression than healthy people (Camilleri, 2001; Camilleri and Williams, 2000; Chey et al., 2015; Enck et al., 2016; Grundy and Brierley, 2017).

In IBD, which is a chronic, relapsing inflammatory disorder of the gastrointestinal tract, a disturbed mucosal epithelial barrier, combined with the release of inflammatory mediators, sensitises peripheral nerve endings within the gut wall, resulting in disturbed visceral sensory perception and abdominal pain. In contrast, CVP in IBS occurs in the absence of overt colon pathology (Enck et al., 2016). Whilst the aetiology of IBS is multi-factorial and additional risk factors may be required for development (Chey et al., 2015; Spiller and Garsed, 2009), there is a strong correlation between a prior exposure of the patient to gut infection and symptom occurrence. This includes a preceding bout of gastroenteritis induced by pathogens such as *Escherichia coli*, *Salmonella*, *Campylobacter* and *Giardia lamblia* (Spiller and Garsed, 2009). Notably, the duration and severity of the initial illness is one of the strongest associated risk factors for the development of IBS symptoms (Marshall et al., 2010). Such bouts of acute gastroenteritis can trigger IBS symptoms in patients that persist for at least 8 years after the initial infection (Marshall et al., 2010). Biopsy and blood samples from IBS patients, in addition to mechanistic pre-clinical studies, are now shedding light on the potential reasons why persistent symptoms occur in the absence of overt pathology to the intestinal mucosa (Bashashati et al., 2018; Brierley and Linden, 2014; Hughes et al., 2013b). These studies suggest that infection or inflammation of the intestine releases mediators that sensitise afferents leading to inflammation-induced visceral hypersensitivity. In post-inflammatory states this hypersensitivity persists, resulting in chronic visceral hypersensitivity (CVH), or the long-term enhanced perception of stimuli originating from our internal organs (Brierley and Linden, 2014; Grundy and Brierley, 2017). It is this CVH of sensory pathways innervating the colon that leads to the development and maintenance of chronic abdominal pain in patients with IBS in the absence of overt intestinal pathology (Azpiroz et al., 2007; Lembo et al., 1994; Ritchie, 1973).

In this article, we'll first briefly outline how sensory information at the level of the colorectum is encoded and conveyed to the central nervous system (CNS). For an in-depth discussion on the neuroanatomy, we refer the readers to some of the excellent reviews on this topic (Brookes et al., 2013; Sengupta, 2009; Vermeulen et al., 2014). In the remaining sections, we will discuss how different membrane receptors and ion channels contribute to colorectal hypersensitivity in animal models relevant to IBS and IBD and finish by discussing anti-nociceptive mechanisms, which may provide additional novel candidates for therapeutic targeting.

2: NEUROANATOMY OF THE GASTROINTESTINAL TRACT

2.1 Intrinsic innervation

The enteric nervous system (ENS) contains over 10^8 neurons and acts autonomously to generate basic motor and secretory patterns independent of the CNS. Accordingly, it is often referred to as the 'little brain' in the gut. Arranged into web-like plexuses (the myenteric/Auerbach's plexus and the submucosal/Meissner's plexus) its main roles are to regulate gastrointestinal motility and control secretion, absorption and blood supply (Furness et al., 2013; Lomax et al., 2005). The involvement of the ENS in pain signalling is unclear, but most likely lies in the release of neurotransmitters, such as substance P, calcitonin gene-related peptide (CGRP) and serotonin that in turn can activate and sensitise adjacent sensory nerves to cause aberrant intestinal contractility. In support of such a mechanism, mediators released from mucosal biopsies from IBS patients, but not healthy controls can activate human and guinea-pig submucosal neurons. The sensitisation of these neurons correlates with the degree of visceral hypersensitivity experienced by IBS patients (Buhner et al., 2014; Buhner et al., 2012; Buhner et al., 2011; Buhner et al., 2009).

2.2 Extrinsic sensory innervation

The extrinsic nervous system, through vagal and spinal afferent signalling, allows the CNS to consciously or unconsciously perceive our gastrointestinal environment. The basic anatomy of extrinsic sensory afferent innervation of the colorectum is shown in Figure 1. These extrinsic sensory afferents also allow modulation of gastrointestinal motility and coordination

of gut reflexes through efferent pathways. Vagal afferents extend from the oesophagus down to the proximal colon and part of the transverse colon (Berthoud and Neuhuber, 2000). Their cell bodies reside within the nodose and jugular ganglia, from which they project centrally to the nucleus of the solitary tract in the brain stem (Berthoud and Neuhuber, 2000). In contrast, the cell bodies of spinal splanchnic and pelvic afferents are located in the dorsal root ganglia (DRG). The central axons of spinal afferents terminate within the dorsal horn of the thoracic, lumbar and sacral spinal cord, where they synapse onto second order neurons. The predominant function of vagal afferents is the conduction of sensory information within the physiological range (Grundy, 2002). By contrast, spinal afferents are specifically equipped to convey information on physiological events, as well as higher-threshold sensations, such as pain, discomfort, bloating and urgency. This occurs through specialised functional subclasses of sensory afferents that respond to either i) low-intensity stimuli to the mucosal surface, ii) low levels of distension or stretch to the intestine, or iii) high levels of distension or stretch to the intestine (Brierley et al., 2009; Brierley et al., 2004; Brierley et al., 2008; Hughes et al., 2009). Many of these subclasses have subsequently been discovered in human intestinal tissue (Hockley et al., 2016; Jiang et al., 2011; McGuire et al., 2018; Peiris et al., 2011; Yu et al., 2016). Studies in animals reveal that nerve endings terminate within different layers of the colon wall, including the mucosa, muscle layers, enteric ganglia and blood vessels in the submucosa, serosa and mesentery (Brookes et al., 2013; Spencer et al., 2016a; Spencer et al., 2016b; Zagorodnyuk et al., 2010). Sensitisation of these nerve endings, which, as discussed below, express a large array of receptors and ion channels, is a major contributor to visceral hypersensitivity in CVP states (Brierley, 2016; Brierley and Linden, 2014; Grundy, 2002). Furthermore, sprouting of the central projections of these colonic afferent neurons occurs within the dorsal horn of the spinal cord. This is associated with secondary increased activation of dorsal horn neurons in animal models of CVP, suggesting that neuroanatomical remodelling at the level of the spinal cord also contributes to central sensitisation and facilitation of CVP (Benson et al., 2014; Harrington et al., 2012; Xia et al., 2011). There is also evidence that cross-organ sensitisation can occur via the existence of dichotomising afferents, which are a sub-population of DRG neurons that have a single cell soma, but axons that project to multiple visceral organs (Christianson et al., 2007; Grundy and Brierley, 2017; Winnard et al., 2006). This may also contribute to the relatively poor localization of visceral pain compared to that of cutaneous pain.

2.3 Central visceral pain pathways

Sensory information is relayed to the CNS via ascending spinal pathways, which include the spinoreticular, spinomesencephalic, spinohypothalamic and spinothalamic tracts, as well as the dorsal funiculus, all of which play an important role in the conscious or unconscious processing of visceral perceptions (Almeida et al., 2004; Anand et al., 2007). The thalamus plays a principal role in central processing of pain (Mayer et al., 2009). From here, the signal is conveyed to different brain regions involved in somatosensory perception. This includes the somatosensory cortices (SI and SII) as well as areas that regulate the cognitive, affective and motivational dimensions of pain perception, such as the dorsal anterior cingulate cortex, the amygdala, the nucleus accumbens, the ventral striatum and the prefrontal cortex. Interestingly, structural abnormalities have been reported in brain regions involved in central pain processing in IBS patients, such as reduced cortical thickness of the dorsolateral prefrontal cortex and increased thickness of the anterior insulate cortex, which correlated with maladaptive coping and IBS duration respectively (Blankstein et al., 2010; Mayer et al., 2015). Furthermore, functional imaging studies have indicated altered brain activity in these regions in IBS patients (Mayer et al., 2015; Tillisch et al., 2011).

In addition to ascending pathways, afferent sensory input from the spinal cord can be regulated in a 'top-down' fashion by several supraspinal regions. The periaqueductal gray, located in the midbrain, is probably the most important regulatory pathway in this regard, receiving input from the anterior cingulate cortex and the amygdala (Anand et al., 2007; Schweinhardt and Bushnell, 2010). This can result in either inhibition or facilitation of transmission, depending on the mediators involved. Dysfunction of descending inhibitory control and enhanced descending facilitatory influences have both been linked to visceral hypersensitivity in chronic pain states (Gebhart, 2004; Piche et al., 2011; Ringel et al., 2008; Urban et al., 1999; Wilder-Smith et al., 2004).

It has been suggested that the changes in neuronal signalling of sensory information from the gut to the CNS are, at least partially, due to alterations in the expression and function of ion channels and receptors on neuronal cell bodies or nerve terminals of extrinsic sensory afferents. These changes result in neuronal hyperexcitability, which may result in tonic or

enhanced impulse input from the periphery to the CNS (Gracely et al., 1992). Therefore, identifying such changes in sensory afferents during CVP is crucial for determining the underlying basis of abnormal signalling and ultimately for finding specific treatments for IBS and IBD.

3. PRO-NOCICEPTIVE MECHANISMS

A summary of key pro-nociceptive mechanisms within extrinsic sensory afferent neurons innervating the colorectum is shown in Figure 2.

3.1: Voltage-gated sodium channels

Voltage-gated sodium (Na_V) channels are essential for the propagation of action potentials and regulation of cell excitability. Na_V channels are composed of a pore-forming α subunit and can associate with auxiliary β subunits via covalent or non-covalent interactions (Catterall, 2014). There are nine mammalian sodium channels, $Na_V1.1$–$Na_V1.9$, which collectively mediate fast, slow and persistent sodium currents. The β subunits regulate kinetic properties and voltage-dependence of Na_V channel activation and inactivation, and can also affect Na_V expression levels (Catterall, 2011, 2014; Erickson et al., 2018).

Intrarectal administration of lidocaine, which inhibits fast sodium currents, reduces rectal sensitivity and abdominal pain in IBS patients, suggesting that localised Na_V channel inhibition may be a therapeutic strategy for alleviation of IBS pain (Verne et al., 2005). The particular Na_V isoforms that effectively mediate this effect are unknown; however, up-regulation of $Na_V1.7$ expression in rectal sensory fibres from biopsies of patients with rectal hypersensitivity has been reported and may be an important isoform in pathological pain signalling (Yiangou et al., 2007). In contrast, the simultaneous activation of all Na_V channels during ciguatoxin poisoning causes abdominal pain in humans and induces pain behaviours in mice (Inserra et al., 2017). Co-administration of a $Na_V1.8$-selective antagonist with ciguatoxin significantly reduced visceral pain responses in mice, an effect which was greater than co-administration of ciguatoxin and tetrodotoxin (TTX; which blocks $Na_V1.1$–$Na_V1.4$, $Na_V1.6$ and $Na_V1.7$), suggesting that $Na_V1.8$ has a predominant role in visceral pain signalling (Erickson et al., 2018; Inserra et al., 2017; Jami et al., 2018). Correspondingly, $Na_V1.8$ has been suggested to play a role in pathological pain signalling from the viscera in cases of colitis, as well as bladder

hypersensitivity and pancreatitis (Beyak et al., 2004; Chen et al., 2012; Gebhart et al., 2002; King et al., 2009; La and Gebhart, 2011; Yoshimura et al., 2001).

More recently, functional studies of colonic afferents in mice have revealed that $Na_V1.1$ plays a crucial role in the signalling of mechanical hypersensitivity (Osteen et al., 2016). Application of the highly selective $Na_V1.1$-agonist, δ-theraphotoxin-Hm1a (Hm1a), enhanced mechanically evoked firing in a subpopulation of high-threshold colonic nociceptors, which was blocked by incubation with the $Na_V1.1$/$Na_V1.3$ antagonist ICA-121431 (Osteen et al., 2016). Furthermore, Hm1a also induced hyper-excitability of isolated colon-innervating DRG neurons from healthy control mice (Osteen et al., 2016). Importantly, colon-innervating DRG neurons isolated from mice with chronic visceral hypersensitivity (CVH) showed significantly enhanced responsiveness to Hm1a compared to healthy control mice, suggesting that $Na_V1.1$ may be essential for the development and maintenance of CVP (Osteen et al., 2016). Accordingly, antagonism of $Na_V1.1$ may be a future target for the treatment of CVP.

More recent studies show a key role for $Na_V1.3$ in non-neuronal tissues, specifically within enterochromaffin cells located within the intestinal epithelium (Bellono et al., 2017; Strege et al., 2017). Voltage-gated sodium currents generated by $Na_V1.3$ likely allow enterochromaffin cells to respond to the detection of mechanical and chemical stimuli within the lumen of the intestine (Bellono et al., 2017; Strege et al., 2017).

3.2: Voltage-gated potassium channels

Voltage-gated potassium (K_V) channels are divided into twelve subfamilies and play important roles in repolarising the membrane following action potential firing, regulation of the refractory period, and resting membrane potential. The precise roles of K_V isoforms in pain signalling is not well understood, and is further complicated by heteromultimerisation of subunits (Wulff et al., 2009); however, the study of specific potassium-type currents has been able to identify roles of certain K_V families in visceral pain signalling.

A-type potassium current (I_A) density is downregulated in IB4-positive colon-innervating neurons from rats with colonic hypersensitivity, and in rodent sensory neurons

during inflammatory colonic hyperalgesia, functional dyspepsia or pancreatitis (Ibeakanma et al., 2009; Li and Chen, 2014; Qian et al., 2009; Xu et al., 2006). Delayed rectifier potassium current (I_K) is downregulated alongside I_A in several visceral pain models, and in colonic neurons from rats with stress-induced visceral hypersensitivity (Luo et al., 2011). However, in the case of post-infectious colonic hypersensitivity, I_K density suppression can recover, whereas the reduction in I_A density is maintained (Ibeakanma et al., 2009). In contrast, in the case of a rat model of pancreatitis, I_A density was reduced without accompanying changes in I_K density (Xu et al., 2006). While the specific isoforms responsible for the suppression of these potassium currents remain to be investigated, it has been reported that both $K_V4.3$ (I_A) and $K_V1.2$ (I_K) expression were reduced in colonic neurons from rats with colonic hypersensitivity (Luo et al., 2011; Qian et al., 2009). Administration of retigabine, which activates $K_V7.2$–$K_V7.5$ of the K_V7 family, was effective in reducing capsaicin-induced colonic pain in rats (Hirano et al., 2007) and bradykinin-induced visceral nociceptive firing in mice (Peiris et al., 2017). Inhibition of K_V7 using XE991 potentiated the visceral afferent firing, further supporting a role of K_V7 channels in visceral signalling (Peiris et al., 2017). Involvement of other potassium channels, such as the mechanosensitive two-pore domain K^+ (K2P) channels have also been implicated in pain (Mathie and Veale, 2015). This has been inferred for colonic hypersensitivity by the altered expression profile of K2P channels within colon-innervating DRG neurons in mice with colitis (La and Gebhart, 2011). However, the precise functional contribution of K2P channels in colonic hypersensitivity remains to be investigated.

3.3: Voltage-gated calcium channels

Voltage-gated calcium (Ca_V) channels mediate depolarisation-induced calcium influx into neurons and other excitable cells. Ca_V channels are composed of a pore-forming α1 subunit (Ca_V1, Ca_V2, and Ca_V3) and can associate with auxiliary β and α2δ subunits (Zamponi et al., 2015). Ca_V channels are an important target for pain management due to their regulatory role in neurotransmitter release at nociceptive synapses. For example, Ca_V channels can initiate numerous signalling events, including gene transcription, neurotransmitter release and activation of calcium-dependent kinases, such as calcium calmodulin-dependent kinase II (CaMKII) and protein kinase C (PKC), in addition to modulating the membrane potential (Catterall, 2011; Clapham, 2007; Simms and Zamponi, 2014). These Ca_V channels have been

classified based on their current properties into low voltage-activated (LVA: T-type) channels that require moderate membrane depolarisation to open, and high voltage-activated (HVA) channels that require greater depolarisation and are sub-classified into L- ($Ca_V1.1$–4), P/Q- ($Ca_V2.1$), N- ($Ca_V2.2$) and R- ($Ca_V2.3$) type channels.

$Ca_V2.2$ and $Ca_V2.3$ are co-expressed in more than 80% of colon-innervating DRG neurons (Castro et al., 2017b). During colitis, increased mRNA expression of $Ca_V1.2$ and $Ca_V2.3$, but not $Ca_V2.2$, four days after induction of colitis has been reported (Qian et al., 2013), whereas in a CVH mouse model, up-regulation of the $Ca_V2.2$ exon 37a variant has been identified in colon-innervating DRG neurons (Castro et al., 2017b). This is particularly interesting, as exon 37a plays a crucial role in neuropathic and inflammatory pain (Altier et al., 2007). Correspondingly, blockers of $Ca_V2.2$ have increased inhibitory actions on colonic afferents in CVP states (Castro et al., 2017b). Increased $Ca_V3.2$ expression in a model of butyrate-induced colonic hypersensitivity has been reported and may contribute to the maintenance of visceral pain (Marger et al., 2011). Voltage-gated calcium channels also couple to various G-protein coupled receptors, as detailed below, which means that they can be inhibited via indirect mechanisms (Castro et al., 2017b).

Gabapentin and pregabalin, both ligands of the α2δ subunit of Ca_V channels, reduces visceral hypersensitivity to colorectal distention in IBS patients as well as in animal visceral pain models (Diop et al., 2002; Feng et al., 2003; Houghton et al., 2007; Lee et al., 2005; Million et al., 2007; Ravnefjord et al., 2008). These effects are likely due to expression of α2δ subunits in intestinal smooth muscle, and neurons within the DRG and CNS (Taylor and Garrido, 2008). Whilst clinical studies show that gabapentin and pregabalin improved symptoms of abdominal pain, bloating and urgency, the central side effects of these compounds may limit broader therapeutic applications.

3.4: Transient receptor potential channels: Primary and secondary integrators for CVP

Transient receptor potential (TRP) channels constitute a superfamily of non-selective ion channels that can be characterised as ankyrin repeat (TRPA), canonical (TRPC), melastatin (TRPM), mucolipin (TRPML), polycystin (TRPP) or vanilloid (TRPV). Whilst their overall role in

gastrointestinal function has been expertly reviewed elsewhere (Balemans et al., 2017), we highlight the importance of TRPV1, TRPA1, and TRPV4 members in the pathogenesis of visceral hypersensitivity (Balemans et al., 2017; Blackshaw et al., 2010; Zielinska et al., 2015b). Importantly, these TRPs contribute directly to visceral hypersensitivity but are also downstream integrators of signalling pathways resulting from activation of several classes of receptor (as discussed in detail below).

3.4.1: TRPV1

TRPV1 is highly expressed on extrinsic afferents and on small and medium diameter neurons in the DRG that give rise to thinly myelinated Aδ-fibres and unmyelinated C-fibres. This is an important functional distinction, as these fibres represent key classes of nociceptive afferents, particularly within cutaneous pathways. Within sensory pathways innervating the colon, C-fibres represent the vast majority of spinal afferents (Brierley et al., 2009; Brierley et al., 2008; Jones et al., 2005). In line with the expression profile of TRPV1, responses to colorectal distension are reduced in TRPV1 knock-out ($^{-/-}$) mice and by TRPV1 receptor antagonists in animal models of visceral hypersensitivity (De Schepper et al., 2008; Jones et al., 2005; van den Wijngaard et al., 2009; Vermeulen et al., 2013; Wiskur et al., 2010). In addition, TRPV1 responses are potentiated by a myriad of endogenous mediators, including histamine, 5-HT, proteases, leukotriene B_4, adenosine triphosphate, bradykinin and prostaglandins (Amadesi et al., 2004; Holzer, 2008; Kajihara et al., 2010; Sugiuar et al., 2004; Szallasi et al., 2007; Wouters et al., 2016). Therefore, TRPV1 is often regarded as the integrator or orchestrator of peripheral sensory input, serving as a common target in the modulation of visceral hypersensitivity. TRPV1 involvement is further corroborated by findings of increased expression of TRPV1-positive sensory fibres in colonic biopsies of patients with IBS or quiescent IBD with IBS-like symptoms, which correlated with patient abdominal pain scores (Akbar et al., 2010; Akbar et al., 2008; Zhou et al., 2016). Recently, it was reported that increased colonic TRPV1 expression in patients with diarrhoea–predominant IBS (IBS-D) results from decreased colonic levels of the microRNA miR-199 (Zhou et al., 2016). Moreover, administration of a lentivirus expressing an miR-199 precursor subsequently reversed visceral nociception in an animal model of visceral hypersensitivity, indicating the potential of miRNA-based therapy to modulate downstream targets such as TRPV1 (Zhou et al., 2016). This might provide a viable

alternative approach as clinical trials with TRPV1 antagonists have been hampered by severe adverse events such as malignant hyperthermia (Brederson et al., 2013).

3.4.2: TRPA1

TRPA1 is preferentially expressed on a subset of small and medium diameter neurons of the vagal, splanchnic, and pelvic afferent pathways (Brierley et al., 2009), and therefore represents a more specific pharmacological target than TRPV1. Preclinical results show that TRPA1 mRNA expression is up-regulated in biopsies of IBD patients, whilst TRPA1 antagonists or deletion of TRPA1 effectively reduced visceral nociception in animal models (Brierley et al., 2009; Cattaruzza et al., 2010; Kun et al., 2014; Mueller-Tribbensee et al., 2015; Vermeulen et al., 2013). Conversely, TRPA1 agonists induce mechanical hypersensitivity of colonic afferents (Brierley et al., 2009) and both TNF-α and bradykinin induce colonic afferent mechanical hypersensitivity via a TRPA1-dependent mechanism (Brierley et al., 2009; Hughes et al., 2013a). In addition to these effects on neurons, TRPA1 can also contribute to the inflammatory response itself, via neurogenic inflammation, as activation and sensitisation of TRPA1 and release of substance P induces and maintains colitis in mice (Engel et al., 2011), which correspondingly re-sensitises nociceptors. TRPA1 is also expressed by intestinal enterochromaffin cells, where it serves as the primary detector of luminal irritants prior to direct sub-mucosal damage (Bellono et al., 2017).

3.4.3: TRPV4

TRPV4 is another important sensory integrator present on colonic sensory fibres and DRG neurons. Mechanosensory responses of colonic high-threshold afferents, but not vagal gastro-oesophageal afferents, can be enhanced by TRPV4 agonists (5,6-EET) and reduced by targeted deletion or antagonism of TRPV4 channels in animal models (Brierley et al., 2008; Sipe et al., 2008). Correspondingly, colonic high-threshold afferents, but not vagal gastro-oesophageal afferents from TRPV4$^{-/-}$ mice display deficits in mechanosensory function (Sipe et al., 2008). TRPV4 expression and responses can be potentiated by pro-nociceptive mediators, such as histamine and serotonin (5-HT), in addition to protease-activated receptor 2 (PAR_2) modulation (Cenac et al., 2010; Cenac et al., 2007; Cenac et al., 2015; Sipe et al., 2008). Specifically, 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). PAR_2-activating peptides evoke mechanical hypersensitivity of colonic afferents and visceral hyperalgesia *in vivo*, which is lost in $TRPV4^{-/-}$ mice (Sipe et al., 2008). Endogenous levels of TRPV4 agonists, such as 5,6-EET, are increased in colonic biopsies from IBS patients and correlate with their abdominal pain scores (Cenac et al., 2015). When administered in an animal model, supernatants from colonic biopsies from IBS patients resulted in further production of endogenous TRPV4 agonists involving a PAR_2-mediated mechanism, and activation of sensory neurons and visceral hypersensitivity *in vivo* that could be inhibited by silencing TRPV4 signalling (Cenac et al., 2015). TRPV4 can also contribute to the inflammatory response itself by inducing neurogenic inflammation, with neuronal TRPV4 activation stimulating neuropeptide release from peripheral afferent terminals (Vergnolle et al., 2010). TRPV4 is also expressed on intestinal epithelial cells, where its activation induces chemokine release and colitis (D'Aldebert et al., 2011).

3.5: Protease-activated receptor 2

Protease-activated receptors (PARs) are a family of four GPCRs that are typically activated by proteolytic cleavage of their N-terminal extracellular domain, releasing a new motif from the N-terminal tail that binds to the receptor in an agonist-like fashion. However, many proteases, including cathepsins and trypsin, can cleave PARs at divergent sites resulting in biased agonism that can lead to unique pathophysiological outcomes (Zhao et al., 2014). Colon-innervating DRG neurons express PAR_2 and activation of PAR_2 enhances visceral sensory signalling (Auge et al., 2009). Elevated luminal serine protease activity as well as increased tryptase release has been reported in colonic samples from IBS patients (Barbara et al., 2004; Cenac et al., 2007; Tooth et al., 2014). When administered into the colon of healthy mice, these proteases induced visceral allodynia and hyperalgesia via a PAR_2-dependent mechanism, with hypersensitivity prevented by a PAR_2 antagonist, and absent in $PAR_2^{-/-}$ mice (Cenac et al., 2007; Coelho et al., 2002; Kawabata et al., 2006). That PAR_2-mediated effects sensitise afferent nerve endings at the peripheral level is further corroborated by recent findings demonstrating that supernatants derived from IBS-D as well as constipation-predominant IBS (IBS-C) biopsies increased excitability of colonic sensory DRG neurons in mice, again an effect that was absent in $PAR_2^{-/-}$ animals (Valdez-Morales et al., 2013). Also in animal models of IBD, increased luminal levels of PAR_2-agonists, particularly cathepsin-S, have been reported, resulting in increased

PAR_2-mediated activation of spinal nociceptors and visceral hypersensitivity (Cattaruzza et al., 2011). More recently, in IBS it has been shown that the intestinal epithelium produces and releases the active protease trypsin-3, which is able to signal to human submucosal enteric neurons and mouse sensory neurons, and induce visceral hypersensitivity *in vivo*, all by a PAR_2-dependent mechanism (Rolland-Fourcade et al., 2017).

3.6: Histamine receptors

Histamine, the main mast cell mediator, is a powerful modulator of sensory afferent activity in the gut wall, contributing to visceral hypersensitivity in CVP (Barbara et al., 2007; Brunsden and Grundy, 1999; Buhner et al., 2009; Kajihara et al., 2010; Kreis et al., 1998). IBS biopsy supernatant-induced excitation of rat jejunal afferents and DRG neurons is reduced by a histamine H_1 receptor (H1R) antagonist, indicating that the pro-nociceptive effect of histamine is, at least partially, mediated by H1R expressed on sensory afferent nerves (Barbara et al., 2007). Coupling of H1R to TRPV1 and TRPV4 in turn modulates sensory signalling to the spinal cord (Cenac et al., 2010; Wouters et al., 2016). *In vivo*, selective H1R antagonists significantly reduce visceral hypersensitivity in animal models of IBS and IBD (Deiteren et al., 2014; Goldhill et al., 1998; Stanisor et al., 2013). Moreover, the clinical potential of specific H1R-targeted therapy was recently confirmed in a randomised, double-blind, placebo-controlled, single-centre clinical trial in which ebastine, a selective H1R antagonist, reduced abdominal pain and improved quality of life in IBS patients (Wouters et al., 2016).

In addition to H1R, histamine H_4 receptors (H4R) most likely also participate in the modulation of visceral sensation as the H4R agonist 4-methylhistamine excites human submucous plexus neurons, an effect that was inhibited by the selective H4R antagonist JNJ7777120 (Breunig et al., 2007). Moreover, this antagonist dose-dependently reduced visceral hypersensitivity in a post-inflammatory rat model of IBS (Deiteren et al., 2014). Interestingly, simultaneous administration of H1 and H4R antagonists *in vivo* potentiates their anti-nociceptive effects, indicating a functional interaction between the receptor subtypes that warrants further investigation (Deiteren et al., 2014).

3.7: Serotonin

The majority of 5-HT in the body is found in the gastrointestinal tract, primarily contained within enterochromaffin cells and is released following ingestion of a meal or by toxins and chemotherapeutic agents (Andrews et al., 1990; Bearcroft et al., 1998). More recently, it has been demonstrated that microbial metabolites, as well as norepinephrine, results in the release of 5-HT from enterochromaffin cells in the intestine and colon that activates afferents innervating the mucosa via a 5-HT_3 receptor mechanism (Bellono et al., 2017). These findings demonstrate that enterochromaffin cells are gut chemosensors that couple to sensory neural pathways via serotonergic signalling (Bellono et al., 2017).

In the rat, 56% of high-threshold splanchnic colonic afferents respond to 5-HT, via both 5-HT_3 and non-5-HT_3 receptors (Hicks et al., 2002). In the rat, 5-HT_1, 5-HT_2 and 5-HT_3 receptor subtypes have been demonstrated to modulate responses to noxious colorectal distension (Danzebrink and Gebhart, 1991), and serotonergic activation of viscera sensory neurons may increase their sensitivity to other sensory modalities (Sugiura et al., 2004). Following intestinal inflammation, the contribution of 5-HT_3 receptors is decreased, yet the responsiveness to 5-HT is increased, indicating other 5-HT receptor subtypes play an increased role in disease states (Coldwell et al., 2007). Correspondingly, later studies demonstrated that peripherally administered 5-HT_4 receptor agonists have anti-nociceptive actions whilst also enhancing gastrointestinal motility (prokinetic actions) and reducing colonic inflammation (Hoffman et al., 2012; Spohn et al., 2016). In terms of translation to humans, metabolism of 5-HT appears disrupted in both IBS and IBD patients (Coates et al., 2004). The involvement of 5-HT in IBS patient symptomatology is implicated by increased numbers of enterochromaffin cells (Spiller et al., 2000), increased mast cell populations (Barbara et al., 2004; Ziino et al., 2010), increased post-prandial 5-HT release (Bearcroft et al., 1998; Houghton et al., 2003), and a decrease in symptoms with use of 5-HT_3 receptor or 5-HT_4 receptor antagonists (Camilleri, 2001; Camilleri et al., 2000).

3.8: Tachykinin Receptors

Tachykinins are endogenous peptides that activate the G protein-coupled tachykinin receptors (NK_1R, NK_2R, and NK_3R) and include substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) (Holzer and Holzer-Petsche, 2001). NK receptors are widely expressed in

the central, peripheral, and enteric nervous systems, as well as in non-neuronal cells of the gastrointestinal tract. In addition to sensory signalling, these receptors have roles in both gut motility and secretion (Corsetti et al., 2015). $NK_1R^{-/-}$ mice displayed deficits in spontaneous behavioural responses to intracolonic capsaicin (TRPV1 agonist), but not intracolonic mustard oil (TRPA1 agonist), and developed referred hyperalgesia and tissue oedema only after exposure to mustard oil (Laird et al., 2000). This suggest that TRPV1-dependent visceral pain signalling requires NK_1R, whereas TRPA1-dependent visceral nociception does not. Antagonism of the three NK receptors showed efficacy in animal models of visceral pain and inflammatory-induced hyperalgesia (Giamberardino, 2009; Sanger, 2004; Sengupta, 2009), and selective antagonism of NK_2R resulted in a reduction in intestinal tissue damage following intracolonic administration of chemical irritants in rodents (Lecci et al., 2004). Altered expression of tachykinins and NKRs in the gastrointestinal tract during functional gastrointestinal diseases has been reported (Holzer, 1998). Positron emission tomography (PET) studies suggest that patients with IBD and IBS have reduced NK_1R availability in specific brain areas, which resembles that seen in individuals with injury-related chronic pain, suggesting central changes involving tachykininergic signalling in CVP conditions (Jarcho et al., 2013).

3.9: Purine receptors

Ligand-gated P2X, and G-protein-coupled P2Y receptors, as well as their endogenous agonists, ATP, UTP and ADP are ubiquitously expressed within the extrinsic and enteric sensory structures of the gastrointestinal tract and are essential to immune cell function. As such, a role for purinergic signalling in the modulation and maintenance of CVP states has been explored by a number of research groups. In an animal model of trinitrobenzene sulfonic acid (TNBS)-induced acute colitis there was up-regulation of P2X3 and P2X2/3 receptors on colon-innervating DRG neurons, and an increase in the release of ATP, which was more pronounced during distension (Wynn et al., 2004). Similarly, in an animal model of IBS, there was increased P2X3 expression in colonic sensory neurons, potentiation of ATP-evoked responses (Xu et al., 2008) and altered purinergic signalling (Shinoda et al., 2010). This is associated with visceral hypersensitivity that can be reduced by a selective P2X3 antagonist (Deiteren et al., 2015). Also, in rodent models of post-inflammatory IBS, intestinal afferent hypersensitivity may be due to the P2X2/3 receptor activation (Deiteren et al., 2014; Rong et al., 2009).

Activation of P2Y receptors can sensitise both mouse and human colonic nociceptors. The application of UTP (P2Y2 and P2Y4 agonist) sensitised colonic sensory neurons by increasing action potential firing in response to current injection and depolarising the membrane potential. The application of ADP (P2Y1, P2Y12 and P2Y13 agonist) also increased action potential firing, an effect blocked by a selective P2Y1 receptor antagonist. P2Y1 and P2Y2 transcripts were detected in 80% and 56% of colon-innervating DRG neurons, respectively, and were predominately co-localised with $Na_V1.9$ (Hockley et al., 2016).

3.10: Glutamate receptors

Glutamate is a major excitatory neurotransmitter in the CNS that is best known for its effect on the ionotropic N-methyl-D-aspartate (NMDA) receptor. The calcium permeable NMDA receptors are also found on the peripheral terminals of primary afferent neurons that innervate the colon and release substance P and calcitonin gene-related peptide when activated (McRoberts et al., 2001). In addition, blocking of NMDA receptors by peripherally administered ketamine, an NMDA antagonist, reduced visceromotor pain responses to noxious colorectal distension in healthy control rats (Shafton et al., 2007). NMDA receptors also contribute to the phenomenon of 'wind-up', which refers to a progressively increasing activity of the dorsal horn neurons in response to afferent C-fibre stimulation that results in central sensitisation. Development of central sensitisation can manifest clinically as somatic as well as visceral hypersensitivity in a subset of IBS patients and can be blocked by an NMDA-antagonist (Eide, 2000; Zhou et al., 2010).

In addition to NMDA receptors, glutamate activates two other ionotropic receptor subtypes, the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and the kainate receptors, as well as eight types of metabotropic glutamate (mGlu) receptors. While their involvement in neuropathic and/or somatic pain has been investigated, studies on their role in visceral pain are scarce (Kannampalli and Sengupta, 2015). However, there is evidence for the involvement of peripheral mGluR5 in mediating mechanically evoked colonic pain, as mGluR5 antagonists inhibit action potential firing from colonic afferent endings and reduce colorectal distension-induced visceromotor responses *in vivo* (Lindstrom et al., 2008).

4. ANTI-NOCICEPTIVE MECHANISMS

A summary of key anti-nociceptive mechanisms within extrinsic sensory afferent neurons innervating the colorectum and epithelial cells is shown in Figure 3.

4.1: Oxytocin receptors

The central analgesic effect of the nine-amino acid peptide hormone oxytocin is well known, involving several brain regions implicated in the pathophysiology of CVP, such as the periaqueductal gray and the nucleus of the solitary tract (Black et al., 2009; Meyer-Lindenberg et al., 2011). However, there is strong evidence that oxytocin also reduces visceral sensory perception through modulation of afferent nerve activity at the level of the gut wall. First, oxytocin receptors are significantly up-regulated in colon-innervating thoracolumbar DRG neurons of mice in a model of CVH, whereas oxytocin receptors are absent from these neurons in healthy states (de Araujo et al., 2014). Second, oxytocin and synthetic oxytocin analogues inhibit colonic nociceptors in *ex vivo* preparations from CVH mice, but not healthy control mice (de Araujo et al., 2014; Wan et al., 2016). Notably, these inhibitory effects of oxytocin can be blocked by pre-application of an oxytocin receptor antagonist (de Araujo et al., 2014). Third, intracolonic administration of synthetic oxytocin analogues *in vivo* inhibits colonic nociceptor mechanosensitivity, resulting in reduced nociceptive signalling in the spinal cords of CVH mice (de Araujo et al., 2014).

These findings of analgesic oxytocin action during CVH are in keeping with a small trial of 26 IBS patients. In these patients, continuous intravenous infusion of oxytocin increased thresholds of colonic perception, an analgesic effect suggested, but not confirmed, to be mediate by oxytocin acting at the level of visceral afferents (Louvel et al., 1996). Similarly, in a placebo-controlled pilot trial in chronic constipation patients, nasal oxytocin administration had no effect on constipation itself, but had a positive effect on abdominal pain and discomfort and depressed mood (Ohlsson et al., 2005). In contrast, and in support of pre-clinical data showing no effect of oxytocin in healthy mice, healthy subjects infused with oxytocin exhibited no change in sensory thresholds to colonic distension (Ohlsson et al., 2004). Oxytocin also has other potential analgesic affects within the viscera, as it has been shown to reduce jejunal mesenteric afferent mechanosensitivity *in vitro,* an effect that was shown to be mediated through the nNOS-K_{ATP} pathway (Li et al., 2015). Central oxytocin administration in animal

models has varying effects on gut motility, depending on the species and region studied (Matsunaga et al., 2009; Qin et al., 2009), and also inhibits chronic colitis (Welch et al., 2010) by a neutrophil-dependent mechanism (Iseri et al., 2005).

4.2: GABA Receptors

Gamma-aminobutyric acid (GABA) is an inhibitory neurotransmitter that plays a crucial role in the processing of visceral, neuropathic and somatic pain, via ionotropic $GABA_A$/$GABA_C$ and metabotropic $GABA_B$ receptors that are distributed along the neuraxis (McCarson and Enna, 2014). An increase in the number of activated GABAergic neurons in the dorsal horn of the spinal cord in CVH mice suggests that neuroplasticity of the GABAergic system may contribute to CVP (Harrington et al., 2012). Whether these changes were specific to a particular GABA receptor subtype remains unknown. In a mouse model of colonic hypersensitivity resulting from cross-sensitisation after acute zymosan-induced cystitis, intrathecal administration of a $GABA_A$ agonist reduced pain behaviour responses (Sengupta et al., 2013). However, the bulk of the evidence implicating GABA receptors in the modulation of CVP focuses on $GABA_B$ receptors ($GABA_BR$). Multiple studies have demonstrated anti-nociceptive effects of baclofen, the archetypal $GABA_B$ receptor agonist, on colonic afferent nerve firing *in vitro* and visceromotor responses *in vivo* (Brusberg et al., 2009; Castro et al., 2017b; Lindstrom et al., 2011; Sengupta et al., 2002). The α-conotoxin Vc1.1, isolated from the venom of the marine cone snail *Conus victoriae* and a potent activator of $GABA_B$ receptors, inhibits nociceptive signalling of colonic afferents from healthy mice (Carstens et al., 2016; Castro et al., 2017a; Castro et al., 2017b), but has greater efficacy in a model of CVH (Castro et al., 2017a; Castro et al., 2017b). Human DRG neurons express $GABA_B$ receptors and the voltage-gated calcium channels $Ca_V2.2$, and $Ca_V2.3$, which are the direct and downstream targets of Vc1.1. Colonic DRG neurons from CVH mice exhibit up-regulation of the $Ca_V2.2$ exon-37a variant, which may explain the increased inhibitory effect of Vc1.1 in hypersensitivity compared with control mice (Castro et al., 2017b). Correspondingly, Vc1.1 inhibits neuronal excitability in a sub-population of human DRG neurons (Castro et al., 2017b). Enhancing the potency of endogenous GABA through positive allosteric modulation of the GABA receptor (McCarson and Enna, 2014) has also been shown to affectively reduce the visceromotor response to colorectal distension in healthy control rats (Brusberg et al., 2009).

4.3: Opioid receptors

Opioid receptors μ, δ, and κ (μ-OPR, δ-OPR and κ-OPR) mediate pain relief when activated by endogenous enkephalins, β-endorphin, and dynorphin, and exogenous opioids, such as morphine, oxycodone, and fentanyl. Typically, activation of these receptors results in inhibition of adenylyl cyclase, downstream inhibition of $Ca_V2.2$ channels and activation of inwardly rectifying potassium channels, resulting in decreased neuronal excitability. However, the use of opioids such as morphine, oxycodone, and fentanyl for the treatment of chronic pain is plagued by addiction, tolerance and significant side effects, such as constipation. In the gut, μ, δ and κ opioid receptors are present in the enteric nervous system (Wood and Galligan, 2004), pacemaker cells (Bagnol et al., 1997) and smooth muscle cells (Kuemmerle and Makhlouf, 1992). Enteric neurons expressing μ-OPR contribute to opioid-induced constipation where local μ-OPR activation reduces secretion and motility (Sternini et al., 1996). δ-OPR expression is localized to submucosal and myenteric neurons, which likely account for the ability of DOR agonists to inhibit intestinal secretion and motility (Galligan and Sternini, 2017; Hughes et al., 2016; Poole et al., 2011). Moreover, κ-OPRs are also essential in modulating gastrointestinal motility, and secretion (Galligan and Sternini, 2017; Hughes et al., 2016).

Up-regulation of μ-opioid receptors and β-endorphin in a mouse model of chronic colitis (Verma-Gandhu et al., 2007), may explain why some IBD patients do not report pain, depending on the type and the severity of inflammation. In contrast, there is evidence for a decrease in the mRNA expression of μ-OPR and κ-OPR in intestinal biopsies from IBS-D patients (Zielinska et al., 2015a), whilst immune-derived opioidergic inhibition of viscerosensory afferents is decreased in IBS patients (Hughes et al., 2014b). In contrast, κ-OPRs are functionally up-regulated in colon-innervating afferents during and following recovery from TNBS-induced colitis in mice (Brust et al., 2016; Hughes et al., 2014a; Sengupta et al., 1999). κ-OPR agonism reduces colonic afferent responses in a mouse model of chronic visceral hypersensitivity, (Brust et al., 2016; Hughes et al., 2014a) and nociceptive responses in animal models of colitis (Davis, 2012; Gebhart et al., 2000; Sengupta et al., 1999). Peripherally restricted κ-OPR agonists, may be suitable for IBS pain treatment, as asimadoline was shown to effectively reduce pain, bowel movement frequency and urgency in IBS patients (Camilleri, 2008), and decrease pain perception in response to colonic distension (Delvaux et al., 2004; Mangel et al., 2008), without altering colonic distension tolerances in healthy volunteers

(Delgado-Aros et al., 2003). More recently, eluxadoline, a mixed μ-OPR and κ-OPR agonist/δ-OPR antagonist improved the global symptoms, stool consistency, frequency and urgency in IBS-D patients (Lembo et al., 2016).

4.4: TRPM8

TRPM8 is activated naturally by cold temperatures, menthol, a constituent of peppermint, as well as synthetic TRPM8 agonists such as icilin. Several products containing peppermint are reported to reduce symptoms of bowel hypersensitivity (Brierley and Kelber, 2011). Whilst part of this action may be due to an anti-spasmodic effect, a key mechanism is the expression of TRPM8 on colonic afferents, where it is co-expressed with TRPV1 (Harrington et al., 2011). TRPM8 was shown to couple to TRPV1 and TRPA1 to inhibit their downstream chemosensitivity and mechanosensitivity, thus reducing overall afferent excitability (Harrington et al., 2011). Furthermore, TRPM8 activation attenuates inflammatory responses in a mouse model of colitis, which is due in part to inhibition of neuropeptide release from colonic tissue (Ramachandran et al., 2013).

4.5: PAR_4

In contrast to the pro-nociceptive action of PAR_2 activation, activation of PAR_4 exerts anti-nociceptive effects. Intracolonic infusion of a PAR_4 agonist reduced visceral pain perception to colorectal distention, whilst activation of PAR_4 reduced PAR_2- and TRPV4-induced Ca^{2+} mobilisation in DRG neurons (Annahazi et al., 2009; Auge et al., 2009). In addition, visceral hyposensitivity induced by the intracolonic infusion of supernatant from colonic biopsies harvested from patients with ulcerative colitis is mimicked by PAR_4-activating peptides and inhibited by a cocktail of anti-proteases (Annahazi et al., 2009). Recent findings of decreased expression of PAR_4 in IBS patients further supports the role of PAR_4 in modulating visceral pain (Han et al., 2012; Zhao et al., 2012).

4.6: Cannabinoid receptors

Cannabinoid (CB) receptors comprise CB1 and CB2, which are both GPCRs. CB1 receptors are primarily distributed within the CNS but are also found in both the enteric nervous system and in primary afferents innervating the gastrointestinal tract. CB2 receptors

are expressed in most enteric neurons and peripheral immune cells (Duncan et al., 2008; Storr et al., 2008b). The endogenous agonists of these receptors, endocannabinoids, are involved in controlling motility, secretion and intestinal inflammation (Duncan et al., 2005; Massa et al., 2005). DRG neurons from rats with stress-induced visceral hypersensitivity levels have increased levels of anandamide, and decreased levels of endocannabinoid degradation enzymes COX2 and FAAH, decreased CB1 receptor expression, but reciprocal increases in TRPV1 expression (Hong et al., 2009).

CB1 and CB2 receptors are also implicated in regulating inflammation; mice lacking CB1 or CB2 receptors experience more severe colitis than wild-type mice (Engel et al., 2011; Massa et al., 2004; Sibaev et al., 2006). Conversely, activation of CB1 or CB2 receptors has a protective effect in colitis animal models (Kimball et al., 2006; Sibaev et al., 2006; Storr et al., 2009). Furthermore, TNBS-induced colitis severity in mice is significantly reduced in the presence of a FAAH inhibitor (URB597) and a membrane transporter inhibitor (VDM11), which elevate endocannabinoid levels (Storr et al., 2008a). FAAH inhibitors have also been shown to reduce mouse colonic motility and decrease visceral pain in a mouse model of IBD (Fichna et al., 2014).

4.7: Guanylate cyclase-C

Guanylate cyclase-C (GC-C) is a transmembrane receptor predominantly found in the intestinal epithelium. Linaclotide, a synthetic and minimally absorbed, 14-amino acid peptide, is a GC-C agonist related to guanylin and uroguanylin, members of a family of naturally-occurring peptide hormones (Schulz et al., 1990). These hormones regulate intestinal fluid and electrolyte homeostasis and consequently bowel function, through GC-C-mediated production of cyclic-guanosine-3′,5′-monophosphate (cGMP)(Pfeifer et al., 1996). Linaclotide acts via the same mechanism as the endogenous hormones, through binding and activating GC-C located on the luminal surface of intestinal epithelial cells, but it is not expressed by sensory DRG neurons (Castro et al., 2013). This interaction elevates intracellular and extracellular levels of cGMP, inducing fluid secretion and accelerating intestinal transit in animal models (Brierley, 2012; Bryant et al., 2010; Busby et al., 2010). In addition, linaclotide has been shown to elicit anti-hyperalgesic effects in several animal models of visceral pain (Eutamene et al., 2010). This effect appears to be independent of changes in colonic compliance or motility. Subsequently,

linaclotide has been demonstrated to inhibit colonic nociceptors with greater efficacy in a mouse model of CVH (Castro et al., 2013). Furthermore, intra-colonic administration of linaclotide reduces signalling of noxious colorectal distention to the spinal cord. (Castro et al., 2013). The downstream effector of GC-C, cGMP, is released following administration of linaclotide and also inhibits nociceptors (Castro et al., 2013). The effects of linaclotide are lost in Gucy2c$^{-/-}$ mice and prevented by inhibiting cGMP transporters or removing the mucosa. This suggests the anti-nociceptive actions of linaclotide are due to activation of GC-C expressed on mucosal epithelial cells, resulting in the production and release of cGMP. This extracellular cGMP acts on and inhibits nociceptors, thereby reducing nociception (Castro et al., 2013). These pharmacological effects of linaclotide have translated into the clinic. In Phase II and Phase III studies, linaclotide accelerated colonic transit and improved abdominal pain and constipation associated with IBS-C and chronic idiopathic constipation (Castro et al., 2013; Chey et al., 2012; Johnston et al., 2010; Rao et al., 2012).

CONCLUSIONS

A variety of membrane receptors and ion channels contribute to the signalling of visceral pain in normal, inflammatory and chronic visceral pain states. The upregulation of specific pro-nociceptive mechanisms during CVP states helps us understand the underlying basis of visceral hypersensitivity. Accordingly, targeting receptors or ion channels involved in visceral pain at the periphery would be beneficial in terms of reducing side effects and providing novel opportunities for the pharmacological treatment of CVP and clinical conditions such as IBD and IBS. Correspondingly, the increase in anti-nociceptive mechanisms also present options for pharmacological treatment. Increased understanding of the full complement of changes in receptors and ion channels during CVP would further aid in this process.

REFERENCES:

Akbar, A., Yiangou, Y., Facer, P., Brydon, W.G., Walters, J.R., Anand, P., Ghosh, S., 2010. Expression of the TRPV1 receptor differs in quiescent inflammatory bowel disease with or without abdominal pain. Gut 59(6), 767-774.

Akbar, A., Yiangou, Y., Facer, P., Walters, J.R., Anand, P., Ghosh, S., 2008. Increased capsaicin receptor TRPV1-expressing sensory fibres in irritable bowel syndrome and their correlation with abdominal pain. Gut 57(7), 923-929.

Almeida, T.F., Roizenblatt, S., Tufik, S., 2004. Afferent pain pathways: a neuroanatomical review. Brain research 1000(1-2), 40-56.

Altier, C., Dale, C.S., Kisilevsky, A.E., Chapman, K., Castiglioni, A.J., Matthews, E.A., Evans, R.M., Dickenson, A.H., Lipscombe, D., Vergnolle, N., Zamponi, G.W., 2007. Differential role of N-type calcium channel splice isoforms in pain. J Neurosci 27(24), 6363-6373.

Amadesi, S., Nie, J., Vergnolle, N., Cottrell, G.S., Grady, E.F., Trevisani, M., Manni, C., Geppetti, P., McRoberts, J.A., Ennes, H., Davis, J.B., Mayer, E.A., Bunnett, N.W., 2004. Protease-activated receptor 2 sensitizes the capsaicin receptor transient receptor potential vanilloid receptor 1 to induce hyperalgesia. J Neurosci 24(18), 4300-4312.

Anand, P., Aziz, Q., Willert, R., van Oudenhove, L., 2007. Peripheral and central mechanisms of visceral sensitization in man. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 19(1 Suppl), 29-46.

Andrews, P.L., Davis, C.J., Bingham, S., Davidson, H.I., Hawthorn, J., Maskell, L., 1990. The abdominal visceral innervation and the emetic reflex: pathways, pharmacology, and plasticity. Can J Physiol Pharmacol 68(2), 325-345.

Annahazi, A., Gecse, K., Dabek, M., Ait-Belgnaoui, A., Rosztoczy, A., Roka, R., Molnar, T., Theodorou, V., Wittmann, T., Bueno, L., Eutamene, H., 2009. Fecal proteases from diarrheic-IBS and ulcerative colitis patients exert opposite effect on visceral sensitivity in mice. Pain 144(1-2), 209-217.

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. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 21(11), 1189-e1107.

Azpiroz, F., Bouin, M., Camilleri, M., Mayer, E.A., Poitras, P., Serra, J., Spiller, R.C., 2007. Mechanisms of hypersensitivity in IBS and functional disorders. Neurogastroenterol Motil 19(1 Suppl), 62-88.

Balemans, D., Boeckxstaens, G.E., Talavera, K., Wouters, M.M., 2017. Transient receptor potential ion channel function in sensory transduction and cellular signaling cascades underlying visceral hypersensitivity. Am J Physiol Gastrointest Liver Physiol 312(6), G635-G648.

Barbara, G., Stanghellini, V., De Giorgio, R., Cremon, C., Cottrell, G.S., Santini, D., Pasquinelli, G., Morselli-Labate, A.M., Grady, E.F., Bunnett, N.W., Collins, S.M., Corinaldesi, R., 2004. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterology 126(3), 693-702.

Barbara, G., Wang, B., Stanghellini, V., de Giorgio, R., Cremon, C., Di Nardo, G., Trevisani, M., Campi, B., Geppetti, P., Tonini, M., Bunnett, N.W., Grundy, D., Corinaldesi, R., 2007. Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. Gastroenterology 132(1), 26-37.

Bashashati, M., Moossavi, S., Cremon, C., Barbaro, M.R., Moraveji, S., Talmon, G., Rezaei, N., Hughes, P.A., Bian, Z.X., Choi, C.H., Lee, O.Y., Coeffier, M., Chang, L., Ohman, L., Schmulson, M.J., McCallum, R.W., Simren, M., Sharkey, K.A., Barbara, G., 2018. Colonic immune cells in irritable bowel syndrome: A systematic review and meta-analysis. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 30(1).
Bearcroft, C.P., Perrett, D., Farthing, M.J., 1998. Postprandial plasma 5-hydroxytryptamine in diarrhoea predominant irritable bowel syndrome: a pilot study. Gut 42(1), 42-46.
Bellono, N.W., Bayrer, J.R., Leitch, D.B., Castro, J., Zhang, C., O'Donnell, T.A., Brierley, S.M., Ingraham, H.A., Julius, D., 2017. Enterochromaffin Cells Are Gut Chemosensors that Couple to Sensory Neural Pathways. Cell 170(1), 185-198 e116.
Benson, J.R., Xu, J., Moynes, D.M., Lapointe, T.K., Altier, C., Vanner, S.J., Lomax, A.E., 2014. Sustained neurochemical plasticity in central terminals of mouse DRG neurons following colitis. Cell and tissue research 356(2), 309-317.
Berthoud, H.R., Neuhuber, W.L., 2000. Functional and chemical anatomy of the afferent vagal system. Autonomic neuroscience : basic & clinical 85(1-3), 1-17.
Beyak, M.J., Ramji, N., Krol, K.M., Kawaja, M.D., Vanner, S.J., 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(4), G845-855.
Black, L.V., Ness, T.J., Robbins, M.T., 2009. Effects of oxytocin and prolactin on stress-induced bladder hypersensitivity in female rats. The journal of pain : official journal of the American Pain Society 10(10), 1065-1072.
Blackshaw, L.A., Brierley, S.M., Hughes, P.A., 2010. TRP channels: new targets for visceral pain. Gut 59(1), 126-135.
Blankstein, U., Chen, J., Diamant, N.E., Davis, K.D., 2010. Altered brain structure in irritable bowel syndrome: potential contributions of pre-existing and disease-driven factors. Gastroenterology 138(5), 1783-1789.
Brederson, J.D., Kym, P.R., Szallasi, A., 2013. Targeting TRP channels for pain relief. European journal of pharmacology 716(1-3), 61-76.
Breunig, E., Michel, K., Zeller, F., Seidl, S., Weyhern, C.W., Schemann, M., 2007. Histamine excites neurones in the human submucous plexus through activation of H1, H2, H3 and H4 receptors. The Journal of physiology 583(Pt 2), 731-742.
Brierley, S.M., 2012. Guanylate cyclase-C receptor activation: unexpected biology. Current opinion in pharmacology 12(6), 632-640.
Brierley, S.M., 2016. Altered Ion Channel/Receptor Expression and Function in Extrinsic Sensory Neurons: The Cause of and Solution to Chronic Visceral Pain? Adv Exp Med Biol 891, 75-90.
Brierley, S.M., Hughes, P.A., Page, A.J., Kwan, K.Y., Martin, C.M., O'Donnell, T.A., Cooper, N.J., Harrington, A.M., Adam, B., Liebregts, T., Holtmann, G., Corey, D.P., Rychkov, G.Y., Blackshaw, L.A., 2009. The ion channel TRPA1 is required for normal mechanosensation and is modulated by algesic stimuli. Gastroenterology 137(6), 2084-2095 e2083.
Brierley, S.M., Jones, R.C., 3rd, Gebhart, G.F., Blackshaw, L.A., 2004. Splanchnic and pelvic mechanosensory afferents signal different qualities of colonic stimuli in mice. Gastroenterology 127(1), 166-178.
Brierley, S.M., Kelber, O., 2011. Use of natural products in gastrointestinal therapies. Current opinion in pharmacology 11(6), 604-611.
Brierley, S.M., Linden, D.R., 2014. Neuroplasticity and dysfunction after gastrointestinal inflammation. Nature reviews. Gastroenterology & hepatology 11(10), 611-627.

Brierley, S.M., Page, A.J., Hughes, P.A., Adam, B., Liebregts, T., Cooper, N.J., Holtmann, G., Liedtke, W., Blackshaw, L.A., 2008. Selective role for TRPV4 ion channels in visceral sensory pathways. Gastroenterology 134(7), 2059-2069.
Brookes, S.J., Spencer, N.J., Costa, M., Zagorodnyuk, V.P., 2013. Extrinsic primary afferent signalling in the gut. Nature reviews. Gastroenterology & hepatology 10(5), 286-296.
Brunsden, A.M., Grundy, D., 1999. Sensitization of visceral afferents to bradykinin in rat jejunum in vitro. The Journal of physiology 521 Pt 2(Pt 2), 517-527.
Brusberg, M., Ravnefjord, A., Martinsson, R., Larsson, H., Martinez, V., Lindstrom, E., 2009. The GABA(B) receptor agonist, baclofen, and the positive allosteric modulator, CGP7930, inhibit visceral pain-related responses to colorectal distension in rats. Neuropharmacology 56(2), 362-367.
Brust, A., Croker, D.E., Colless, B., Ragnarsson, L., Andersson, A., Jain, K., Garcia-Caraballo, S., Castro, J., Brierley, S.M., Alewood, P.F., Lewis, R.J., 2016. Conopeptide-Derived kappa-Opioid Agonists (Conorphins): Potent, Selective, and Metabolic Stable Dynorphin A Mimetics with Antinociceptive Properties. J Med Chem 59(6), 2381-2395.
Bryant, A.P., Busby, R.W., Bartolini, W.P., Cordero, E.A., Hannig, G., Kessler, M.M., Pierce, C.M., Solinga, R.M., Tobin, J.V., Mahajan-Miklos, S., Cohen, M.B., Kurtz, C.B., Currie, M.G., 2010. Linaclotide is a potent and selective guanylate cyclase C agonist that elicits pharmacological effects locally in the gastrointestinal tract. Life Sci 86(19-20), 760-765.
Buhner, S., Braak, B., Li, Q., Kugler, E.M., Klooker, T., Wouters, M., Donovan, J., Vignali, S., Mazzuoli-Weber, G., Grundy, D., Boeckxstaens, G., Schemann, M., 2014. Neuronal activation by mucosal biopsy supernatants from irritable bowel syndrome patients is linked to visceral sensitivity. Experimental physiology 99(10), 1299-1311.
Buhner, S., Li, Q., Berger, T., Vignali, S., Barbara, G., De Giorgio, R., Stanghellini, V., Schemann, M., 2012. Submucous rather than myenteric neurons are activated by mucosal biopsy supernatants from irritable bowel syndrome patients. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 24(12), 1134-e1572.
Buhner, S., Li, Q., Braak, B., Klooker, T.K., Vignali, S., Schemann, M., Boeckxstaens, G.E., 2011. Excitation of enteric neurons by supernatans of colonic biopsies from irritable bowel syndrome patients (IBS) is linked to visceral hypersensitivity. Gastroenterology 140(5 Suppl 1), S521.
Buhner, S., Li, Q., Vignali, S., Barbara, G., De Giorgio, R., Stanghellini, V., Cremon, C., Zeller, F., Langer, R., Daniel, H., Michel, K., Schemann, M., 2009. Activation of human enteric neurons by supernatants of colonic biopsy specimens from patients with irritable bowel syndrome. Gastroenterology 137(4), 1425-1434.
Busby, R.W., Bryant, A.P., Bartolini, W.P., Cordero, E.A., Hannig, G., Kessler, M.M., Mahajan-Miklos, S., Pierce, C.M., Solinga, R.M., Sun, L.J., Tobin, J.V., Kurtz, C.B., Currie, M.G., 2010. Linaclotide, through activation of guanylate cyclase C, acts locally in the gastrointestinal tract to elicit enhanced intestinal secretion and transit. European journal of pharmacology 649(1-3), 328-335.
Camilleri, M., 2001. Management of the Irritable Bowel Syndrome. Gastroenterology 120(3), 652-668.
Camilleri, M., 2008. Novel pharmacology: asimadoline, a kappa-opioid agonist, and visceral sensation. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 20(9), 971-979.

Camilleri, M., Northcutt, A.R., Kong, S., Dukes, G.E., McSorley, D., Mangel, A.W., 2000. Efficacy and safety of alosetron in women with irritable bowel syndrome: a randomised, placebo-controlled trial. Lancet 355(9209), 1035-1040.
Camilleri, M., Williams, D.E., 2000. Economic burden of irritable bowel syndrome. Proposed strategies to control expenditures. Pharmacoeconomics 17(4), 331-338.
Carstens, B.B., Berecki, G., Daniel, J.T., Lee, H.S., Jackson, K.A., Tae, H.S., Sadeghi, M., Castro, J., O'Donnell, T., Deiteren, A., Brierley, S.M., Craik, D.J., Adams, D.J., Clark, R.J., 2016. Structure-Activity Studies of Cysteine-Rich alpha-Conotoxins that Inhibit High-Voltage-Activated Calcium Channels via GABA(B) Receptor Activation Reveal a Minimal Functional Motif. Angew Chem Int Ed Engl 55(15), 4692-4696.
Castro, J., Grundy, L., Deiteren, A., Harrington, A.M., O'Donnell, T., Maddern, J., Moore, J., Garcia-Caraballo, S., Rychkov, G.Y., Yu, R., Kaas, Q., Craik, D.J., Adams, D.J., Brierley, S.M., 2017a. Cyclic analogues of alpha-conotoxin Vc1.1 inhibit colonic nociceptors and provide analgesia in a mouse model of chronic abdominal pain. British journal of pharmacology doi: 10.1111/bph.14115. [Epub ahead of print], n/a-n/a.
Castro, J., Harrington, A.M., Garcia-Caraballo, S., Maddern, J., Grundy, L., Zhang, J., Page, G., Miller, P.E., Craik, D.J., Adams, D.J., Brierley, S.M., 2017b. alpha-Conotoxin Vc1.1 inhibits human dorsal root ganglion neuroexcitability and mouse colonic nociception via GABAB receptors. Gut 66(6), 1083-1094.
Castro, J., Harrington, A.M., Hughes, P.A., Martin, C.M., Ge, P., Shea, C.M., Jin, H., Jacobson, S., Hannig, G., Mann, E., Cohen, M.B., MacDougall, J.E., Lavins, B.J., Kurtz, C.B., Silos-Santiago, I., Johnston, J.M., Currie, M.G., Blackshaw, L.A., Brierley, S.M., 2013. Linaclotide inhibits colonic nociceptors and relieves abdominal pain via guanylate cyclase-C and extracellular cyclic guanosine 3',5'-monophosphate. Gastroenterology 145(6), 1334-1346 e1331-1311.
Cattaruzza, F., Lyo, V., Jones, E., Pham, D., Hawkins, J., Kirkwood, K., Valdez-Morales, E., Ibeakanma, C., Vanner, S.J., Bogyo, M., Bunnett, N.W., 2011. Cathepsin S is activated during colitis and causes visceral hyperalgesia by a PAR2-dependent mechanism in mice. Gastroenterology 141(5), 1864-1874 e1861-1863.
Cattaruzza, F., Spreadbury, I., Miranda-Morales, M., Grady, E.F., Vanner, S., Bunnett, N.W., 2010. Transient receptor potential ankyrin-1 has a major role in mediating visceral pain in mice. Am J Physiol Gastrointest Liver Physiol 298(1), G81-91.
Catterall, W.A., 2011. Voltage-gated calcium channels. Cold Spring Harbor perspectives in biology 3(8), a003947.
Catterall, W.A., 2014. Structure and function of voltage-gated sodium channels at atomic resolution. Experimental physiology 99(1), 35-51.
Cenac, N., Altier, C., Motta, J.P., d'Aldebert, E., Galeano, S., Zamponi, G.W., Vergnolle, N., 2010. Potentiation of TRPV4 signalling by histamine and serotonin: an important mechanism for visceral hypersensitivity. Gut 59(4), 481-488.
Cenac, N., Andrews, C.N., Holzhausen, M., Chapman, K., Cottrell, G., Andrade-Gordon, P., Steinhoff, M., Barbara, G., Beck, P., Bunnett, N.W., Sharkey, K.A., Ferraz, J.G., Shaffer, E., Vergnolle, N., 2007. Role for protease activity in visceral pain in irritable bowel syndrome. J Clin Invest 117(3), 636-647.
Cenac, N., Bautzova, T., Le Faouder, P., Veldhuis, N.A., Poole, D.P., Rolland, C., Bertrand, J., Liedtke, W., Dubourdeau, M., Bertrand-Michel, J., Zecchi, L., Stanghellini, V., Bunnett, N.W., Barbara, G., Vergnolle, N., 2015. Quantification and Potential Functions of Endogenous Agonists of Transient Receptor Potential Channels in Patients With Irritable Bowel Syndrome. Gastroenterology 149(2), 433-444 e437.

Chen, J., Gong, Z.H., Yan, H., Qiao, Z., Qin, B.Y., 2012. Neuroplastic alteration of TTX-resistant sodium channel with visceral pain and morphine-induced hyperalgesia. Journal of pain research 5, 491-502.
Chey, W.D., Kurlander, J., Eswaran, S., 2015. Irritable bowel syndrome: a clinical review. JAMA 313(9), 949-958.
Chey, W.D., Lembo, A.J., Lavins, B.J., Shiff, S.J., Kurtz, C.B., Currie, M.G., MacDougall, J.E., Jia, X.D., Shao, J.Z., Fitch, D.A., Baird, M.J., Schneier, H.A., Johnston, J.M., 2012. Linaclotide for irritable bowel syndrome with constipation: a 26-week, randomized, double-blind, placebo-controlled trial to evaluate efficacy and safety. The American journal of gastroenterology 107(11), 1702-1712.
Christianson, J.A., Liang, R., Ustinova, E.E., Davis, B.M., Fraser, M.O., Pezzone, M.A., 2007. Convergence of bladder and colon sensory innervation occurs at the primary afferent level. Pain 128(3), 235-243.
Clapham, D.E., 2007. Calcium signaling. Cell 131(6), 1047-1058.
Coates, M.D., Mahoney, C.R., Linden, D.R., Sampson, J.E., Chen, J., Blaszyk, H., Crowell, M.D., Sharkey, K.A., Gershon, M.D., Mawe, G.M., Moses, P.L., 2004. Molecular defects in mucosal serotonin content and decreased serotonin reuptake transporter in ulcerative colitis and irritable bowel syndrome. Gastroenterology 126(7), 1657-1664.
Coelho, A.M., Vergnolle, N., Guiard, B., Fioramonti, J., Bueno, L., 2002. Proteinases and proteinase-activated receptor 2: a possible role to promote visceral hyperalgesia in rats. Gastroenterology 122(4), 1035-1047.
Coldwell, J.R., Phillis, B.D., Sutherland, K., Howarth, G.S., Blackshaw, L.A., 2007. Increased responsiveness of rat colonic splanchnic afferents to 5-HT after inflammation and recovery. The Journal of physiology 579(Pt 1), 203-213.
Corsetti, M., Akyuz, F., Tack, J., 2015. Targeting tachykinin receptors for the treatment of functional gastrointestinal disorders with a focus on irritable bowel syndrome. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 27(10), 1354-1370.
Costigan, M., Scholz, J., Woolf, C.J., 2009. Neuropathic pain: a maladaptive response of the nervous system to damage. Annual review of neuroscience 32, 1-32.
D'Aldebert, E., Cenac, N., Rousset, P., Martin, L., Rolland, C., Chapman, K., Selves, J., Alric, L., Vinel, J.P., Vergnolle, N., 2011. Transient receptor potential vanilloid 4 activated inflammatory signals by intestinal epithelial cells and colitis in mice. Gastroenterology 140(1), 275-285.
Danzebrink, R.M., Gebhart, G.F., 1991. Evidence that spinal 5-HT1, 5-HT2 and 5-HT3 receptor subtypes modulate responses to noxious colorectal distension in the rat. Brain research 538(1), 64-75.
Davis, M.P., 2012. Drug management of visceral pain: concepts from basic research. Pain research and treatment 2012, 265605.
de Araujo, A.D., Mobli, M., Castro, J., Harrington, A.M., Vetter, I., Dekan, Z., Muttenthaler, M., Wan, J., Lewis, R.J., King, G.F., Brierley, S.M., Alewood, P.F., 2014. Selenoether oxytocin analogues have analgesic properties in a mouse model of chronic abdominal pain. Nat Commun 5, 3165.
De Schepper, H.U., De Winter, B.Y., Van Nassauw, L., Timmermans, J.P., Herman, A.G., Pelckmans, P.A., De Man, J.G., 2008. TRPV1 receptors on unmyelinated C-fibres mediate colitis-induced sensitization of pelvic afferent nerve fibres in rats. The Journal of physiology 586(21), 5247-5258.

Deiteren, A., De Man, J.G., Ruyssers, N.E., Moreels, T.G., Pelckmans, P.A., De Winter, B.Y., 2014. Histamine H4 and H1 receptors contribute to postinflammatory visceral hypersensitivity. Gut 63(12), 1873-1882.
Delgado-Aros, S., Chial, H.J., Camilleri, M., Szarka, L.A., Weber, F.T., Jacob, J., Ferber, I., McKinzie, S., Burton, D.D., Zinsmeister, A.R., 2003. Effects of a kappa-opioid agonist, asimadoline, on satiation and GI motor and sensory functions in humans. Am J Physiol Gastrointest Liver Physiol 284(4), G558-566.
Delvaux, M., Beck, A., Jacob, J., Bouzamondo, H., Weber, F.T., Frexinos, J., 2004. Effect of asimadoline, a kappa opioid agonist, on pain induced by colonic distension in patients with irritable bowel syndrome. Alimentary pharmacology & therapeutics 20(2), 237-246.
Diop, L., Raymond, F., Fargeau, H., Petoux, F., Chovet, M., Doherty, A.M., 2002. Pregabalin (CI-1008) inhibits the trinitrobenzene sulfonic acid-induced chronic colonic allodynia in the rat. J Pharmacol Exp Ther 302(3), 1013-1022.
Duncan, M., Davison, J.S., Sharkey, K.A., 2005. Review article: endocannabinoids and their receptors in the enteric nervous system. Alimentary pharmacology & therapeutics 22(8), 667-683.
Duncan, M., Mouihate, A., Mackie, K., Keenan, C.M., Buckley, N.E., Davison, J.S., Patel, K.D., Pittman, Q.J., Sharkey, K.A., 2008. Cannabinoid CB2 receptors in the enteric nervous system modulate gastrointestinal contractility in lipopolysaccharide-treated rats. Am J Physiol Gastrointest Liver Physiol 295(1), G78-G87.
Eide, P.K., 2000. Wind-up and the NMDA receptor complex from a clinical perspective. European journal of pain 4(1), 5-15.
Enck, P., Aziz, Q., Barbara, G., Farmer, A.D., Fukudo, S., Mayer, E.A., Niesler, B., Quigley, E.M., Rajilic-Stojanovic, M., Schemann, M., Schwille-Kiuntke, J., Simren, M., Zipfel, S., Spiller, R.C., 2016. Irritable bowel syndrome. Nat Rev Dis Primers 2, 16014.
Engel, M.A., Leffler, A., Niedermirtl, F., Babes, A., Zimmermann, K., Filipovic, M.R., Izydorczyk, I., Eberhardt, M., Kichko, T.I., Mueller-Tribbensee, S.M., Khalil, M., Siklosi, N., Nau, C., Ivanovic-Burmazovic, I., Neuhuber, W.L., Becker, C., Neurath, M.F., Reeh, P.W., 2011. TRPA1 and substance P mediate colitis in mice. Gastroenterology 141(4), 1346-1358.
Erickson, A., Deiteren, A., Harrington, A.M., Garcia-Caraballo, S., Castro, J., Caldwell, A., Grundy, L., Brierley, S.M., 2018. Voltage-gated sodium channels: (NaV)igating the field to determine their contribution to visceral nociception. The Journal of physiology doi: 10.1113/JP273461. [Epub ahead of print].
Eutamene, H., Bradesi, S., Larauche, M., Theodorou, V., Beaufrand, C., Ohning, G., Fioramonti, J., Cohen, M., Bryant, A.P., Kurtz, C., Currie, M.G., Mayer, E.A., Bueno, L., 2010. Guanylate cyclase C-mediated antinociceptive effects of linaclotide in rodent models of visceral pain. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 22(3), 312-e384.
Feng, Y., Cui, M., Willis, W.D., 2003. Gabapentin markedly reduces acetic acid-induced visceral nociception. Anesthesiology 98(3), 729-733.
Fichna, J., Salaga, M., Stuart, J., Saur, D., Sobczak, M., Zatorski, H., Timmermans, J.P., Bradshaw, H.B., Ahn, K., Storr, M.A., 2014. Selective inhibition of FAAH produces antidiarrheal and antinociceptive effect mediated by endocannabinoids and cannabinoid-like fatty acid amides. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 26(4), 470-481.
Furness, J.B., Rivera, L.R., Cho, H.J., Bravo, D.M., Callaghan, B., 2013. The gut as a sensory organ. Nature reviews. Gastroenterology & hepatology 10(12), 729-740.

Galligan, J.J., Sternini, C., 2017. Insights into the Role of Opioid Receptors in the GI Tract: Experimental Evidence and Therapeutic Relevance, in: Greenwood-Van Meerveld, B. (Ed.) Gastrointestinal Pharmacology. Springer International Publishing, Cham, pp. 363-378.
Gebhart, G.F., 2004. Descending modulation of pain. Neuroscience and biobehavioral reviews 27(8), 729-737.
Gebhart, G.F., Bielefeldt, K., Ozaki, N., 2002. Gastric hyperalgesia and changes in voltage gated sodium channel function in the rat. Gut 51 Suppl 1, i15-18.
Gebhart, G.F., Su, X., Joshi, S., Ozaki, N., Sengupta, J.N., 2000. Peripheral opioid modulation of visceral pain. Annals of the New York Academy of Sciences 909, 41-50.
Giamberardino, M.A., 2009. Visceral Pain: Clinical, Pathophysiological and Therapeutic Aspects, illustrated ed. OUP Oxford, 2009.
Goldhill, J., Pichat, P., Roome, N., Angel, I., Arbilla, S., 1998. Effect of mizolastine on visceral sensory afferent sensitivity and inflammation during experimental colitis. Arzneimittelforschung 48(2), 179-184.
Gracely, R.H., Lynch, S.A., Bennett, G.J., 1992. Painful neuropathy: altered central processing maintained dynamically by peripheral input. Pain 51(2), 175-194.
Grundy, D., 2002. Neuroanatomy of visceral nociception: vagal and splanchnic afferent. Gut 51 Suppl 1, i2-5.
Grundy, L., Brierley, S.M., 2017. Cross-organ sensitisation between the colon and bladder: To pee, or not to pee? Am J Physiol Gastrointest Liver Physiol doi: 10.1152/ajpgi.00272.2017. [Epub ahead of print].
Han, W., Wang, Z., Lu, X., Guo, C., 2012. Protease activated receptor 4 status of mast cells in post infectious irritable bowel syndrome. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 24(2), 113-119, e182.
Harrington, A.M., Brierley, S.M., Isaacs, N., Hughes, P.A., Castro, J., Blackshaw, L.A., 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(10), 2241-2255.
Harrington, A.M., Hughes, P.A., Martin, C.M., Yang, J., Castro, J., Isaacs, N.J., Blackshaw, L.A., Brierley, S.M., 2011. A novel role for TRPM8 in visceral afferent function. Pain 152(7), 1459-1468.
Hicks, G.A., Coldwell, J.R., Schindler, M., Ward, P.A., Jenkins, D., Lynn, P.A., Humphrey, P.P., Blackshaw, L.A., 2002. Excitation of rat colonic afferent fibres by 5-HT(3) receptors. The Journal of physiology 544(Pt 3), 861-869.
Hirano, K., Kuratani, K., Fujiyoshi, M., Tashiro, N., Hayashi, E., Kinoshita, M., 2007. Kv7.2-7.5 voltage-gated potassium channel (KCNQ2-5) opener, retigabine, reduces capsaicin-induced visceral pain in mice. Neuroscience letters 413(2), 159-162.
Hockley, J.R., Tranter, M.M., McGuire, C., Boundouki, G., Cibert-Goton, V., Thaha, M.A., Blackshaw, L.A., Michael, G.J., Baker, M.D., Knowles, C.H., Winchester, W.J., Bulmer, D.C., 2016. P2Y Receptors Sensitize Mouse and Human Colonic Nociceptors. J Neurosci 36(8), 2364-2376.
Hoffman, J.M., Tyler, K., MacEachern, S.J., Balemba, O.B., Johnson, A.C., Brooks, E.M., Zhao, H., Swain, G.M., Moses, P.L., Galligan, J.J., Sharkey, K.A., Greenwood-Van Meerveld, B., Mawe, G.M., 2012. Activation of colonic mucosal 5-HT(4) receptors accelerates propulsive motility and inhibits visceral hypersensitivity. Gastroenterology 142(4), 844-854 e844.
Holzer, P., 1998. Tachykinins as targets of gastroenterological pharmacotherapy. Drug news & perspectives 11(7), 394-401.

Holzer, P., 2008. TRPV1: a new target for treatment of visceral pain in IBS? Gut 57(7), 882-884.
Holzer, P., Holzer-Petsche, U., 2001. Tachykinin receptors in the gut: physiological and pathological implications. Current opinion in pharmacology 1(6), 583-590.
Hong, S., Fan, J., Kemmerer, E.S., Evans, S., Li, Y., Wiley, J.W., 2009. Reciprocal changes in vanilloid (TRPV1) and endocannabinoid (CB1) receptors contribute to visceral hyperalgesia in the water avoidance stressed rat. Gut 58(2), 202-210.
Houghton, L.A., Atkinson, W., Whitaker, R.P., Whorwell, P.J., Rimmer, M.J., 2003. Increased platelet depleted plasma 5-hydroxytryptamine concentration following meal ingestion in symptomatic female subjects with diarrhoea predominant irritable bowel syndrome. Gut 52(5), 663-670.
Houghton, L.A., Fell, C., Whorwell, P.J., Jones, I., Sudworth, D.P., Gale, J.D., 2007. Effect of a second-generation alpha2delta ligand (pregabalin) on visceral sensation in hypersensitive patients with irritable bowel syndrome. Gut 56(9), 1218-1225.
Hughes, P.A., Brierley, S.M., Martin, C.M., Brookes, S.J., Linden, D.R., Blackshaw, L.A., 2009. Post-inflammatory colonic afferent sensitisation: different subtypes, different pathways and different time courses. Gut 58(10), 1333-1341.
Hughes, P.A., Castro, J., Harrington, A.M., Isaacs, N., Moretta, M., Hicks, G.A., Urso, D.M., Brierley, S.M., 2014a. Increased kappa-opioid receptor expression and function during chronic visceral hypersensitivity. Gut 63(7), 1199-1200.
Hughes, P.A., Costello, S.P., Bryant, R.V., Andrews, J.M., 2016. Opioidergic effects on enteric and sensory nerves in the lower GI tract: basic mechanisms and clinical implications. Am J Physiol Gastrointest Liver Physiol 311(3), G501-513.
Hughes, P.A., Harrington, A.M., Castro, J., Liebregts, T., Adam, B., Grasby, D.J., Isaacs, N.J., Maldeniya, L., Martin, C.M., Persson, J., Andrews, J.M., Holtmann, G., Blackshaw, L.A., Brierley, S.M., 2013a. Sensory neuro-immune interactions differ between irritable bowel syndrome subtypes. Gut 62(10), 1456-1465.
Hughes, P.A., Moretta, M., Lim, A., Grasby, D.J., Bird, D., Brierley, S.M., Liebregts, T., Adam, B., Blackshaw, L.A., Holtmann, G., Bampton, P., Hoffmann, P., Andrews, J.M., Zola, H., Krumbiegel, D., 2014b. Immune derived opioidergic inhibition of viscerosensory afferents is decreased in Irritable Bowel Syndrome patients. Brain Behav Immun 42, 191-203.
Hughes, P.A., Zola, H., Penttila, I.A., Blackshaw, L.A., Andrews, J.M., Krumbiegel, D., 2013b. Immune activation in irritable bowel syndrome: can neuroimmune interactions explain symptoms? The American journal of gastroenterology 108(7), 1066-1074.
Ibeakanma, C., Miranda-Morales, M., Richards, M., Bautista-Cruz, F., Martin, N., Hurlbut, D., Vanner, S., 2009. Citrobacter rodentium colitis evokes post-infectious hyperexcitability of mouse nociceptive colonic dorsal root ganglion neurons. The Journal of physiology 587(Pt 14), 3505-3521.
Inserra, M.C., Israel, M.R., Caldwell, A., Castro, J., Deuis, J.R., Harrington, A.M., Keramidas, A., Garcia-Caraballo, S., Maddern, J., Erickson, A., Grundy, L., Rychkov, G.Y., Zimmermann, K., Lewis, R.J., Brierley, S.M., Vetter, I., 2017. Multiple sodium channel isoforms mediate the pathological effects of Pacific ciguatoxin-1. Sci Rep 7, 42810.
Iseri, S.O., Sener, G., Saglam, B., Gedik, N., Ercan, F., Yegen, B.C., 2005. Oxytocin ameliorates oxidative colonic inflammation by a neutrophil-dependent mechanism. Peptides 26(3), 483-491.
Jami, S., Erickson, A., Brierley, S.M., Vetter, I., 2018. Pain-Causing Venom Peptides: Insights into Sensory Neuron Pharmacology. Toxins 10(1), 15.

Jarcho, J.M., Feier, N.A., Bert, A., Labus, J.A., Lee, M., Stains, J., Ebrat, B., Groman, S.M., Tillisch, K., Brody, A.L., London, E.D., Mandelkern, M.A., Mayer, E.A., 2013. Diminished neurokinin-1 receptor availability in patients with two forms of chronic visceral pain. Pain 154(7), 987-996.
Jiang, W., Adam, I.J., Kitsanta, P., Tiernan, J., Hill, C., Shorthouse, A., Grundy, D., 2011. 'First-in-man': characterising the mechanosensitivity of human colonic afferents. Gut 60(2), 281-282.
Johnston, J.M., Kurtz, C.B., Macdougall, J.E., Lavins, B.J., Currie, M.G., Fitch, D.A., O'Dea, C., Baird, M., Lembo, A.J., 2010. Linaclotide improves abdominal pain and bowel habits in a phase IIb study of patients with irritable bowel syndrome with constipation. Gastroenterology 139(6), 1877-1886 e1872.
Jones, R.C., 3rd, Xu, L., Gebhart, G.F., 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(47), 10981-10989.
Kajihara, Y., Murakami, M., Imagawa, T., Otsuguro, K., Ito, S., Ohta, T., 2010. Histamine potentiates acid-induced responses mediating transient receptor potential V1 in mouse primary sensory neurons. Neuroscience 166(1), 292-304.
Kannampalli, P., Sengupta, J.N., 2015. Role of principal ionotropic and metabotropic receptors in visceral pain. J Neurogastroenterol Motil 21(2), 147-158.
Kaplan, G.G., 2015. The global burden of IBD: from 2015 to 2025. Nature reviews. Gastroenterology & hepatology 12(12), 720-727.
Kawabata, A., Kawao, N., Kitano, T., Matsunami, M., Satoh, R., Ishiki, T., Masuko, T., Kanke, T., Saito, N., 2006. Colonic hyperalgesia triggered by proteinase-activated receptor-2 in mice: involvement of endogenous bradykinin. Neuroscience letters 402(1-2), 167-172.
Kimball, E.S., Schneider, C.R., Wallace, N.H., Hornby, P.J., 2006. Agonists of cannabinoid receptor 1 and 2 inhibit experimental colitis induced by oil of mustard and by dextran sulfate sodium. Am J Physiol Gastrointest Liver Physiol 291(2), G364-371.
King, D.E., Macleod, R.J., Vanner, S.J., 2009. Trinitrobenzenesulphonic acid colitis alters Na 1.8 channel expression in mouse dorsal root ganglia neurons. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 21(8), 880-e864.
Kreis, M.E., Haupt, W., Kirkup, A.J., Grundy, D., 1998. Histamine sensitivity of mesenteric afferent nerves in the rat jejunum. Am J Physiol 275(4 Pt 1), G675-680.
Kun, J., Szitter, I., Kemeny, A., Perkecz, A., Kereskai, L., Pohoczky, K., Vincze, A., Godi, S., Szabo, I., Szolcsanyi, J., Pinter, E., Helyes, Z., 2014. Upregulation of the transient receptor potential ankyrin 1 ion channel in the inflamed human and mouse colon and its protective roles. PLoS One 9(9), e108164.
La, J.H., Gebhart, G.F., 2011. Colitis decreases mechanosensitive K2P channel expression and function in mouse colon sensory neurons. Am J Physiol Gastrointest Liver Physiol 301(1), G165-174.
Laird, J.M., Olivar, T., Roza, C., De Felipe, C., Hunt, S.P., Cervero, F., 2000. Deficits in visceral pain and hyperalgesia of mice with a disruption of the tachykinin NK1 receptor gene. Neuroscience 98(2), 345-352.
Lecci, A., Capriati, A., Maggi, C.A., 2004. Tachykinin NK2 receptor antagonists for the treatment of irritable bowel syndrome. British journal of pharmacology 141(8), 1249-1263.
Lee, K.J., Kim, J.H., Cho, S.W., 2005. Gabapentin reduces rectal mechanosensitivity and increases rectal compliance in patients with diarrhoea-predominant irritable bowel syndrome. Alimentary pharmacology & therapeutics 22(10), 981-988.

Lembo, A.J., Lacy, B.E., Zuckerman, M.J., Schey, R., Dove, L.S., Andrae, D.A., Davenport, J.M., McIntyre, G., Lopez, R., Turner, L., Covington, P.S., 2016. Eluxadoline for Irritable Bowel Syndrome with Diarrhea. N Engl J Med 374(3), 242-253.
Lembo, T., Munakata, J., Mertz, H., Niazi, N., Kodner, A., Nikas, V., Mayer, E.A., 1994. Evidence for the hypersensitivity of lumbar splanchnic afferents in irritable bowel syndrome. Gastroenterology 107(6), 1686-1696.
Li, J., Xue, B., Han, T., Huang, K., Gong, L., Ma, X., Liu, K., Cui, S., Zhang, M., Kunze, W., Liu, C., 2015. Oxytocin down-regulates mesenteric afferent sensitivity via the enteric OTR/nNOS/NO/KATP pathway in rat. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 27(1), 51-62.
Li, S., Chen, J.D., 2014. Down-regulation of A-type potassium channel in gastric-specific DRG neurons in a rat model of functional dyspepsia. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 26(7), 962-970.
Lindstrom, E., Brusberg, M., Hughes, P.A., Martin, C.M., Brierley, S.M., Phillis, B.D., Martinsson, R., Abrahamsson, C., Larsson, H., Martinez, V., Blackshaw, L.A., 2008. Involvement of metabotropic glutamate 5 receptor in visceral pain. Pain 137(2), 295-305.
Lindstrom, E., Brusberg, M., Ravnefjord, A., Kakol-Palm, D., Pahlman, I., Noven, A., Larsson, H., Martinez, V., 2011. Oral baclofen reduces visceral pain-related pseudo-affective responses to colorectal distension in rats: relation between plasma exposure and efficacy. Scand J Gastroenterol 46(6), 652-662.
Lomax, A.E., Fernandez, E., Sharkey, K.A., 2005. Plasticity of the enteric nervous system during intestinal inflammation. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 17(1), 4-15.
Louvel, D., Delvaux, M., Felez, A., Fioramonti, J., Bueno, L., Lazorthes, Y., Frexinos, J., 1996. Oxytocin increases thresholds of colonic visceral perception in patients with irritable bowel syndrome. Gut 39(5), 741-747.
Luo, J.L., Qin, H.Y., Wong, C.K., Tsang, S.Y., Huang, Y., Bian, Z.X., 2011. Enhanced excitability and down-regulated voltage-gated potassium channels in colonic drg neurons from neonatal maternal separation rats. The journal of pain : official journal of the American Pain Society 12(5), 600-609.
Mangel, A.W., Bornstein, J.D., Hamm, L.R., Buda, J., Wang, J., Irish, W., Urso, D., 2008. Clinical trial: asimadoline in the treatment of patients with irritable bowel syndrome. Alimentary pharmacology & therapeutics 28(2), 239-249.
Marger, F., Gelot, A., Alloui, A., Matricon, J., Ferrer, J.F., Barrere, C., Pizzoccaro, A., Muller, E., Nargeot, J., Snutch, T.P., Eschalier, A., Bourinet, E., Ardid, D., 2011. T-type calcium channels contribute to colonic hypersensitivity in a rat model of irritable bowel syndrome. Proceedings of the National Academy of Sciences of the United States of America 108(27), 11268-11273.
Marshall, J.K., Thabane, M., Garg, A.X., Clark, W.F., Moayyedi, P., Collins, S.M., Walkerton Health Study, I., 2010. Eight year prognosis of postinfectious irritable bowel syndrome following waterborne bacterial dysentery. Gut 59(5), 605-611.
Massa, F., Marsicano, G., Hermann, H., Cannich, A., Monory, K., Cravatt, B.F., Ferri, G.L., Sibaev, A., Storr, M., Lutz, B., 2004. The endogenous cannabinoid system protects against colonic inflammation. J Clin Invest 113(8), 1202-1209.
Massa, F., Storr, M., Lutz, B., 2005. The endocannabinoid system in the physiology and pathophysiology of the gastrointestinal tract. Journal of molecular medicine 83(12), 944-954.
Mathie, A., Veale, E.L., 2015. Two-pore domain potassium channels: potential therapeutic targets for the treatment of pain. Pflugers Arch 467(5), 931-943.

Matsunaga, M., Konagaya, T., Nogimori, T., Yoneda, M., Kasugai, K., Ohira, H., Kaneko, H., 2009. Inhibitory effect of oxytocin on accelerated colonic motility induced by water-avoidance stress in rats. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 21(8), 856-e859.
Mayer, E.A., Aziz, Q., Coen, S., Kern, M., Labus, J.S., Lane, R., Kuo, B., Naliboff, B., Tracey, I., 2009. Brain imaging approaches to the study of functional GI disorders: a Rome working team report. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 21(6), 579-596.
Mayer, E.A., Gupta, A., Kilpatrick, L.A., Hong, J.Y., 2015. Imaging brain mechanisms in chronic visceral pain. Pain 156 Suppl 1, S50-63.
McCarson, K.E., Enna, S.J., 2014. GABA pharmacology: the search for analgesics. Neurochem Res 39(10), 1948-1963.
McGuire, C., Boundouki, G., Hockley, J.R.F., Reed, D., Cibert-Goton, V., Peiris, M., Kung, V., Broad, J., Aziz, Q., Chan, C., Ahmed, S., Thaha, M.A., Sanger, G.J., Blackshaw, L.A., Knowles, C.H., Bulmer, D.C., 2018. Ex vivo study of human visceral nociceptors. Gut 67(1), 86-96.
McRoberts, J.A., Coutinho, S.V., Marvizon, J.C., Grady, E.F., Tognetto, M., Sengupta, J.N., Ennes, H.S., Chaban, V.V., Amadesi, S., Creminon, C., Lanthorn, T., Geppetti, P., Bunnett, N.W., Mayer, E.A., 2001. Role of peripheral N-methyl-D-aspartate (NMDA) receptors in visceral nociception in rats. Gastroenterology 120(7), 1737-1748.
Meyer-Lindenberg, A., Domes, G., Kirsch, P., Heinrichs, M., 2011. Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. Nat Rev Neurosci 12(9), 524-538.
Million, M., Wang, L., Adelson, D.W., Roman, F., Diop, L., Tache, Y., 2007. Pregabalin decreases visceral pain and prevents spinal neuronal activation in rats. Gut 56(10), 1482-1484.
Mueller-Tribbensee, S.M., Karna, M., Khalil, M., Neurath, M.F., Reeh, P.W., Engel, M.A., 2015. Differential Contribution of TRPA1, TRPV4 and TRPM8 to Colonic Nociception in Mice. PLoS One 10(7), e0128242.
Ohlsson, B., Ringstrom, G., Abrahamsson, H., Simren, M., Bjornsson, E.S., 2004. Oxytocin stimulates colonic motor activity in healthy women. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 16(2), 233-240.
Ohlsson, B., Truedsson, M., Bengtsson, M., Torstenson, R., Sjolund, K., Bjornsson, E.S., Simren, M., 2005. Effects of long-term treatment with oxytocin in chronic constipation; a double blind, placebo-controlled pilot trial. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 17(5), 697-704.
Osteen, J.D., Herzig, V., Gilchrist, J., Emrick, J.J., Zhang, C., Wang, X., Castro, J., Garcia-Caraballo, S., Grundy, L., Rychkov, G.Y., Weyer, A.D., Dekan, Z., Undheim, E.A., Alewood, P., Stucky, C.L., Brierley, S.M., Basbaum, A.I., Bosmans, F., King, G.F., Julius, D., 2016. Selective spider toxins reveal a role for the Nav1.1 channel in mechanical pain. Nature 534(7608), 494-499.
Peiris, M., Bulmer, D.C., Baker, M.D., Boundouki, G., Sinha, S., Hobson, A., Lee, K., Aziz, Q., Knowles, C.H., 2011. Human visceral afferent recordings: preliminary report. Gut 60(2), 204-208.
Peiris, M., Hockley, J.R., Reed, D.E., Smith, E.S.J., Bulmer, D.C., Blackshaw, L.A., 2017. Peripheral KV7 channels regulate visceral sensory function in mouse and human colon. Mol Pain 13, 1744806917709371.

Pfeifer, A., Aszodi, A., Seidler, U., Ruth, P., Hofmann, F., Fassler, R., 1996. Intestinal secretory defects and dwarfism in mice lacking cGMP-dependent protein kinase II. Science 274(5295), 2082-2086.
Piche, M., Bouin, M., Arsenault, M., Poitras, P., Rainville, P., 2011. Decreased pain inhibition in irritable bowel syndrome depends on altered descending modulation and higher-order brain processes. Neuroscience 195, 166-175.
Poole, D.P., Pelayo, J.C., Scherrer, G., Evans, C.J., Kieffer, B.L., Bunnett, N.W., 2011. Localization and regulation of fluorescently labeled delta opioid receptor, expressed in enteric neurons of mice. Gastroenterology 141(3), 982-991 e918.
Qian, A., Song, D., Li, Y., Liu, X., Tang, D., Yao, W., Yuan, Y., 2013. Role of voltage gated Ca2+ channels in rat visceral hypersensitivity change induced by 2,4,6-trinitrobenzene sulfonic acid. Mol Pain 9, 15.
Qian, A.H., Liu, X.Q., Yao, W.Y., Wang, H.Y., Sun, J., Zhou, L., Yuan, Y.Z., 2009. Voltage-gated potassium channels in IB4-positive colonic sensory neurons mediate visceral hypersensitivity in the rat. The American journal of gastroenterology 104(8), 2014-2027.
Qin, J., Feng, M., Wang, C., Ye, Y., Wang, P.S., Liu, C., 2009. Oxytocin receptor expressed on the smooth muscle mediates the excitatory effect of oxytocin on gastric motility in rats. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 21(4), 430-438.
Ramachandran, R., Hyun, E., Zhao, L., Lapointe, T.K., Chapman, K., Hirota, C.L., Ghosh, S., McKemy, D.D., Vergnolle, N., Beck, P.L., Altier, C., Hollenberg, M.D., 2013. TRPM8 activation attenuates inflammatory responses in mouse models of colitis. Proceedings of the National Academy of Sciences of the United States of America 110(18), 7476-7481.
Rao, S., Lembo, A.J., Shiff, S.J., Lavins, B.J., Currie, M.G., Jia, X.D., Shi, K., MacDougall, J.E., Shao, J.Z., Eng, P., Fox, S.M., Schneier, H.A., Kurtz, C.B., Johnston, J.M., 2012. A 12-week, randomized, controlled trial with a 4-week randomized withdrawal period to evaluate the efficacy and safety of linaclotide in irritable bowel syndrome with constipation. The American journal of gastroenterology 107(11), 1714-1724; quiz p 1725.
Ravnefjord, A., Brusberg, M., Larsson, H., Lindstrom, E., Martinez, V., 2008. Effects of pregabalin on visceral pain responses and colonic compliance in rats. British journal of pharmacology 155(3), 407-416.
Ringel, Y., Drossman, D.A., Leserman, J.L., Suyenobu, B.Y., Wilber, K., Lin, W., Whitehead, W.E., Naliboff, B.D., Berman, S., Mayer, E.A., 2008. Effect of abuse history on pain reports and brain responses to aversive visceral stimulation: an FMRI study. Gastroenterology 134(2), 396-404.
Ritchie, J., 1973. Pain from distention of the pelvic colon by inflating a balloon in the irritable bowel syndrome. Gut 6, 105–112.
Rolland-Fourcade, C., Denadai-Souza, A., Cirillo, C., Lopez, C., Jaramillo, J.O., Desormeaux, C., Cenac, N., Motta, J.P., Larauche, M., Tache, Y., Berghe, P.V., Neunlist, M., Coron, E., Kirzin, S., Portier, G., Bonnet, D., Alric, L., Vanner, S., Deraison, C., Vergnolle, N., 2017. Epithelial expression and function of trypsin-3 in irritable bowel syndrome. Gut 66(10), 1767-1778.
Rong, W., Keating, C., Sun, B., Dong, L., Grundy, D., 2009. Purinergic contribution to small intestinal afferent hypersensitivity in a murine model of postinfectious bowel disease. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 21(6), 665-671, e632.

Sandler, R.S., Stewart, W.F., Liberman, J.N., Ricci, J.A., Zorich, N.L., 2000. Abdominal pain, bloating, and diarrhea in the United States: prevalence and impact. Digestive diseases and sciences 45(6), 1166-1171.
Sanger, G.J., 2004. Neurokinin NK1 and NK3 receptors as targets for drugs to treat gastrointestinal motility disorders and pain. British journal of pharmacology 141(8), 1303-1312.
Schulz, S., Green, C.K., Yuen, P.S.T., Garbers, D.L., 1990. Guanylyl cyclase is a heat-stable enterotoxin receptor. Cell 63(5), 941-948.
Schweinhardt, P., Bushnell, M.C., 2010. Pain imaging in health and disease--how far have we come? J Clin Invest 120(11), 3788-3797.
Sengupta, J.N., 2009. Visceral pain: the neurophysiological mechanism. Handbook of experimental pharmacology(194), 31-74.
Sengupta, J.N., Medda, B.K., Shaker, R., 2002. Effect of GABA(B) receptor agonist on distension-sensitive pelvic nerve afferent fibers innervating rat colon. Am J Physiol Gastrointest Liver Physiol 283(6), G1343-1351.
Sengupta, J.N., Pochiraju, S., Kannampalli, P., Bruckert, M., Addya, S., Yadav, P., Miranda, A., Shaker, R., Banerjee, B., 2013. MicroRNA-mediated GABA Aalpha-1 receptor subunit down-regulation in adult spinal cord following neonatal cystitis-induced chronic visceral pain in rats. Pain 154(1), 59-70.
Sengupta, J.N., Snider, A., Su, X., Gebhart, G.F., 1999. Effects of kappa opioids in the inflamed rat colon. Pain 79(2-3), 175-185.
Shafton, A.D., Bogeski, G., Kitchener, P.D., Sanger, G.J., Furness, J.B., Shimizu, Y., 2007. Effects of NMDA receptor antagonists on visceromotor reflexes and on intestinal motility, in vivo. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 19(7), 617-624.
Shinoda, M., La, J.H., Bielefeldt, K., Gebhart, G.F., 2010. Altered purinergic signaling in colorectal dorsal root ganglion neurons contributes to colorectal hypersensitivity. Journal of neurophysiology 104(6), 3113-3123.
Sibaev, A., Massa, F., Yuce, B., Marsicano, G., Lehr, H.A., Lutz, B., Goke, B., Allescher, H.D., Storr, M., 2006. CB1 and TRPV1 receptors mediate protective effects on colonic electrophysiological properties in mice. Journal of molecular medicine 84(6), 513-520.
Simms, B.A., Zamponi, G.W., 2014. Neuronal voltage-gated calcium channels: structure, function, and dysfunction. Neuron 82(1), 24-45.
Sipe, W.E., Brierley, S.M., Martin, C.M., Phillis, B.D., Cruz, F.B., Grady, E.F., Liedtke, W., Cohen, D.M., Vanner, S., Blackshaw, L.A., Bunnett, N.W., 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(5), G1288-1298.
Spencer, N.J., Kyloh, M., Beckett, E.A., Brookes, S., Hibberd, T., 2016a. Different types of spinal afferent nerve endings in stomach and esophagus identified by anterograde tracing from dorsal root ganglia. J Comp Neurol 524(15), 3064-3083.
Spencer, N.J., Zagorodnyuk, V., Brookes, S.J., Hibberd, T., 2016b. Spinal afferent nerve endings in visceral organs: recent advances. Am J Physiol Gastrointest Liver Physiol 311(6), G1056-G1063.
Spiller, R., Garsed, K., 2009. Postinfectious irritable bowel syndrome. Gastroenterology 136(6), 1979-1988.
Spiller, R.C., Jenkins, D., Thornley, J.P., Hebden, J.M., Wright, T., Skinner, M., Neal, K.R., 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(6), 804-811.
Spohn, S.N., Bianco, F., Scott, R.B., Keenan, C.M., Linton, A.A., O'Neill, C.H., Bonora, E., Dicay, M., Lavoie, B., Wilcox, R.L., MacNaughton, W.K., De Giorgio, R., Sharkey, K.A., Mawe, G.M., 2016. Protective Actions of Epithelial 5-Hydroxytryptamine 4 Receptors in Normal and Inflamed Colon. Gastroenterology 151(5), 933-944 e933.
Stanisor, O.I., van Diest, S.A., Yu, Z., Welting, O., Bekkali, N., Shi, J., de Jonge, W.J., Boeckxstaens, G.E., van den Wijngaard, R.M., 2013. Stress-induced visceral hypersensitivity in maternally separated rats can be reversed by peripherally restricted histamine-1-receptor antagonists. PLoS One 8(6), e66884.
Storr, M.A., Keenan, C.M., Emmerdinger, D., Zhang, H., Yuce, B., Sibaev, A., Massa, F., Buckley, N.E., Lutz, B., Goke, B., Brand, S., Patel, K.D., Sharkey, K.A., 2008a. Targeting endocannabinoid degradation protects against experimental colitis in mice: involvement of CB1 and CB2 receptors. Journal of molecular medicine 86(8), 925-936.
Storr, M.A., Keenan, C.M., Zhang, H., Patel, K.D., Makriyannis, A., Sharkey, K.A., 2009. Activation of the cannabinoid 2 receptor (CB2) protects against experimental colitis. Inflamm Bowel Dis 15(11), 1678-1685.
Storr, M.A., Yuce, B., Andrews, C.N., Sharkey, K.A., 2008b. The role of the endocannabinoid system in the pathophysiology and treatment of irritable bowel syndrome. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 20(8), 857-868.
Strege, P.R., Knutson, K.R., Eggers, S.J., Wang, F., Li, J., Leiter, A., Farrugia, G., Beyder, A., 2017. SCN3A-encoded Voltage-gated Sodium Channel NaV1.3 is Specifically Expressed in Human and Mouse Gastrointestinal Enterochromaffin Cells and is Important for Enterochromaffin Cell Excitability. The FASEB Journal 31(1 Supplement), 1007.1040.
Sugiuar, T., Bielefeldt, K., Gebhart, G.F., 2004. TRPV1 function in mouse colon sensory neurons is enhanced by metabotropic 5-hydroxytryptamine receptor activation. J Neurosci 24(43), 9521-9530.
Sugiura, T., Bielefeldt, K., Gebhart, G.F., 2004. TRPV1 function in mouse colon sensory neurons is enhanced by metabotropic 5-hydroxytryptamine receptor activation. J. Neurosci. 24(43), 9521-9530.
Szallasi, A., Cortright, D.N., Blum, C.A., Eid, S.R., 2007. The vanilloid receptor TRPV1: 10 years from channel cloning to antagonist proof-of-concept. Nature reviews. Drug discovery 6(5), 357-372.
Taylor, C.P., Garrido, R., 2008. Immunostaining of rat brain, spinal cord, sensory neurons and skeletal muscle for calcium channel alpha2-delta (alpha2-delta) type 1 protein. Neuroscience 155(2), 510-521.
Tillisch, K., Mayer, E.A., Labus, J.S., 2011. Quantitative meta-analysis identifies brain regions activated during rectal distension in irritable bowel syndrome. Gastroenterology 140(1), 91-100.
Tooth, D., Garsed, K., Singh, G., Marciani, L., Lam, C., Fordham, I., Fields, A., Banwait, R., Lingaya, M., Layfield, R., Hastings, M., Whorwell, P., Spiller, R., 2014. Characterisation of faecal protease activity in irritable bowel syndrome with diarrhoea: origin and effect of gut transit. Gut 63(5), 753-760.
Urban, M.O., Zahn, P.K., Gebhart, G.F., 1999. Descending facilitatory influences from the rostral medial medulla mediate secondary, but not primary hyperalgesia in the rat. Neuroscience 90(2), 349-352.

Valdez-Morales, E.E., Overington, J., Guerrero-Alba, R., Ochoa-Cortes, F., Ibeakanma, C.O., Spreadbury, I., Bunnett, N.W., Beyak, M., Vanner, S.J., 2013. Sensitization of peripheral sensory nerves by mediators from colonic biopsies of diarrhea-predominant irritable bowel syndrome patients: a role for PAR2. The American journal of gastroenterology 108(10), 1634-1643.
van den Wijngaard, R.M., Klooker, T.K., Welting, O., Stanisor, O.I., Wouters, M.M., van der Coelen, D., Bulmer, D.C., Peeters, P.J., Aerssens, J., de Hoogt, R., Lee, K., de Jonge, W.J., Boeckxstaens, G.E., 2009. Essential role for TRPV1 in stress-induced (mast cell-dependent) colonic hypersensitivity in maternally separated rats. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 21(10), 1107-e1194.
Vergnolle, N., Cenac, N., Altier, C., Cellars, L., Chapman, K., Zamponi, G.W., Materazzi, S., Nassini, R., Liedtke, W., Cattaruzza, F., Grady, E.F., Geppetti, P., Bunnett, N.W., 2010. A role for transient receptor potential vanilloid 4 in tonicity-induced neurogenic inflammation. British journal of pharmacology 159(5), 1161-1173.
Verma-Gandhu, M., Verdu, E.F., Bercik, P., Blennerhassett, P.A., Al-Mutawaly, N., Ghia, J.E., Collins, S.M., 2007. Visceral pain perception is determined by the duration of colitis and associated neuropeptide expression in the mouse. Gut 56(3), 358-364.
Vermeulen, W., De Man, J.G., De Schepper, H.U., Bult, H., Moreels, T.G., Pelckmans, P.A., De Winter, B.Y., 2013. Role of TRPV1 and TRPA1 in visceral hypersensitivity to colorectal distension during experimental colitis in rats. European journal of pharmacology 698(1-3), 404-412.
Vermeulen, W., De Man, J.G., Pelckmans, P.A., De Winter, B.Y., 2014. Neuroanatomy of lower gastrointestinal pain disorders. World journal of gastroenterology : WJG 20(4), 1005-1020.
Verne, G.N., Sen, A., Price, D.D., 2005. Intrarectal lidocaine is an effective treatment for abdominal pain associated with diarrhea-predominant irritable bowel syndrome. The journal of pain : official journal of the American Pain Society 6(8), 493-496.
Wan, J., Mobli, M., Brust, A., Muttenthaler, M., Andersson, A., Ragnarsson-McGrath, L., Castro, J., Vetter, I., Nilsson, M., Huang, J., Brierley, S.M., Cooper, M., Lewis, R.J., Alewood, P., 2016. Synthesis of multivalent [lys8]-oxytocin dendrimers that inhibit visceral nociceptive responses. Australian Journal of Chemistry. http://dx.doi.org/10.1071/CH16407.
Welch, M.G., Anwar, M., Chang, C.Y., Gross, K.J., Ruggiero, D.A., Tamir, H., Gershon, M.D., 2010. Combined administration of secretin and oxytocin inhibits chronic colitis and associated activation of forebrain neurons. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 22(6), 654-e202.
Wilder-Smith, C.H., Schindler, D., Lovblad, K., Redmond, S.M., Nirkko, A., 2004. Brain functional magnetic resonance imaging of rectal pain and activation of endogenous inhibitory mechanisms in irritable bowel syndrome patient subgroups and healthy controls. Gut 53(11), 1595-1601.
Winnard, K.P., Dmitrieva, N., Berkley, K.J., 2006. Cross-organ interactions between reproductive, gastrointestinal, and urinary tracts: modulation by estrous stage and involvement of the hypogastric nerve. American journal of physiology. Regulatory, integrative and comparative physiology 291(6), R1592-1601.
Wiskur, B.J., Tyler, K., Campbell-Dittmeyer, K., Chaplan, S.R., Wickenden, A.D., Greenwood-Van Meerveld, B., 2010. A novel TRPV1 receptor antagonist JNJ-17203212 attenuates colonic hypersensitivity in rats. Methods Find Exp Clin Pharmacol 32(8), 557-564.
Wouters, M.M., Balemans, D., Van Wanrooy, S., Dooley, J., Cibert-Goton, V., Alpizar, Y.A., Valdez-Morales, E.E., Nasser, Y., Van Veldhoven, P.P., Vanbrabant, W., Van der Merwe, S.,

Mols, R., Ghesquiere, B., Cirillo, C., Kortekaas, I., Carmeliet, P., Peetermans, W.E., Vermeire, S., Rutgeerts, P., Augustijns, P., Hellings, P.W., Belmans, A., Vanner, S., Bulmer, D.C., Talavera, K., Vanden Berghe, P., Liston, A., Boeckxstaens, G.E., 2016. Histamine Receptor H1-Mediated Sensitization of TRPV1 Mediates Visceral Hypersensitivity and Symptoms in Patients With Irritable Bowel Syndrome. Gastroenterology 150(4), 875-887 e879.
Wulff, H., Castle, N.A., Pardo, L.A., 2009. Voltage-gated potassium channels as therapeutic targets. Nature reviews. Drug discovery 8(12), 982-1001.
Wynn, G., Ma, B., Ruan, H.Z., Burnstock, G., 2004. Purinergic component of mechanosensory transduction is increased in a rat model of colitis. Am J Physiol Gastrointest Liver Physiol 287(3), G647-657.
Xia, C.M., Colomb, D.G., Jr., Akbarali, H.I., Qiao, L.Y., 2011. Prolonged sympathetic innervation of sensory neurons in rat thoracolumbar dorsal root ganglia during chronic colitis. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 23(8), 801-e339.
Xu, G.Y., Shenoy, M., Winston, J.H., Mittal, S., Pasricha, P.J., 2008. P2X receptor-mediated visceral hyperalgesia in a rat model of chronic visceral hypersensitivity. Gut 57(9), 1230-1237.
Xu, G.Y., Winston, J.H., Shenoy, M., Yin, H., Pasricha, P.J., 2006. Enhanced excitability and suppression of A-type K+ current of pancreas-specific afferent neurons in a rat model of chronic pancreatitis. Am J Physiol Gastrointest Liver Physiol 291(3), G424-431.
Yiangou, Y., Facer, P., Chessell, I.P., Bountra, C., Chan, C., Fertleman, C., Smith, V., Anand, P., 2007. Voltage-gated ion channel Nav1.7 innervation in patients with idiopathic rectal hypersensitivity and paroxysmal extreme pain disorder (familial rectal pain). Neuroscience letters 427(2), 77-82.
Yoshimura, N., Seki, S., Novakovic, S.D., Tzoumaka, E., Erickson, V.L., Erickson, K.A., Chancellor, M.B., de Groat, W.C., 2001. The involvement of the tetrodotoxin-resistant sodium channel Na(v)1.8 (PN3/SNS) in a rat model of visceral pain. J Neurosci 21(21), 8690-8696.
Yu, Y., Daly, D.M., Adam, I.J., Kitsanta, P., Hill, C.J., Wild, J., Shorthouse, A., Grundy, D., Jiang, W., 2016. Interplay between mast cells, enterochromaffin cells, and sensory signaling in the aging human bowel. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society 28(10), 1465-1479.
Zagorodnyuk, V.P., Brookes, S.J., Spencer, N.J., 2010. Structure-function relationship of sensory endings in the gut and bladder. Autonomic neuroscience : basic & clinical 153(1-2), 3-11.
Zamponi, G.W., Striessnig, J., Koschak, A., Dolphin, A.C., 2015. The Physiology, Pathology, and Pharmacology of Voltage-Gated Calcium Channels and Their Future Therapeutic Potential. Pharmacol Rev 67(4), 821-870.
Zhao, J.H., Dong, L., Shi, H.T., Wang, Z.Y., Shi, H.Y., Ding, H., 2012. The expression of protease-activated receptor 2 and 4 in the colon of irritable bowel syndrome patients. Digestive diseases and sciences 57(1), 58-64.
Zhao, P., Metcalf, M., Bunnett, N.W., 2014. Biased signaling of protease-activated receptors. Front Endocrinol (Lausanne) 5, 67.
Zhou, Q., Fillingim, R.B., Riley, J.L., 3rd, Malarkey, W.B., Verne, G.N., 2010. Central and peripheral hypersensitivity in the irritable bowel syndrome. Pain 148(3), 454-461.
Zhou, Q., Yang, L., Larson, S., Basra, S., Merwat, S., Tan, A., Croce, C., Verne, G.N., 2016. Decreased miR-199 augments visceral pain in patients with IBS through translational upregulation of TRPV1. Gut 65(5), 797-805.

Zielinska, M., Chen, C., Mokrowiecka, A., Cygankiewicz, A.I., Zakrzewski, P.K., Salaga, M., Malecka-Panas, E., Wlaz, P., Krajewska, W.M., Fichna, J., 2015a. Orally administered novel cyclic pentapeptide P-317 alleviates symptoms of diarrhoea-predominant irritable bowel syndrome. The Journal of pharmacy and pharmacology 67(2), 244-254.
Zielinska, M., Jarmuz, A., Wasilewski, A., Salaga, M., Fichna, J., 2015b. Role of transient receptor potential channels in intestinal inflammation and visceral pain: novel targets in inflammatory bowel diseases. Inflamm Bowel Dis 21(2), 419-427.
Ziino, G., Nibali, V., Panebianco, A., 2010. Bacteriological investigation on "Mauro" sold in Catania. Vet Res Commun 34 Suppl 1(5), S157-161.

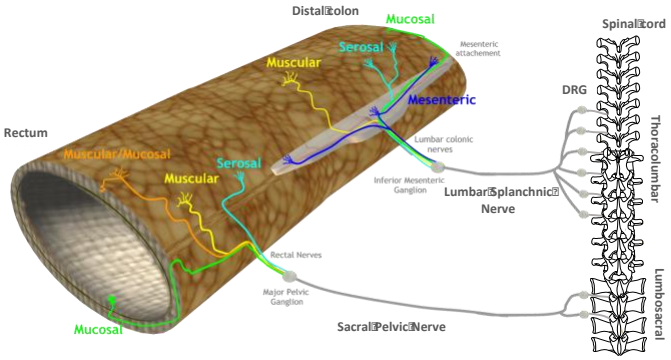

Figure 1: Extrinsic sensory afferent innervation of the colorectum.
The colon and rectum receive dual spinal innervation via the lumbar splanchnic nerve and the sacral pelvic nerves. The cell bodies of splanchnic and pelvic afferents are located within the thoracolumbar (T10-L1) and lumbosacral (L6-S1) dorsal root ganglia (DRG), respectively. 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 and muscle, and wrap around blood vessels within the submucosa and on the mesenteric attachment. This gives rise to distinct classes of afferents; muscular, mucosal and serosal (splanchnic and pelvic pathway) and mesenteric (splanchnic only) and muscular/mucosal (pelvic only). These afferents allow the full range of mechanical and chemical stimuli occurring within the colorectum to be detected. Mucosal afferents detect low-threshold distortion of the intestinal mucosa and respond to endogenous mediators, whereas muscular afferents respond to low-threshold distension and contraction and have wide dynamic ranges of response, including into the noxious range, and respond to endogenous mediators. Muscular/mucosal afferents display the combined properties of muscular and mucosal afferents. Mesenteric and serosal (also known as vascular afferents) have high thresholds to mechanical stimuli and respond to a wide array of endogenous mediators, particularly, inflammatory and immune mediators.

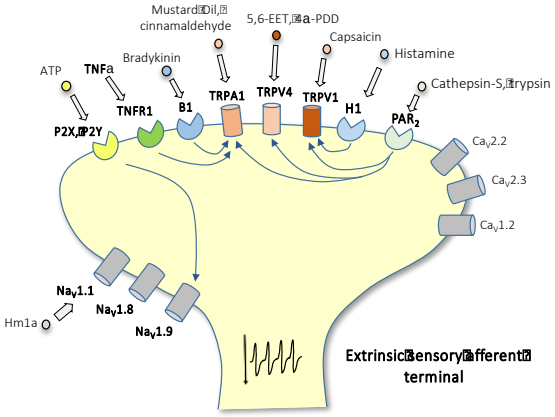

Figure 2: Key pro-nociceptive mechanisms within extrinsic sensory afferent neurons innervating the colorectum.

The peripheral afferent terminal of colorectal afferents express a wide array of pro-nociceptive ion channels and receptors that can enhance afferent excitability and increase action potential firing. These include the voltage-gated sodium (Na_V) channels, $Na_V1.1$ (which contributes to mechanical pain), $Na_V1.8$ and $Na_V1.9$ (which can be sensitised by activation of an array of receptors, including P2X, P2Y). The transient receptor potential (TRP) channels TRPA1, TRVP4, and TRPV1 play primary and integrative roles in sensory function, either being activated directly by exogenous and endogenous stimuli, or by being sensitised in response to receptor activation (including bradykinin B1, TRPA1, histamine, TNF-α, and protease-activated receptor 2 (PAR_2)). Several voltage-gated calcium (Ca_V) channels play key roles in regulating neuronal excitability, including $Ca_V2.2$ (N-type), $Ca_V2.3$ (R-type), $Ca_V1.2$ (L-type) and $Ca_V3.2$ (T-type).

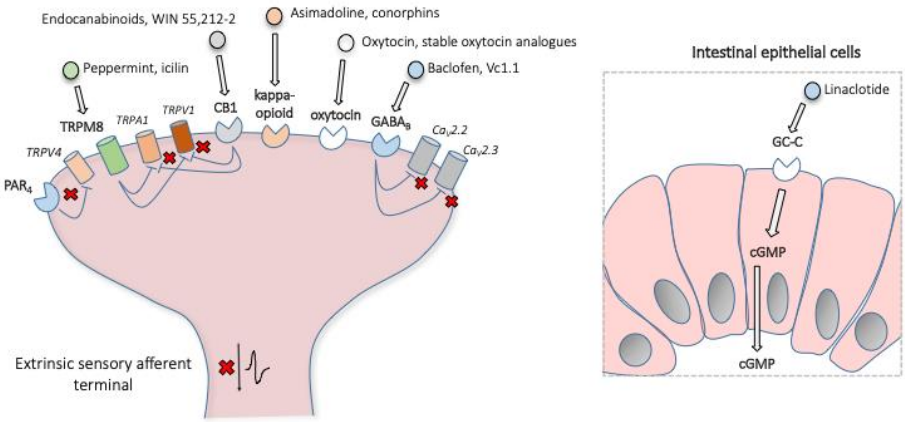

Figure 3: Key anti-nociceptive mechanisms within extrinsic sensory afferent neurons innervating the colorectum and colonic epithelial cells.

Numerous anti-nociceptive mechanisms exist and are up-regulated during inflammatory or chronic visceral hypersensitivity (CVH) states. This includes expression of TRPM8, which upon activation inhibits TRPA1 and TRPV1 function. Activation of the cannabinoid receptor 1 (CB1) also reduces TRPV1 function on colonic afferents. Activation of the κ-opioid receptor inhibits colonic nociceptors, but only in inflammatory and CVH states. Activation of the oxytocin receptor inhibits colonic nociceptors, but only in CVH states, due to a large increase in oxytocin receptor expression. Activation of the $GABA_B$ receptor inhibits colonic afferents in both healthy and CVH states via down-stream inhibition of the voltage-gated calcium channels $Ca_V2.2$ and $Ca_V2.3$. Activation of PAR_4 has been shown to subsequently decrease TRPV4 function. Activation of GC-C on epithelial cells results in cGMP production, which is pumped out of the cell basolaterally, where it then acts upon and inhibits colonic nociceptors.