Neuroplasticity and dysfunction after gastrointestinal inflammation

Stuart M. Brierley and David R. Linden

Visceral Pain Group, Centre for Nutrition and Gastrointestinal Diseases, Discipline of Medicine, University of Adelaide, South Australian Health and Medical Research Institute (SAHMRI), Adelaide, SA 5000 AUSTRALIA (S.M. B)

Department of Physiology and Biomedical Engineering and Enteric NeuroScience Program, Mayo Clinic College of Medicine, Rochester MN 55905 (D.R. L).

Correspondence to:
S.M.B.

stuart.brierley@adelaide.edu.au

Acknowledgements:

The authors wish to thank Ms J. Applequist at the Mayo Clinic College of Medicine and Ms Jessica Maddern at the University of Adelaide for their secretarial assistance. S.M.B. is supported by an NHMRC R.D Wright Biomedical Fellowship and by NHMRC Australia Project grants (1008100, 1049682, 1049928 and 1063803). D.R.L. is supported by NIH grant DK76665.

Competing interests:

S.M.B. receives research support from Ironwood Pharmaceuticals Inc. and Tioga Pharmaceuticals Inc. None of the details relating to this research support are discussed in this review. D.R.L. declares no competing interests.

Abstract

The gastrointestinal tract is innervated by several distinct populations of neurons, whose cell bodies either reside within (intrinsic) or outside (extrinsic) the gastrointestinal wall. Normally, most individuals are completely unaware of the continuous, complicated functions of these neurons. However, for patients with gastrointestinal disorders, such as IBD and IBS, altered gastrointestinal motility, discomfort and pain are common, debilitating symptoms. Although bouts of intestinal inflammation underlie the symptoms associated with IBD, increasing pre-clinical and clinical evidence indicates that infection and inflammation are key risk factors for the development of other gastrointestinal disorders. Notably, in IBS a strong correlation exists between prior exposure to gut infection and symptom occurrence, with the duration and severity of the initial illness the strongest associated risk factors. This Review discusses the current body of evidence for neuroplasticity (the structural, synaptic or intrinsic changes that alter neuronal function) affecting gastrointestinal function. Such changes are evident during inflammation and, in many cases, long after healing of the damaged tissues, when the nervous system fails to reset back to normal. Neuroplasticity within distinct populations of neurons has a fundamental role in the aberrant motility, secretion and sensation associated with common clinical gastrointestinal disorders. To find appropriate therapeutic treatments for these disorders, the extent and time-course of neuroplasticity must be fully appreciated.

Key Points:

- Gastrointestinal infection and inflammation are key risk factors for the development of numerous clinical gastrointestinal disorders, which present with symptoms that include, altered motility or secretion, and abdominal discomfort and pain.

- Neuronal processing along the gut–brain axis is crucial for the function and modulation of key gastrointestinal processes; findings suggest that this processing can be altered by gut inflammation or infection

- Inflammation or infection causes specific changes in enteric neuronal excitability, which can persist long after inflammation has resolved. In some models, inflammation also causes a rapid loss of myenteric neurons and viscerofugal neurons.

- Inflammation causes a specific hypersensitivity of visceromotor sympathetic neurons in prevertebral ganglia, which persists long after inflammation has resolved.

- Extrinsic gut sensory afferents express pro-nociceptive channels and receptors that can be activated in response to inflammatory and immune mediators, leading to acute neuronal hyperexcitability, visceral hypersensitivity and neurogenic inflammation.

- Inflammation leads to the lowering of mechanical activation thresholds of high-threshold or low-threshold afferents; this leads to hyperexcitability in afferent neuronal cells bodies, and increased activation of nociceptive pathways in the central nervous system (CNS).

Explanation Boxes:

Box 1 The Enteric Nervous System (ENS): consists of networks, or plexuses, of neurons. Their axons and neuronal cell bodies coalesce with enteric glia as ganglia within the plexus and interganglionic fibre tracts of varying nerve density. The myenteric, or Auerbach's plexus, resides between the longitudinal and circular muscle layers in the muscularis externa. It is continuous, essentially running from the mid-oesophagus to the anal sphincter. The submucosal, or Meissner's plexus, resides within the submucosa and is absent or sparse in the oesophagus and stomach but present throughout the intestines.

Box 2: Enteric neurons: Individual enteric neurons function either as intrinsic afferents (cells that respond, with or without non-neural transducer cells, to mechanical or chemical stimuli to initiate reflexes), efferents or motor neurons (cells that transmit information to effector cells such as arterioles, glandular epithelium, or smooth muscle), or interneurons (cells that relay, integrate and modify reflexes). Individual enteric ganglia are composed of apparently random, intermingled selections of different classes of neurons. Intrinsic reflex activity that is responsible for co-ordinating intestinal motility is accomplished via overlapping circuits of afferent, interneuronal and efferent neurons located throughout the length of the gut. Based on their electrophysiological properties enteric neurons can be subdivided functionally into either after-hyperpolarization (AH) neurons (intrinsic afferents) or synaptic (S) neurons (interneurons and motor neurons).

Box 3: Viscerofugal neurons have cell bodies within the myenteric plexus, but have projections out of the gut wall, via extrinsic nerve trucks, to prevertebral ganglia (PVG). These viscerofugal neurons sense and receive information regarding mechanical distension of the intestine and transmit this information to postganglionic sympathetic visceromotor neurons in the PVG.

Box 4: ***Sympathetic neurons:*** are an anatomically defined division of the autonomic nervous system. Preganglionic sympathetic neurons involved in regulating gastrointestinal function are located in the intermediate zone (or lamina VII) of the thoracolumbar spinal cord where they cluster with other preganglionic neurons that serve different functions. These neurons integrate synaptic input from sympathetic premotor neurons located in several nuclei of the brain stem and hypothalamus as well as spinal interneurons and primary afferent neurons. The axons of these neurons project through the ventral root and the gray rami to the paravertebral ganglia where a small portion terminates in the paravertebral ganglia. The majority do not synapse, but rather project through the splanchnic nerves and terminate in the prevertebral ganglia. Postganglionic sympathetic fibres fasciculate with sensory neurons and course through the mesentery in close proximity to the many branches of the celiac, superior mesenteric and inferior mesenteric arteries. Axons of postganglionic vasomotor neurons that

extensively innervate the arterioles within the gut wall and mesentery providing direct vasoconstrictor control of gastrointestinal blood flow arise from both paravertebral and prevertebral ganglia. Axons of visceromotor neurons that innervate enteric ganglia, the deep muscular plexus and to a lesser extent the mucosa arise from prevertebral ganglia and alter intestinal motor and secretory function.

Box 5: Parasympathetic neurons: are a separate division of the autonomic nervous system with inputs to the gut concentrated in the oesophagus, stomach and upper small intestine and also the rectum and anal sphincter. There is relatively little parasympathetic innervation of the mid-gut. The dorsal motor nucleus of the vagus (DMV) and the nucleus ambiguous contain the cell bodies of preganglionic parasympathetic neurons innervating the proximal gastrointestinal tract. The parasympathetic nuclei in the lower lumbar and sacral levels of the spinal cord contain the cell bodies of the preganglionic parasympathetic neurons innervating the hindgut. Neuronal tracing studies in animals reveal that the myenteric plexus is the major innervation target of preganglionic parasympathetic neurons with few exceptions indicating the postganglionic parasympathetic neurons are the enteric neurons themselves. Highly branched varicose endings in the ganglia are in close proximity to most myenteric neurons where released acetylcholine activates both excitatory and inhibitory enteric neural pathways to modulate gut function via intrinsic reflex circuitry.

Box 6: Extrinsic sensory afferent neurons: project to the CNS and give rise to perceivable sensations. Vagal afferents innervating the gut have cell bodies in the nodose/jugular ganglia, whilst spinal afferents have cell bodies in multiple dorsal root ganglia (DRG). The latter is further subdivided into splanchnic and pelvic afferents based on the nerves through which they project. Vagal afferent neurons project centrally to the brainstem, whilst spinal afferents project centrally to the spinal cord. As such these extrinsic sensory neurons provide the afferent limb of spinal and brainstem reflexes, provide input to central autonomic processing centres, and result in sensations of visceral origin, including pain. Vagal afferents are generally associated with signalling physiological events, such as fullness and satiety, whereas spinal pathways are generally associated with higher-threshold sensations such as pain, discomfort, bloating and urgency. Importantly, however, these neurons also serve efferent function and utilize axonal reflexes to mediate neurogenic inflammation and modulate synaptic transmission in enteric and prevertebral ganglia. The peripheral projections of vagal and spinal nerve fibres terminate at various levels within the gut wall, including the mucosa, muscle layers, enteric ganglia, and blood vessels in the sub-mucosa, serosa and mesentery. Many of these endings are specialised structures, rather than the traditionally perceived 'free nerve endings'.

Introduction [level 1 heading]

Neural control of gastrointestinal function is provided by distinct populations of neurons, whose cell bodies lie either within or outside the gut wall. Neuronal control involves interactions between the following reflexes: one, local enteric reflexes within the gut wall; two, extraspinal reflexes that originate in the gut wall and pass through prevertebral sympathetic ganglia without involving the central nervous system (CNS); and three, reflexes that pass to and from the gut via the CNS[1] (Figure 1). These neurons and their nerve terminals reside within a complex signalling environment, in which they are subjected to mechanical distortion during distension or contraction and a changing milieu of neuroactive signalling molecules. This environment, and concomitantly neuronal function, can be modulated at numerous levels by stress,[2,3] the immune system,[4] gut microbiota,[5] enteric glia,[6] as well as macronutrients [7], and the circadian [8] and feeding state [9] of the host. In particular, inflammation of the gut, either through abnormal immune responses or via gut infection has been consistently demonstrated to cause neuroplasticity (that is, structural, synaptic or intrinsic changes that alter neuronal function). In turn, neuroplasticity contributes to abnormal secretion, motility and sensation resulting in the development of discomfort, pain and diarrhoea or constipation. This Review focuses on short-term (hours or days) neuroplasticity, the resultant effects of gut inflammation on neuronal function in the gut–brain axis, and the persistent (weeks and months) neuroplasticity and dysfunction that remains after resolution of inflammation. As these studies have largely been conducted in animal models, it is instructive to present an overview of the clinical symptoms of gastrointestinal dysfunction to which neuroplasticity probably contributes. For brevity, we describe the accumulated evidence for dysfunction in IBD, and in part, IBS. Information on other inflammatory diseases of the gastrointestinal tract can be found in the supplementary information.

Clinical manifestations of neuroplasticity [level 1 heading]

IBD [level 2 heading]

IBD includes Crohn's disease and ulcerative colitis, which are characterized by chronic remitting and relapsing inflammations of the intestine. Although the aetiology of IBD is unknown, it is generally accepted that these diseases develop through an inadequately suppressed or exaggerated immune response to luminal antigens, probably derived from the microbiota, in genetically susceptible individuals.[10] Abdominal pain, diarrhoea, gastrointestinal bleeding and malnutrition are clinical symptoms of IBD, and current medical and surgical therapies for IBD seek to resolve mucosal inflammation and reduce symptoms. Although biologic therapies for IBD seem to demonstrate greater mucosal healing than symptom recovery,[11] steroid and 5-aminosalicylic acid therapies tend to provide greater symptom relief than mucosal healing.[12] One interpretation of these findings is that the mechanisms contributing to mucosal inflammation and symptoms are distinct. Interestingly, there seems to be a subset of patients with IBD (20%), whose symptoms do not subside following near complete endoscopic recovery.[13,14] A wealth of studies have demonstrated defined patterns of motor, sensory and autonomic dysfunction as well as neuropathies indicative of neuroplasticity in IBD, which are discussed below.

Motility changes in active IBD [level 3 heading]

Patients with active ulcerative colitis have been shown to display slower whole-gut transit,[15] and although inflammation is localised to the colon—reduced small-bowel transit compared with healthy individuals.[15] Numerous studies indicate that colonic and rectal motor patterns in patients with ulcerative colitis can be different from healthy controls, with reduced rhythmic phasic contractions and increased high-amplitude and low-amplitude propagating

contractions [16,17]. Activity of rythmic phasic contractions inversely correlates with increased stool frequency.[17] Interestingly, simultaneous pressure and myoelectrical activity recordings show reduced concurrency between spike potentials and increases in pressure, especially in response to a meal (the gastrocolonic response).[18] This observation is consistent with intracellular recordings of circular smooth muscle cells from patients with ulcerative colitis, in which contractile activity is coupled more frequently to slow waves rather than spike potentials.[19] Colonic scintigraphy shows increased to-and-fro movements of tracer in both resting and postprandial states in patients with ulcerative colitis compared with controls,[20] as well as proximal colonic stasis and increased sigmoidal transit.[15] The lack of rhythmic phasic contractions is considered consistent with the lack of haustra, or segmenting contractions, in 'lead pipe' radiographic images. Compared to ulcerative colitis, motility studies in patients with active Crohn's disease are lacking. Dynamic MRI assessing small-bowel motility has demonstrated reduced ileal motility in patients with Crohn's disease.[21] Other noninvasive recording methods, such as hydrogen breath tests, have identified decreased orocecal transit time in patients with active Crohn's disease.[22] Furthermore, subsets of patients with Crohn's disease display delayed gastric emptying of solid but not liquid meals.[23,24] By contrast, rectoanal manometry has been inconclusive, with reports of either absent[25] or normal[26] rectoanal inhibitory reflexes with reduced,[27,28] unchanged[25] or increased[29] resting and maximal squeeze anal pressures.

Motility changes in IBD in remission [level 3 heading]

Few studies have investigated motor function during the postinflammatory state of IBD, and conflicting results have been reported. Reports suggest normal[30] or even increased[31] rhythmic phasic contractions compared with controls and either reduced[30] or normal[31] gastrocolonic responses during remission from ulcerative colitis. Reports also indicate an increased incidence of single and clustered propagating contractions compared to controls, which

importantly relates to the reporting of symptoms during remission from Crohn's disease.[32] In patients in remission from either Crohn's disease or ulcerative colitis, duodenal pressure waves initiated by luminal acid are increased, whereas the propagation of lipid-induced duodenal pressure waves is reduced.[33] In addition, a subset of patients in remission from Crohn's disease exhibit delayed gastric emptying.[24] Whether identified motor disturbances during active or remitting IBD are attributable to neuroplasticity or non-neural mechanisms (see Supplementary Side Bar 1) in humans remains unclear. However, studies from animal models (see below) suggest that neuroplasticity might have some role in motility disturbances in IBD in remission.

Enteric neuropathy in active IBD [level 3 heading]

Several lines of evidence indicate that the structure and neurochemical composition of the enteric nervous system (ENS) is altered during active IBD.[34,35] Numbers of mucosal and muscular nerve fibres increase, along with immunoreactivity of neurochemicals including tyrosine hydroxylase, substance P, calcitonin gene-related peptide (CGRP), vasoactive intestinal polypeptide (VIP), 5-hydroxytryptamine (5-HT), and nitric oxide (NO) synthase.[36,37] These findings are by no means unequivocal, as some studies have identified reduced or no change in VIP immunoreactivity,[38,39] no change in immunoreactivity of multiple neuropeptides,[40] and a reduction of tyrosine hydroxylase-immunoreactive nerve fibres.[41] However, evidence exists of axon degeneration in Crohn's disease,[42,43] with cyclo-oxygenase (COX)-2 expression greatly increased in myenteric ganglia during active IBD.[44]

Changes in the number of enteric neurons during active IBD are controversial with studies yielding conflicting reports of increased,[45,46] no change,[45,47] or decreased[48] numbers of myenteric neurons. Mechanisms contributing to changes in nerve structure might include increased apoptosis[49] and increased levels of nerve growth factor (NGF).[46] Future studies that

account for patient stratification and temporal considerations might help resolve these discrepancies. Despite its proximity to the lamina propria, where inflammation is apparent in both Crohn's disease and ulcerative colitis, little evidence exists for altered numbers of ganglion cells in the submucosal plexus of patients with IBD.[37] However, plexitis (inflammatory infiltrate of the submucosal or myenteric plexuses) is common in Crohn's disease [33], [37,40] ulcerative colitis[36,40,43] and other gastrointestinal disorders [40] (see Supplementary Side Bar 2). Notably, major histocompatability complex (MHC) antigens are increased in enteric glia and neurons in both Crohn's disease and ulcerative colitis[50,51] and might contribute to immune infiltration. Importantly, plexitis occurs in otherwise histologically normal tissue outside the involved segments of bowel.[52] In fact, plexitis in marginal resected tissue is predictive of recurrence in Crohn's disease following surgery,[53,54] which suggests that plexitis and perhaps neuroplasticity-induced dysfunction might precede mucosal involvement.

Sensation abnormalities in active IBD [level 3 heading]

Reduced stool volume and increased stool frequency during ulcerative colitis suggests that patients have reduced thresholds for the urge to defecate.[55] The maximal tolerable balloon volume might also be reduced in patients with active ulcerative colitis,[55,56] with pain reported at lower cross-sectional areas of the rectum compared with healthy individuals.[57] This finding might be due to increased muscle tone, as pain responses relative to muscle tension are similar between patients and controls.[57] In Crohn's disease, although a subset of patients experience urgency to defecate,[58] the threshold pressure for discomfort in patients with Crohn's disease is substantially higher than controls.[26] The reporting of abdominal pain ranges from 40–90% of patients with IBD,[55,59,60] although pain in IBD seems to be affected by the age at onset. In particular, paediatric patients with IBD have a higher incidence of pain[61] compared to patients diagnosed >40 years of age.[62]

Levels of the proinflammatory cytokine, TNF-alpha are elevated in colonic biopsy samples from patients with active ulcerative colitis. Supernatants from these biopsy samples and TNF-alpha itself can activate and sensitize colonic dorsal root ganglion (DRG) neurons from mice by enhancing the voltage-gated sodium (Na_V) currents, and suppressing the voltage-gated potassium (K_V) currents, in particular I_A (A-type) and I_K (delayed rectifier) that regulate neuronal excitability.[63] Notably, these are the same currents that are altered in numerous animal models of inflammatory and postinflammatory hyperalgesia (see below), suggesting a potential underlying mechanism for pain during active ulcerative colitis.

Sensation abnormalities in IBD in remission [level 3 heading]

Several studies suggest that subsets of patients with IBD in remission retain altered rectal hypersensitivity and remain intolerant to rectal distension.[56] Overall, 20–60% of patients experience persistent pain during remission from IBD,[60,64,65] whereas other patients with IBD report that rectal sensitivity returns to normal during remission.[55,66] Interestingly, sensation of duodenal acid or lipid is not different than controls during remission from Crohn's disease or ulcerative colitis.[33] One study of patients with ulcerative colitis in remission demonstrated inhibition of activity in the limbic and paralimbic brain regions; conversely, these brain regions were activated by colorectal distension in patients with IBS.[67]

Autonomic dysfunction in IBD [level 3 heading]

During active IBD there is evidence of imbalanced autonomic function with increased sympathetic influence and decreased parasympathetic influence.[52,68,69] Testing numerous autonomic reflexes reveals hyper-reflexia in patients with active IBD that correlates with the degree of systemic inflammation.[70] Interestingly, an α_2-adrenergic receptor agonist can restore normal autonomic cardiovascular function and improve disease activity index scores

[68], which is consistent with previous findings of a beneficial effect of clonidine treatment for IBD.[71] However, conflicting results are reported for IBD in remission, with either parasympathetic predominance,[72] or lower parasympathetic function reported.[73]

IBS [level 2 heading]

IBS is a prevalent chronic functional gastrointestinal disorder that affects 7–14% of the population [74]. This disorder is characterized by abdominal pain or discomfort associated with altered bowel habit, and is subclassified as IBS with constipation (IBS-C), IBS with diarrhoea (IBS-D), alternating or mixed IBS (A/M-IBS) and postinfectious IBS (PI-IBS). Neuroplasticity is well appreciated in IBS,[75] with symptoms (most predominantly abdominal pain) being triggered or worsened by stress, possible via altered central noradrenergic signalling.[76] Persistent neuroplasticity might arise after acute bouts of intestinal inflammation that resolve, or might be the result of low-grade inflammation not identified during routine clinical screening.[77,78] Interestingly, in their original description of IBS, Peters and Bargen[75] wrote with passion for the need to define IBS as distinct from inflammatory diseases. This distinction was necessary because contemporary gastroenterologists were defining functional syndromes with terms such as “membranous enteritis”, “membranous colitis”, “mucous colitis”, “neurogenic mucous colitis” or “spastic colitis”.[79] It is perhaps because of the subsequent whole-hearted adoption of clear boundaries between functional and organic digestive diseases that assertions of the concept that IBS and IBD are on the same clinical spectrum have met with controversy. However, a failure to understand the overlap between IBS and IBD might contribute to delays in proper diagnosis and therapy.[80,81] Approximately 40% of patients with IBD in remission meet symptom criteria for diagnosis of a functional bowel disorder.[80,81] Furthermore, the incidence of IBS is two to three times higher in patients with IBD in remission than the general population.[82] IBS is also a risk factor for IBD.[83] Whether these data reflect the development of new disease, or a delayed diagnosis remains to

be resolved.[80,81] Perhaps a reason for the overlap lies with the gut microbiota, as mucosal barrier function is disturbed in patients with IBD[84] and increased epithelial permeability is evident in the small intestine and colon of patients with IBS.[85,86] These changes might enable bacteria to gain access to the interstitial compartment, initiate innate immune responses[87] and access neurons. Indeed, several animal studies have identified that cell products from *Salmonella typhimurium* and *Escherichia coli NLM28* can activate or sensitise extrinsic sensory afferents.[88-91]

Post-infectious IBS [level 3 heading]

The most compelling case for inflammation-induced persistent neuroplasticity is the development of symptomatic IBS following infectious gastroenteritis, a clinical entity known as PI-IBS.[92-94] Numerous clinical studies attribute IBS symptom development in 3–36% of individuals[93,94] after infection of *Campylobacter*, *Salmonella*, *Shigella*, *E. coli*, or *Giardia lamblia.* [93,94] In the short-term setting of tissue damage, inflammation in response to infection is a protective process that facilitates wound healing. However, these clinical findings, in addition to the pre-clinical findings detailed below, suggest that in these individuals the peripheral and central neuroplasticity induced by infection and inflammation is maintained and fails to reset back to normal, long after healing of the intestinal tissue.

Low-grade inflammation and IBS [level 3 heading]

Several lines of evidence suggest that IBS is associated with a persistent low-grade inflammation within the gut wall[95,96] and altered immunological function.[97-100] Plexitis occurs in 70–100% of patients with IBS or dysmotility symptoms who consent to undergo full-thickness biopsy, and might be a contributing factor in the overlap of IBS and IBD.[101,102] In mucosal biopsy samples from patients with IBS, evidence indicates increased levels of interleukin (IL)-1β,[103] and increased numbers of mucosal mast cells[96] and intraepithelial

lymphocytes.[104] Correspondingly, supernatants from biopsy samples from patients with IBS, but not healthy individuals, activate extrinsic sensory nerve endings and neuronal cell bodies from mice via the histamine H1 receptor and serine protease mechanisms.[105] In addition, these mediators activate human submucosal enteric neurons.[106] Thus, inflammatory mediators potentially contribute to the mechanisms underlying abdominal pain and dysmotility in patients with IBS. Changes are also evident in peripheral blood mononuclear cells (PBMCs) from patients with IBS.[97-100] In particular, evidence indicates increased activation of T lymphocytes,[99,100] and correlation of pain with levels of several proinflammatory cytokines, including TNF, IL-1β and IL-6 in PBMC supernatants from patients with IBS-D. Notably, PBMC supernatants from patients with IBS-D evoke mechanical hypersensitivity of colonic afferents from mice.[97-99] These findings also demonstrate two distinct mechanisms by which cytokines increase nociceptive signalling from the colon. The first example is IL-1β, which causes direct action potential discharge of colonic afferents via a $Na_V1.7$ dependent mechanism [97]. The second is TNF, which causes mechanical hypersensitivity via a transient receptor potential ankyrin-1 (TRPA1) dependent mechanism.[97] Additional evidence suggests that patients with IBS tend to harbour an *IL-10* gene polymorphism associated with reduced production of this anti-inflammatory cytokine.[107] Overall, these findings suggest that neuronal function is a balance between both excitatory and inhibitory mechanisms and that shifting this balance towards excitation results in aberrant neuronal activity. If maintained, this imbalance **[Au:OK?]** results in the development and maintenance of persistent neuroplasticity and gastrointestinal symptoms.

Neuroplasticity in animal models [level 1 heading]

Animal models of gastrointestinal inflammation [level 2 heading]

Animal models of gastrointestinal inflammation have been used to answer the mechanistic questions posed by the clinical studies described above. There are several important points to note regarding pre-clinical animal models of gastrointestinal inflammation. Firstly, although no current animal model recapitulates all aspects of human digestive diseases, these models are essential because they can be tightly controlled for species, age, genetic background, diet, stress and experimental time-course. This level of control is something that cannot be said for the use of human tissue in functional experiments. Secondly, not all experimental models are the same, nor does the same inducing manipulation result in the same immune response and neuroplasticity in different animal species or strains, or even in different laboratories. Overall, experimental animal models of gastrointestinal inflammation can be broadly grouped into four major categories: chemical-induced, infection-induced, adoptive transfer-induced and genetically-induced (Supplementary Table 1). Thirdly, gastrointestinal inflammation is not a single response, but rather a broad spectrum of innate and cell-mediated immune responses. As such, these models, much like the clinical disorders they intend to mimic, differ in immune responses and resultant neuroplasticity based on the inducing manipulation. These factors include the chemical, infectious agent, cell transfer, genetic manipulation, dose, route and time course of the manipulation. Contributing factors during the inducing manipulation also include the species and strain of the animals, the environmental factors that contribute to the state of the animals, especially their stress levels. Overall, data from numerous laboratories using varying inflammatory models suggest that short and long-term neuroplasticity occurs at every level of the brain–gut axis.

Inflammation-induced functional changes [level 2 heading]

Active inflammation [level 3 heading]

Inflammation-induced dysmotility is highly dependent on the model of inflammation used.[108] Hypercontractility and enhanced motility are hallmarks of type-2 T-helper (T_H2) models,

including *Trichinella spiralis* or *Nippostrongylus brasiliensis* infection.[109-112] By contrast, type-1 T-helper (T_H1) models, such as trinitrobenzene-sulphonic acid (TNBS) ileitis and colitis, are characterized by hypocontractility and reduced motility.[113-115] In TNBS-induced colitis, propulsive motility is actually temporarily or completely halted, but only at specific sites of ulceration (Figure 2).[116] Neural and mast cell interactions with the epithelium produce ion-transport abnormalities during infection with *N. brasiliensis.*[111,117] In particular, they produce a shift away from cholinergic to non-cholinergic regulation, with increased amounts of the proinflammatory neuropeptide, substance P,[118] defective mucosal cAMP production and inhibition of ionic secretion.[119] Furthermore, NO derived from inducible nitric oxide synthase (iNOS) mediates, in part, inflammation-induced suppression of neurally evoked electrolyte transport.[120] Notably, TNBS-iduced colitis results in inhibition of calcium-dependent secretion in the noninflamed proximal colon via a reduction of the contribution of the ENS.[121]

Resolved inflammation [level 3 heading]

Many of the alterations observed during inflammation persist into the post inflammatory state (Figure 2). Persistent gut dysfunction is evident in chronic parasitic infection of *Trichuris muris*, which is independent of the continued presence of the parasite and is maintained by an inflammatory process that includes eosinophils.[122] Similarly, gastric and intestinal dysmotility is evident 70-days postinfection with *T. spiralis,* which can be normalized by dexamethasone administration.[123] TNBS-induced colonic dysmotility also persists in the postinflammatory state, but unlike the dysmotility evident during inflammation, cannot be reversed by COX-2 inhibitors.[124] Furthermore, there is evidence of prolonged neurally-mediated ion secretion after resolution of colitis,[125] which might contribute to the dysfunction. In general, these findings of postinflammatory dysmotility are consistent with the altered function reported in patients with IBS and in patients in remission from IBD.

Neuroplasticity in enteric neurons [level 2 heading]

Plexitis and neuron loss [level 3 heading]

Inflammation clearly has profound effects on enteric neural circuits (Box 1). However, much like the contrasting reports of altered numbers of enteric neuron in patients with active IBD, there are also differing reports in animal models of inflammation. In the mouse ileum, evidence indicates considerable myenteric neuron damage, but not loss, during *Schistosoma mansoni* infection[126] and similarly no loss of neurons, but a reduction in levels of NO synthase following TNBS-induced-ileitis.[127] Correspondingly, no evidence exists of neuronal proliferation during ileitis, although mitosis is evident in myenteric glia.[128] By contrast, TNBS-induced colitis is associated with progressive and selective alterations in myenteric plexus structure and function,[129] which seems to consistently cause up to a 20% loss in myenteric neurons, with apoptosis and transglutaminase up-regulation apparent.[130] This neuronal loss is evident as early as 12 h after the inflammatory insult,[131] and is partly mediated by neutrophil granulocyte infiltration[132] and by activation of a neuronal signalling complex composed of purinergic $P2X_7$ receptors and pannexin-1 (PanX1) channels.[48] Loss of myenteric neurons after a single inflammatory event is still evident 2 months later, long after inflammation has resolved,[131] which is also consistent with the observed lack of proliferation. Although this loss of neurons seems to be indiscriminate across different enteric neuronal populations, there is an 80% loss in viscerofugal neurons (Figure 2). [133] Notably, this loss of viscerofugal neurons only occurs in the area of inflammation and does not occur in colitis induced by acetic acid treatment, which again suggests that neuronal loss is associated with neutrophil infiltration and specific immune processes.[133] The loss of viscerofugal neurons in the colon corresponds with a reduced frequency of ongoing synaptic potentials in visceromotor neurons within the prevertebral ganglia. These changes are still present 2 months postinflammation, again highlighting the profound long-term neuroplasticity that can be evoked by a single inflammatory insult.[133] Evidence also suggests that enteric neuron density can contribute to

the overall severity of intestinal inflammation,[134] perhaps via a neurogenic inflammation-mediated mechanism. Such findings are consistent with clinical observations that enteric neuron hyperplasia might be a predisposing factor to subsequent mucosal inflammation.[135]

Enteric neuron excitability and synaptic plasticity [level 3 heading]

There is extensive evidence of hyperexcitability of myenteric AH neurons causing dysmotility after TNBS-inflammation[136] and *T. spiralis*[137] infection (Figure 2). The mechanisms by which this occurs is different as the TNBS-induced effects are COX-2 dependent,[138] whereas the *T. spiralis*-induced hyperexcitability can be suppressed with acute exposure to blockers of histamine, adenylate-cyclase, COX or leukotriene pathways.[137] Colitis also causes enhanced synaptic facilitation in the inflamed myenteric plexus, which involves a presynaptic increase in activity of protein kinase A.[139] AH neuron hyperexcitability and synaptic facilitation is also evident in the colonic submucosal plexus, the latter due to increased noncholinergic transmission.[140] There is evidence for prejunctional α_{2a}-adrenoceptors contributing to enhanced inhibitory control of cholinergic and noradrenergic transmission, both at the inflamed colon and at distant noninflamed sites.[141] Correspondingly, evidence also exists for reduced acetylcholine release from the myenteric plexus of animals with *T. Spiralis*[142] and TNBS-induced[141] inflammation.

Inflammation-induced dysmotility at specific sites of ulceration is due to a transient reduction of inhibitory purinergic neuromuscular transmission, which thereby reduces smooth muscle relaxation and disrupts co-ordinated motility and propulsion.[116] Myenteric neuron loss might contribute to a reduction in neuromuscular transmission as the inhibition of neuronal Panx1 during DNBS-induced colitis, which reduces cell loss, protects against postinflammation deficits in inhibitory neuromuscular transmission in mice.[48] In TNBS – induced colitis, inhibitors of hyperpolarization-activated cyclic nucleotide-gated channels,

which contribute to AH neuron excitability, normalize motility in the ulcerated regions[143] as well as the increased motility at either side of ulcers [116] (Figure 2). These data strongly support a causal relationship between neuronal excitability and dysmotility. As such, these alterations at the site of inflammation or those on either side of ulceration, might explain differing reports of motility changes in human patients with IBD.

Inflammation-induced enteric neuroplasticity is not only localised to the colon—ileitis also alters synaptic transmission and AH neuron excitability.[144] Furthermore, it is also apparent that inflammation in one region of the gut can profoundly influence other noninflamed regions. For instance inflammation of the guinea-pig ileum induces hypersensitivity in submucosal AH neurons and enhances synaptic transmission in the distal colon.[145] These changes are very similar to those induced by colitis itself. However, colitis-induced changes in the submucosal plexus of the ileum are very different; hyperexcitability is largely absent, slow synaptic transmission is profoundly altered and noncholinergic secretion is reduced.[146] Colitis causes an increased excitability and depolarization of myenteric AH neurons in the ileum, which seems mechanistically different than ileitis-induced excitability.[147] Overall, these findings suggest that changes in enteric neural circuits are contributing factors in inflammation-induced dysfunction at sites distant from a localized inflammatory insult. These findings might explain why patients with ulcerative colitis, for instance, display reduced small-bowel transit.[15]

Hyperexcitability of AH neurons, facilitated fast synaptic transmission in both the submucosal and myenteric ganglia and intestinal dysmotility are all still evident in the postinflammatory state.[124,125,147] These findings in animal models might explain the continued dysmotility in patients with IBD in remission, or perhaps the dysmotility of patients with IBS. Collectively, these findings demonstrate the long-term effects of a single inflammatory insult on enteric neurons.

Neuroplasticity in autonomic efferent.neurons [level 2 heading]

Reports of autonomic dysfunction in animal models of gastrointestinal inflammation are numerous, which are consistent with the autonomic dysfunction described in humans. For example in rodents during TNBS-induced, dextran sodium sulphate (DSS)-induced and *T. spiralis*-induced colitis, evidence indicates altered sympathetic function within the gastrointestinal tract, with reduced evoked norepinephrine release occurring in both inflamed and noninflamed gut regions.[148,149] This reduced norepinephrine release occurs via steroid-sensitive and IL-1-mediated processes that do not require T lymphocytes. Reduced evoked release can occur via increased levels of autoinhibitory presynaptic α_{2a}-adrenoreceptors[141] or via reduced voltage-gated calcium currents.[149] Therefore, these changes might contribute to functional alterations in both the inflamed and noninflamed regions of the gastrointestinal tract during inflammation.

Evidence also indicates that colitis impairs sympathetic vasoconstrictor regulation of mesenteric arteries[150] and submucosal arterioles, via changes in blood flow, and reduces purinergic transmission.[151] Extrinsic afferent nerves have also been shown to participate in a cardiovascular depressor reflex in *N. brasiliensis*-sensitized rats,[152] whereby capsaicin evoked arteriolar dilation involves the release of histamine from mast cells, which then dilates the vessels by a NO-dependent mechanism.[153] Sympathetic vasoconstriction of submucosal arterioles is impaired during TNBS-induced or DSS-induced colitis because of increased ectonuclease-mediated reduction of purinergic neurotransmission.[125,154] Taken together these findings suggest that a disruption in the balance between vasoconstrictor and vasodilator influences might lead to altered blood flow and altered intestinal motility.

In prevertebral ganglia (PVG), hyperexcitability of visceromotor neurons is evident

from 12 h after initiation of TNBS colitis. This hyperexcitability seems to be mediated via enhanced activity of Na_V channels,[155] and can be maintained for at least 2 months after the inflammatory insult. By contrast, PVG vasomotor neurons exhibit normal excitability during inflammation (Figure 3).[155] These data indicate that enhanced sympathetic drive from hyperexcitable visceromotor neurons might protect the colon by reducing secretion and motility, but might also contribute to intestinal dysfunction during colitis.[155] In fact, reduced small-bowel transit is temporally correlated with hyperexcitability of visceromotor neurons.[147] Furthermore, similar hyperexcitability also occurs in visceromotor neurons, but not in vasomotor neurons, in the celiac ganglion following acute TNBS-induced ileitis (Figure 3).[156] Reduced evoked release of norepinephrine from these neurons, described above, might compensate for the observed hyperexcitability. These primary and compensatory effects might explain the varying reports of motility changes in patients with IBD described above.

To our knowledge, no study has investigated preganglionic sympathetic neurons in the spinal cord during intestinal inflammation. However, several studies have demonstrated changes in parasympathetic brainstem nuclei after inflammation [153–155]. c-Fos expression, a widely used marker for neuronal activation, is increased in the nucleus of the solitary tract following *N. brasiliensis* infection, which might reflect increased afferent activity (see below), and is likely to influence vagal outflow.[157] In addition, levels of c-Fos are increased in the brainstem after DSS-induced[158] or iodoacetamide-induced[159] gastritis. The vagus nerve seems to have a crucial role in regulating immune responses by dampening immune cell activation via an α7 nicotinic receptor mechanism.[160-162] This newly appreciated interaction between the CNS and the immune system in modulating gastrointestinal inflammation through both neuronal and humoral pathways is expertly reviewed elsewhere.[160-162]

Neuroplasticity in extrinsic afferent pathways [level 2 heading]

Acute neuroplasticity (level 3 heading)

Extrinsic afferents innervating the gastrointestinal tract provide sensory input to central processing centres, resulting in sensations of visceral origin, including pain. Experimental studies can be focussed on nerve terminals in the periphery (receptive fields) or the cell bodies of afferents in vagal or spinal ganglia, or determining downstream effects of afferent activation by studying central pathways or pain-related behaviours. Overall, it is clear that experimentally-induced inflammation or infection causes afferent hypersensitivity, neuronal hyperexcitability and, correspondingly, hyperalgesia and allodynia in animal models. These studies also indicate that the mechanisms underlying inflammatory and chronic long-term postinflammatory neuroplasticity are varied, but originate from the periphery.[95,96]

Receptive fields [level 4 heading]

Acid and pepsin-induced oesophagitis in ferrets causes a decrease in basal mechanosensitivity, although this decrease is offset by a sensitizing response to P_2X purinoceptor agonists.[163] In eosinophilic oesophagitis, which is characterized by increased infiltration and degranulation of eosinophils in the oesophagus, eosinophil-derived cationic proteins do not activate guinea-pig oesophageal vagal afferents, but instead cause sensitization to oesophageal distension.[164] By contrast, in a guinea-pig model of mast-cell-induced oesophagitis, sensitization of TRPA1, via a protease activated receptor 2 (PAR_2)-dependent mechanism, has an important role in vagal afferent mechanical hypersensitivity.[165] Similarly, experimentally induced gastritis in rats causes sensitization of mechanosensitive vagal afferents in the stomach.[166] Evidence indicates that TNBS-induced inflammation in mice causes mechanical hypersensitivity of both low-threshold and high-threshold afferents in the colon,[167,168,169] with high-threshold afferents also displaying reduced activation thresholds to mechanical stimuli.[167] The time course of this hypersensitivity can vary between splanchnic

and pelvic pathways,[167] but when present in mice is mediated by nociceptive ion channels including the acid sensing ion channel 3 (ASIC3),[170] TRPA1,[171,172] TRPV4[173,174] and TRPV1.[168,175,176] These channels can be sensitized by mediators released during inflammation including bradykinin,[171,177] 5-HT,[178] histamine,[179] PAR_2[174] and the PAR_2-dependent mediator cathepsin-S,[180] to cause neuronal hyperexcitability and hyperalgesia (Figure 4). Notably, TRPA1, TRPV4 and TRPV1 can also contribute to the inflammatory response themselves, by inducing neuropeptide release from peripheral afferent terminals and subsequent neurogenic inflammation.[181,182] As TRPV4 is also expressed on intestinal epithelial cells, its activation induces chemokine release and induces colitis.[183] Hypersensitivity is also likely to be induced via sensitization of mechanically insensitive afferents, also known as 'silent afferents'.[184,185]

Afferent cell bodies [level 4 heading]

Most studies utilizing chemical (for example, acetic acid, iodoacetamide, or TNBS), nematode (*T. Spiralis* and *N. brasiliensis*) or bacterial models (*Citrobacter rodentium*) of inflammation show that afferent neurons innervating the stomach,[186,187] small intestine[188,189] and colon[190-192] display pronounced hyperexcitability during gastrointestinal inflammation (Figure 5). Increases in Na_V currents, in particular $Na_V1.8$,[190,192] and suppression of K_V currents, in particular I_A and I_K currents[187,191] are implicated in this hyperexcitability.

Central pathways and pain-related behaviours [level 4 heading]

Crucially, neuroplasticity in vagal peripheral afferent endings and vagal neuronal function during inflammation translates to hypersensitive responses to gastric distension *in vivo*,[158,159,193] and increased neuronal activation in the brainstem following *N. brasiliensis* infection or DSS-induced[158,159] or iodoacetamide-induced gastritis.[157,158] Similarly, neuroplasticity in colonic peripheral afferent endings and DRG neuronal function during inflammation translates to hypersensitive responses to colonic distension[168,175] and increased

neuronal activation within the thoracolumbar and lumbosacral spinal cord *in vivo*[194,195] (Figure 5). Overall, this inflammation-induced neuroplasticity might underlie the sensory abnormalities experienced by patients with IBD and IBS and those patients with other clinical gastrointestinal disorders during inflammatory episodes.

Persistent neuroplasticity in extrinsic afferent pathways [level 3 heading]

Persistent neuroplasticity of sensory pathways is evident across a range of different experimental models. However, much like the clinical gastrointestinal disorders they intend to mimic, some inflammatory models produce contrasting effects with different afferent subtypes, different neuronal pathways and time courses involved in this process.[167] For example, although mouse jejunal afferents do not display changes in mechanosensitivity in postinfectious *N. brasiliensis*, they do show changes in chemosensitivity to somatostatin-receptor-2 stimulation.[196] Furthermore, these mouse jejunal afferents also display neuronal hyperexcitability, which is mediated by $Na_V1.8$, but not $Na_V1.9$.[188] Although purinergic mechanisms have no role in healthy small intestinal mechanosensitivity, they do contribute to mechanical hypersensitivity after *T. spiralis* infection .[197] The development of long-term mechanical hypersensitivity at 1 and 2 months postinfection[198] is dependent upon a process involving a $P2X_7$ receptor-dependent increase in immune cell expression and release of IL-1β. Notably, $P2X_7$ receptor knockout animals display attenuated innate inflammatory responses and no postinfectious mechanical hypersensitivity at any time point,[199] again demonstrating the causal link between long-term neuroplasticity and inflammation.

Long-term neuroplasticity is also evident in colonic pathways with suppression of K_V I_A currents contributing to postinfectious *C. rodentium*-induced neuronal hyperexcitability in mice[191] In another rat model, deoxycholic acid, an unconjugated secondary bile acid, induces a mild, transient colitis that causes exaggerated long-term visceromotor responses to colorectal

distension, referred pain to mechanical stimulation, and increased dorsal horn neuron activity.[200] A postinflammatory model of *T. spiralis* demonstrates colonic hypersensitivity and gut dysmotility that can be normalized by administration of an anti-inflammatory treatment.[123] Although there are some inconsistencies in the reporting of persistent TNBS-induced neuroplasticity, these discrepancies probably relate to the precise time-point studied and the severity of mucosal inflammation, which is a predictor for alterations of visceral sensory function in rodents[201] and in human patients.[202] However, evidence also exists for mild, asymptomatic colitis inducing long-lasting visceral hyperalgesia in the presence of additional stimuli.[201] Overall, TNBS-induced postinflammatory hyperalgesia has been identified from between 3 weeks to 4 months after resolution of inflammation.[203,204] The extent of mechanical hypersensitivity in high-threshold afferents is increased in postinflammatory states, whereas in some studies pelvic high-threshold afferents only become hypersensitive postinflammation, again suggesting that distinct immune processes contribute to the development of neuroplasticity.[167] Evidence also supports the emergence of increased proportions of 'silent afferents' in postinflammatory states.[185] Postinflammatory afferent hypersensitivity is also correlated with an increased density and sprouting of colonic afferent central terminals in the thoracolumbar spinal cord and an increased number of activated dorsal horn neurons within the spinal cord in response to noxious colorectal distension (Figure 5).[205] Studies have reported potential targets to reverse this hypersensitivity, with the identification of substantial increases in the expression and function of inhibitory kappa-opioid[206] and oxytocin[207] receptors in colonic DRG neurons in the postinflammatory state.

DSS-induced colitis also has contrasting effects on inducing afferent mechanical hypersensitivity or short-term or long-term hyperalgesia.[208] These differences might be dependent on the severity of inflammation and the specific time-course studied. For example,

mild and acute, but not chronic DSS-induced colitis is associated with visceral hypersensitivity.[209] In acute colitis, an increased responsiveness to colorectal distension is observed, which is accompanied by granulocyte infiltrate and increased expression of substance P. In chronic DSS-induced colitis, infiltration by lymphocytes, accompanied by μ-opioid receptor and β-endorphin upregulation, provides an anti-nociceptive mechanism that restores normal visceral perception. [209] Colonic supernatants from chronic DSS-treated mice with chronic colitis have a 14-fold increase in β-endorphin levels, which suppresses the excitability of nociceptive colonic DRG neurons.[210] These findings might provide insight as to why some patients with IBD do not report pain, depending on the type and degree of inflammation. However, DSS-treated animals do display increased visceral sensitivity to capsaicin and 5-HT,[211,212] thereby providing another example whereby overall neuronal function at any given time-point is a resultant function of the balance in pro-nocieptive and anti-nociceptive influences (Figure 4). Another example of differential effects is acute zymosan treatment, which recruits a different immune response, resulting in brief monocyte infiltration, but no increase in myeloperoxidase activity. This model seems to preferentially affect low-threshold distension sensitive afferents[185] and induce visceral hyperalgesia *in vivo*, which is partially dependent on TRPV1,[175] ASIC3[175] and P2X receptors.[213] Unlike TNBS treatment, zymosan does not seem to alter high-threshold colonic afferent function.[185]

Overall, inflammation-induced neuroplasticity is likely to underlie the increased sensory perception and increased incidence of pain reported in patients with the clinical digestive diseases detailed above, including oesophagitis, gastritis and IBD. Long-term neuroplasticity might also explain why symptoms are apparent in functional gastrointestinal disorders, such as functional dyspepsia and IBS, in which no overt pathology is evident.

Stress plus inflammation [level 3 heading]

Numerous models of stress have been shown to increase visceral pain sensitivity.[3] However, stress in conjunction with colitis induced by either *C. rodentium*,[214] or TNBS[215] results in additive increases in peripheral nociceptive signalling of colonic afferents, their cell bodies and correspondingly visceral hyperalgesia. These processes occur via protease, β-2 adrenergic, glucocorticoid receptor and PAR_2 mechanisms.[214] Other models such as *T. Spiralis* combined with acute restraint stress demonstrate the essential role of mast cells in postinfectious hyperalgesia,[216] with substantial increases in the expression of VGLUT3, a vesicular transporter for presynaptic packaging of the neurotransmitter glutamate, in the lumbosacral spinal dorsal horn.[217] Taken together these interactions might potentially explain why stress can trigger or worsen symptoms in patients with IBS and IBD. Interestingly, this additive hyperalgesia does not occur when combining stress and the DSS model of inflammation,[218] which again suggests that specific neuroimmune interactions and a skewed balance toward pro-nociceptive mechanisms are involved in the development of neuroplasticity and chronic hyperalgesia.

Neonatal insult and long-term neuroplasticity [level 3 heading]

One of the most consistent displays of persistent visceral neuroplasticity occurs after neonatal inflammatory insult. Neonatal colonic irritation results in chronic visceral hypersensitivity, allodynia and hyperalgesia in adult animals, which is associated with central neuronal sensitization in the absence of identifiable peripheral abnormalities. Evidence exists for TRPV1[219] and TRPA1[220] initiating colonic hypersensitivity and early increases in growth factor expression resulting in permanent changes in TRPV1,[219] P2X[221] and TRPA1[220] function, thereby maintaining the hypersensitivity induced by acetic acid or mustard oil colonic irritation in neonatal animals. In some studies, the same dose of irritant is unable to induce visceral hypersensitivity in adult animals, suggesting that the immature nervous system is

more susceptible to inflammatory insult. This finding might potentially explain why pain associated with IBD has a higher incidence in paediatric-onset than in adult-onset disease.[61]

Similar mechanisms of neonatal neuroplasticity in the upper gut, which might be applicable to functional dyspepsia. Neonatal gastric irritation can induce chronic gastric hypersensitivity and gastric motor dysfunction in adults, in the absence of detectable gastric pathology.[222] Gastric hypersensitivity in adult animals can be attenuated by the $GABA_B$ agonist baclofen, although this seems to occur via central rather than peripheral mechanisms.[223] Interestingly, gastric hypersensitivity in adult animals can also be induced by neonatal colonic TNBS administration. This single intervention results in aberrant increases of plasma corticosterone in neonates, increased plasma levels of norepinephrine, increased levels of nerve growth factor in the gastric fundus, increased brain-derived neurotrophic factor in DRG and the spinal cord, and downregulation of the potassium channel $K_V1.1$ in DRG.[224] Overall, these findings again highlight how a single inflammatory insult can induce a cascade of events that results in increased neurotrophic factor expression, epigenetic changes, altered neuronal function and ultimately pronounced long-term neuroplasticity.

CNS neuroplasticity [level 2 heading]

Several reports indicate that chemical and infection-induced gut inflammation is accompanied by changes in behaviour that include anorexia[225,226] and anxiety-like behavior.[227] *T. muris* induced-colitis can lead to decreased levels of hippocampal brain-derived neurotrophic factor and anxiety-like behavior, which can be normalized by administration of the probiotic *Bifidobacterium longum.*[228] Similarly, *C. rodentium* infection causes anxiety-like behaviour in mice and stress-induced memory dysfunction.[227] Notably, administration of probiotics both before and during the infection prevents memory dysfunction, indicating a clear peripheral

aetiology.[229] Changes in food intake, gastric emptying and visceral hypersensitivity are evident during *H. pylori* infection in mice, which improve after bacterial eradication. Interestingly, these mice display a feeding pattern reminiscent of early satiety, which persists after *H. pylori* eradication and is accompanied by increased TNF levels in the brain.[230] Similarly, reduced food intake is apparent during TNBS colitis,[225] with central IL-1 receptors or prostaglandins contributing to suppression of feeding during acute colitis.[226,231] Impaired gastric emptying via sensitized pelvic afferent neurons during colitis[232] might also contribute to reduced food intake. Finally, evidence indicates that colitis causes converging sensitisation of visceral and somatic pathways,[204] with colitis being associated with bladder overactivity and enhanced skin responses to mechanical and thermal stimulation. Overall, these findings again support a role for altered gut–brain pathways in the maintenance of postinfectious or postinflammatory gut dysfunction, which might underlie symptoms within and those extending beyond the gastrointestinal tract.

Conclusions [level 1 heading]

Work towards mechanistic understanding of gastrointestinal inflammation-induced neuroplasticity has burgeoned over the past decade. Despite the absence of perfect pre-clinical models to replicate clinical gastrointestinal disorders, animal models have identified molecular mechanisms that might potentially underlie neuroplasticity in the clinical setting. These models have specific immune responses, which lead to distinct interactions between immune cells and neurons. These neuro-immune interactions cause neuroplasticity either in the form of neuronal loss, altered synaptic transmission or altered neuronal ion channel and receptor expression and ultimately aberrant neuronal function that might be evident in the postinflammatory state. Persistent neuroplasticity might explain the symptoms of functional bowel disorders, in which overt pathology is absent from the gastrointestinal tract. It is also

clear from animal studies that neuroplasticity is a dynamic process. As such, tremendous potential remains for increased understanding of the mechanisms of inflammatory neuroplasticity in human disease. Developing stringent stratification is required for patients with digestive diseases, either organic or functional, and future studies designed to test neuroplasticity in humans should account for the dynamics of the disease. Identifying correlative markers of disease progression or resolution to aid further patient stratification will provide much needed insight to inflammatory neuroplasticity. Overall, this information might reveal that several different therapeutic strategies exist for the treatment and prevention of gastrointestinal dysfunction. Targeting neuronal populations displaying neuroplasticity is the ultimate goal to treat patients experiencing pain or dysmotility. Another therapeutic window of opportunity might also exist, whereby inhibiting inflammatory processes, during the early stages of gastroenteritis, might reduce or prevent the subsequent development of neuroplasticity and dysfunction.

Review criteria:

For this Review we conducted a number of PubMed database searches involving combinations of terms that included "neuroplasticity", “inflammatory bowel disease”, “ulcerative colitis”, “Crohn's disease”, “inflammation”, "remission", "resolved", “IBS”, "animal model", "dysfunction", “motility”, "dysmotility", “secretion”, "sensitivity", “vagal afferent”, “spinal afferent”, “intrinsic afferent”, “AH neuron”, “gastrointestinal”, “intestine”, “stomach”, “colon”, "autonomic nervous system", "sympathetic", "parasympathetic", "central nervous system", "spinal cord", "hypothalamus", and “enteric nervous system”. Additionally, references cited by the literature found by these database searches that did not appear in the original searches, such as those prior to 1966, were also retrieved for information. Together the authors have a combined 33 years of experience in the field of neuroplasticity with much of that work associated with animal models of gastrointestinal inflammation. This experience was also used to form the basis of this Review.

Figure 1: Neural innervation and multiple levels of reflex control of gastrointestinal function (Nat Rev G&H to redraw)

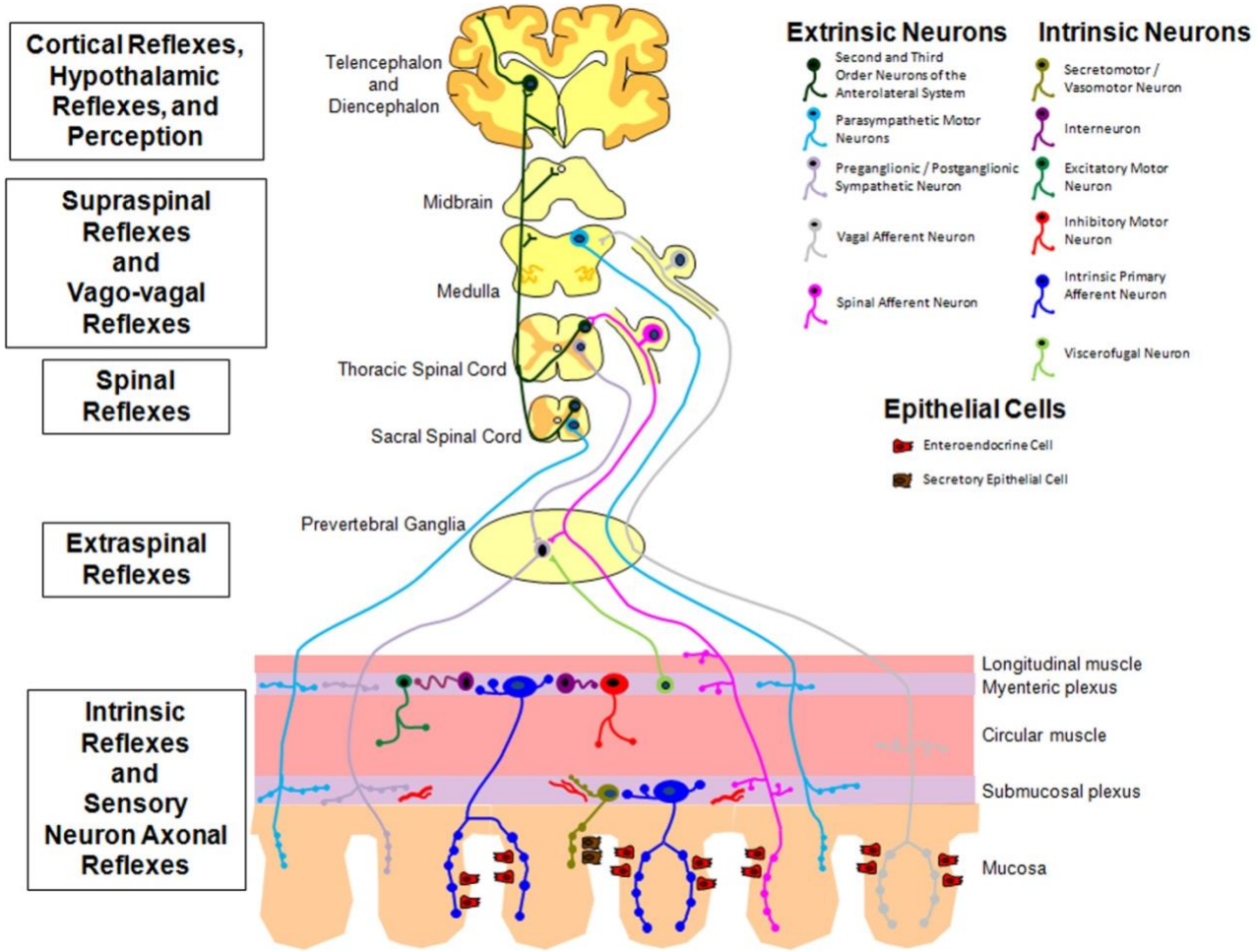

Figure 1: Neural innervation and multiple levels of reflex control of gastrointestinal function.

Intrinsic (enteric) neurons have cell bodies and processes within the gut wall and form neural networks to control motility, secretion and vasodilation. Viscerofugal neurons are enteric neurons that project to postganglionic sympathetic visceromotor neurons in the prevertebral ganglia that lie just ventrolateral to the aorta. Reflexes that are activated by viscerofugal neurons are called extraspinal reflexes, as they do not involve the central nervous system (CNS). Postganglionic sympathetic neurons project to the gut wall to modulate secretion, blood flow and motility. These neurons also receive input from preganglionic sympathetic neurons in the spinal cord that form the efferent limb of central nervous system reflexes. The other efferent limb of CNS reflexes arises from parasympathetic motor neurons in the sacral spinal cord and brainstem. Extrinsic afferent neurons have peripheral endings within the gut wall and communicate with the CNS via vagal and spinal pathways. Vagal afferents have cell bodies in the nodose and jugular ganglia and central projections into the nucleus of the solitary tract, whereas spinal afferents follow the splanchnic and pelvic nerve trucks, have cell bodies in the thoracolumbar and lumbosacral DRG and have central projections into the dorsal horn of the spinal cord. Extrinsic afferents form the afferent limb of central reflexes and pathways contributing to conscious perception, but also have efferent function via axon reflexes that can be local or extraspinal.

Nat Rev G&H to re-draw

Figure 2: Neuroplasticity in enteric neurons during and following gut inflammation.

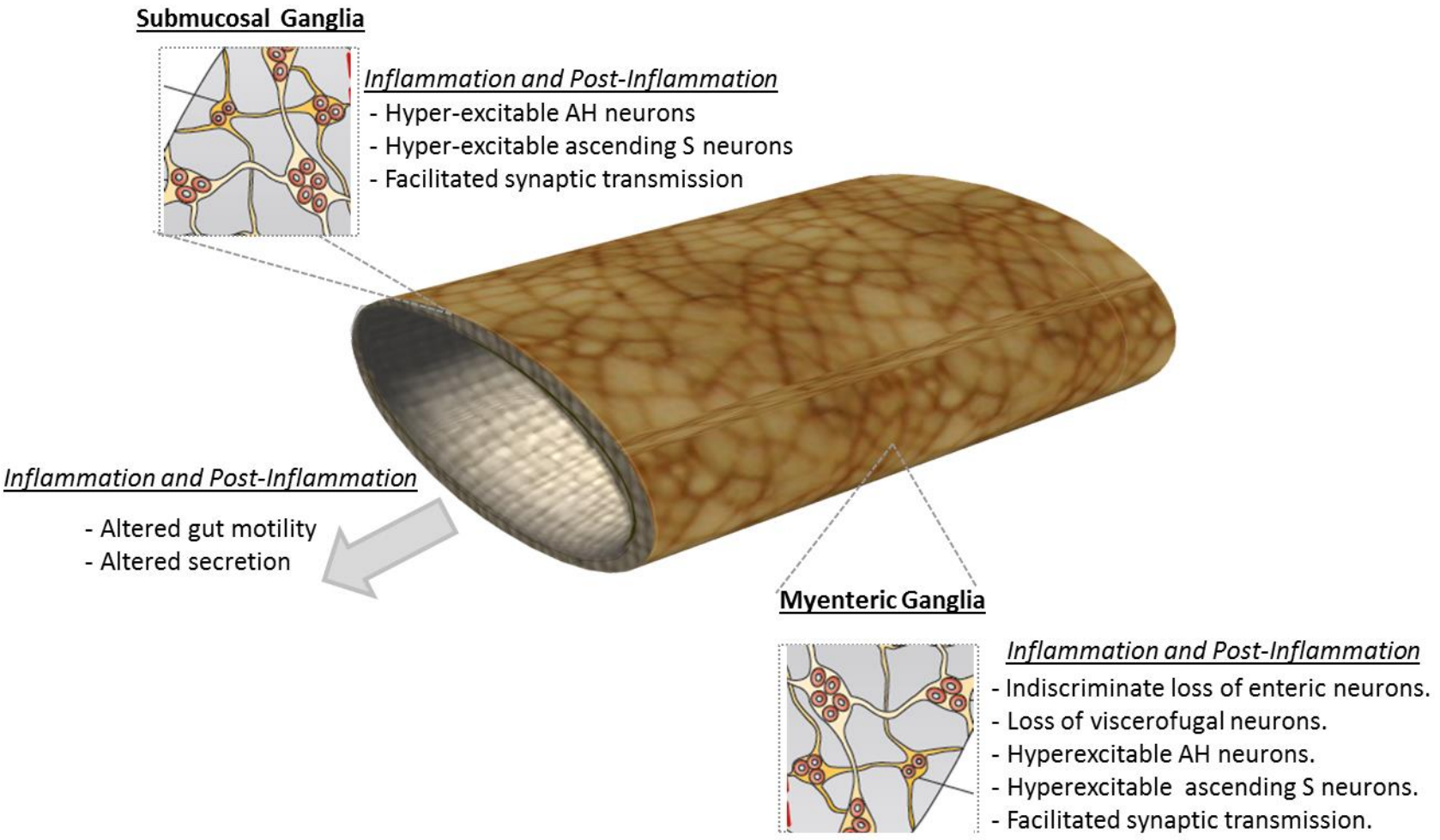

Figure 2: Neuroplasticity in enteric neurons during and following gut inflammation.

In the enteric nervous system, inflammation causes a rapid loss of enteric neurons and viscerofugal neurons. Of the remaining enteric neurons, specific subpopulations located in the myenteric and submucosal ganglia, become hyperexcitable (AH neurons) and synaptic transmission between them is facilitated. These changes in neuronal function result in decreased secretion and disrupted motility. Many of the neuronal changes evident during inflammation are still present after resolution of inflammation, with hyperexcitable enteric neurons and facilitated synaptic transmission still evident in the postinflammatory state.

Figure 3: Neuroplasticity in sympathetic neurons during and following gut inflammation.

Nature Reviews G&H to redraw

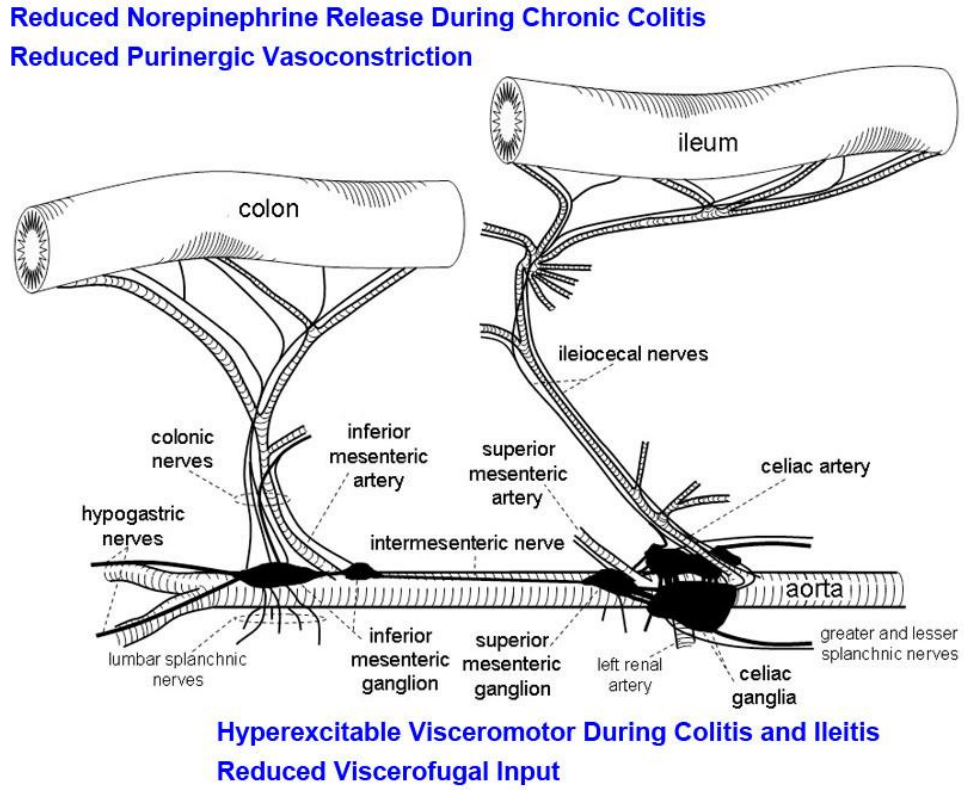

Figure 3: Neuroplasticity in sympathetic neurons during and after gut inflammation. In the prevertebral ganglia, although there is reduced synaptic input from viscerofugal neurons, sympathetic visceromotor neurons are actually hyperexcitable. Enhanced sympathetic outflow results in decreased secretion and reduced motility. Changes in auto-inhibitory adrenergic receptors and voltage-gated calcium channels in sympathetic terminals reduce evoked norepinephrine release and might restore more normal sympathetic drive because neurons remain hyperexcitable following resolution of inflammation. Sympathetic vasomotor neurons are not hyperexcitable, but purinergic vasoconstriction from these neurons is reduced.

Nat Rev G&H to re-draw

Figure 4: Inflammation-induced neuroplasticity: contribution of neuroactive signalling molecules and the channels/receptors they act on.

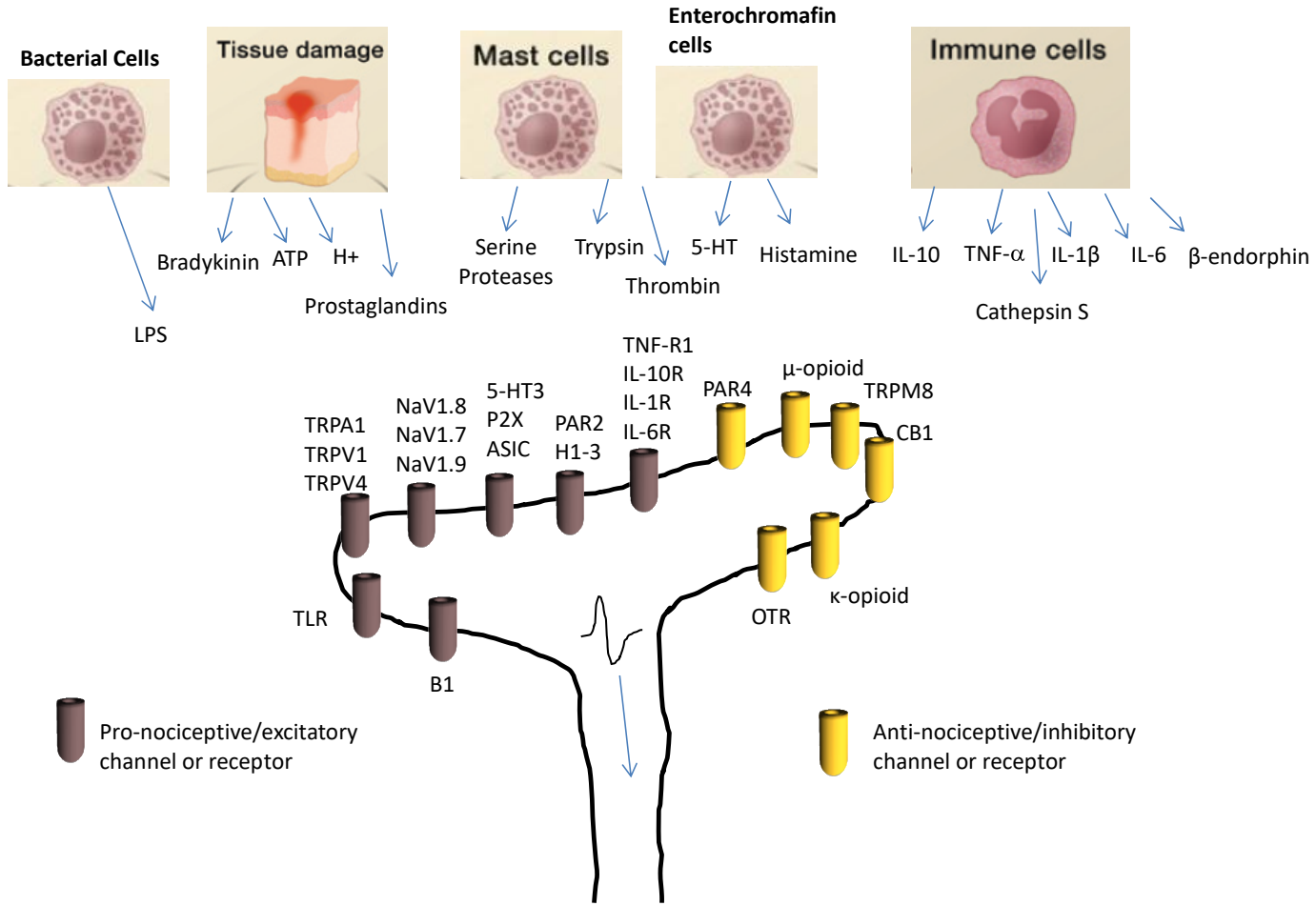

Figure 4: Inflammation-induced neuroplasticity: contribution of neuroactive signalling molecules and the channels/receptors they act on.

Numerous mediators can directly activate gut afferents by binding to the numerous cell surface receptors and channels expressed on their peripheral endings. The majority of the channels and receptors identified here result in afferent activation, sensitisation and neuronal hypersensitivity and hyperexcitability. Furthermore, once activated, the nociceptors themselves can release substance P and CGRP from their peripheral terminals, inducing neurogenic inflammation. TRPA1, TRPV1 and TRPV4 are implicated in this process. By contrast, activation of another set of channels/receptors results in reduced neuronal excitability and resultant anti-nociceptive effects. Pro-nociceptive mechanisms seem to be upregulated during inflammatory and postinflammatory states. Some anti-nociceptive mechanisms are downregulated, whereas some are upregulated in inflammatory and postinflammatory states (κ-opioid and OTR). Abbreviations: calcitonin-gene related peptide (CGRP), Transient Receptor Potential (TRP); Acid Sensing Ion Channel (ASIC); Protease activated receptor 4(PAR-4)[233], Cannabinoid receptor 1 (CB1)[234], tumour necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), interleukin-10 (IL-10), Histamine 1 receptor (H1), Toll-like receptor (TLR), Lipopolysaccharide (LPS), oxytocin receptor (OTR), Transient Receptor Potential Melastatin-8 (TRPM8)[235].

Nat Rev G&H to re-draw

Figure 5: Neuroplasticity in extrinsic sensory afferent pathways during and following resolution of gut inflammation.

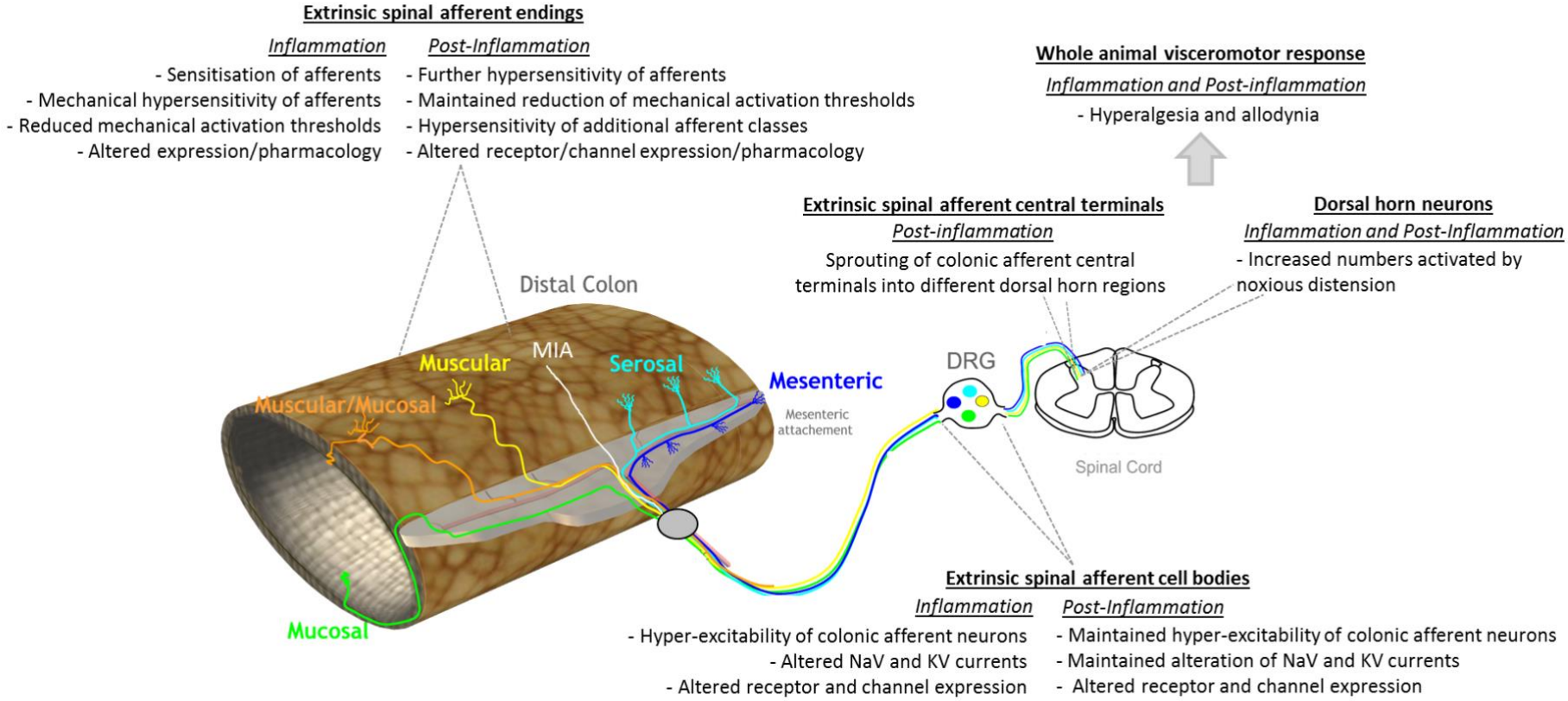

Figure 5: Neuroplasticity in extrinsic sensory afferent pathways during and after resolution of gut inflammation.

During inflammation sensory afferent endings become hypersensitive, are activated at lower stimulus intensities, display enhanced mechanical responsiveness, whilst their cell bodies in the nodose ganglia or DRG display hyperexcitability. This results in increased neuronal activation in the nucleus of the solitary tract or the dorsal horn of the spinal cord respectively. In whole-animal studies this translates to enhanced pain responses to either gastric or colorectal distension. Many of these changes are still present or are even enhanced following resolution of inflammation. Nociceptive sensory afferent endings now display increased mechanical hypersensitivity, and their cell bodies in the nodose and DRG remain hyperexcitable. An increased density of colonic afferent central afferent terminals is now also evident, as is sprouting of these terminals into different regions of the dorsal horn of the spinal cord. This plasticity results in greater numbers of dorsal horn neurons in the spinal cord being activated in response to noxious colorectal distension. There is evidence of enhanced pain responses to gastric and colorectal distension, the extent of which can be dependent upon the experimental model used and influenced by the severity of the initial insult. *(Note: For the purposes of clarity the pathways innervating the colon are illustrated).*

References:

1. Furness, J.B. The enteric nervous system and neurogastroenterology. *Nat Rev Gastroenterol Hepatol* **9**, 286-94 (2012).
2. Kiank, C., Tache, Y. & Larauche, M. Stress-related modulation of inflammation in experimental models of bowel disease and post-infectious irritable bowel syndrome: role of corticotropin-releasing factor receptors. *Brain Behav Immun* **24**, 41-8 (2010).
3. Larauche, M., Mulak, A. & Tache, Y. Stress and visceral pain: from animal models to clinical therapies. *Exp Neurol* **233**, 49-67 (2012).
4. Ohman, L. & Simren, M. Pathogenesis of IBS: role of inflammation, immunity and neuroimmune interactions. *Nat Rev Gastroenterol Hepatol* **7**, 163-73 (2010).
5. Collins, S.M., Surette, M. & Bercik, P. The interplay between the intestinal microbiota and the brain. *Nat Rev Microbiol* **10**, 735-42 (2012).
6. Gulbransen, B.D. & Sharkey, K.A. Novel functional roles for enteric glia in the gastrointestinal tract. *Nat Rev Gastroenterol Hepatol* **9**, 625-32 (2012).
7. Zeltser, L.M., Seeley, R.J. & Tschop, M.H. Synaptic plasticity in neuronal circuits regulating energy balance. *Nat Neurosci* **15**, 1336-42 (2012).
8. Hoogerwerf, W.A. Role of biological rhythms in gastrointestinal health and disease. *Rev Endocr Metab Disord* **10**, 293-300 (2009).
9. Cummings, D.E. & Overduin, J. Gastrointestinal regulation of food intake. *J Clin Invest* **117**, 13-23 (2007).
10. Baumgart, D.C. & Sandborn, W.J. Crohn's disease. *Lancet* **380**, 1590-605 (2012).
11. Colombel, J.F. et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med* **362**, 1383-95 (2010).
12. Mantzaris, G.J. et al. Azathioprine is superior to budesonide in achieving and maintaining mucosal healing and histologic remission in steroid-dependent Crohn's disease. *Inflamm Bowel Dis* **15**, 375-82 (2009).
13. Casellas, F. et al. Mucosal healing restores normal health and quality of life in patients with inflammatory bowel disease. *Eur J Gastroenterol Hepatol* **24**, 762-9 (2012).
14. Higgins, P.D. et al. Patient defined dichotomous end points for remission and clinical improvement in ulcerative colitis. *Gut* **54**, 782-8 (2005).
15. Rao, S.S., Read, N.W., Brown, C., Bruce, C. & Holdsworth, C.D. Studies on the mechanism of bowel disturbance in ulcerative colitis. *Gastroenterology* **93**, 934-40 (1987).
16. Bassotti, G. et al. Twenty-four-hour manometric study of colonic propulsive activity in patients with diarrhea due to inflammatory (ulcerative colitis) and non-inflammatory (irritable bowel syndrome) conditions. *Int J Colorectal Dis* **19**, 493-7 (2004).
17. Kern, F., Jr., Almy, T.P., Abbot, F.K. & Bogdonoff, M.D. The motility of the distal colon in nonspecific ulcerative colitis. *Gastroenterology* **19**, 492-503 (1951).
18. Snape, W.J., Jr., Matarazzo, S.A. & Cohen, S. Abnormal gastrocolonic response in patients with ulcerative colitis. *Gut* **21**, 392-6 (1980).
19. Koch, T.R., Carney, J.A., Go, V.L. & Szurszewski, J.H. Spontaneous contractions and some electrophysiologic properties of circular muscle from normal sigmoid colon and ulcerative colitis. *Gastroenterology* **95**, 77-84 (1988).
20. Reddy, S.N. et al. Colonic motility and transit in health and ulcerative colitis. *Gastroenterology* **101**, 1289-97 (1991).
21. Odille, F. et al. Quantitative assessment of small bowel motility by nonrigid registration of dynamic MR images. *Magn Reson Med* **68**, 783-93 (2012).
22. Tursi, A., Brandimarte, G., Giorgetti, G. & Nasi, G. Assessment of orocaecal transit time in different localization of Crohn's disease and its possible influence on clinical response to therapy. *Eur J Gastroenterol Hepatol* **15**, 69-74 (2003).

23. Keller, J., Beglinger, C., Holst, J.J., Andresen, V. & Layer, P. Mechanisms of gastric emptying disturbances in chronic and acute inflammation of the distal gastrointestinal tract. *Am J Physiol Gastrointest Liver Physiol* **297**, G861-8 (2009).
24. Nobrega, A.C. et al. Dyspeptic symptoms and delayed gastric emptying of solids in patients with inactive Crohn's disease. *BMC Gastroenterol* **12**, 175 (2012).
25. Mueller, M.H. et al. Anorectal functional disorders in the absence of anorectal inflammation in patients with Crohn's disease. *Br J Surg* **89**, 1027-31 (2002).
26. Bernstein, C.N. et al. Rectal afferent function in patients with inflammatory and functional intestinal disorders. *Pain* **66**, 151-61 (1996).
27. Chrysos, E. et al. Rectoanal motility in Crohn's disease patients. *Dis Colon Rectum* **44**, 1509-13 (2001).
28. Kangas, E., Hiltunen, K.M. & Matikainen, M. Anorectal function in Crohn's disease. *Ann Chir Gynaecol* **81**, 43-7 (1992).
29. Andersson, P., Olaison, G., Hallbook, O., Boeryd, B. & Sjodahl, R. Increased anal resting pressure and rectal sensitivity in Crohn's disease. *Dis Colon Rectum* **46**, 1685-9 (2003).
30. Loening-Baucke, V., Metcalf, A.M. & Shirazi, S. Rectosigmoid motility in patients with quiescent and active ulcerative colitis. *Am J Gastroenterol* **84**, 34-9 (1989).
31. Bassotti, G. et al. Colonic propulsive and postprandial motor activity in patients with ulcerative colitis in remission. *Eur J Gastroenterol Hepatol* **18**, 507-10 (2006).
32. Annese, V. et al. Gastrointestinal motility disorders in patients with inactive Crohn's disease. *Scand J Gastroenterol* **32**, 1107-17 (1997).
33. Minderhoud, I.M., Smout, A.J., Oldenburg, B. & Samsom, M. A pilot study on chemospecific duodenal visceral sensitivity in inflammatory bowel disease in remission. *Digestion* **73**, 151-9 (2006).
34. Collins, S.M., Van Assche, G. & Hogaboam, C. Alterations in enteric nerve and smooth-muscle function in inflammatory bowel diseases. *Inflamm Bowel Dis* **3**, 38-48 (1997).
35. Geboes, K. & Collins, S. Structural abnormalities of the nervous system in Crohn's disease and ulcerative colitis. *Neurogastroenterol Motil* **10**, 189-202 (1998).
36. Belai, A., Boulos, P.B., Robson, T. & Burnstock, G. Neurochemical coding in the small intestine of patients with Crohn's disease. *Gut* **40**, 767-74 (1997).
37. Schneider, J. et al. Neurotransmitter coding of enteric neurones in the submucous plexus is changed in non-inflamed rectum of patients with Crohn's disease. *Neurogastroenterol Motil* **13**, 255-64 (2001).
38. Sjolund, K. et al. Peptide-containing nerve fibres in the gut wall in Crohn's disease. *Gut* **24**, 724-33 (1983).
39. Todorovic, V. et al. Colonic vasoactive intestinal polypeptide (VIP) in ulcerative colitis--a radioimmunoassay and immunohistochemical study. *Hepatogastroenterology* **43**, 483-8 (1996).
40. Lee, C.M., Kumar, R.K., Lubowski, D.Z. & Burcher, E. Neuropeptides and nerve growth in inflammatory bowel diseases: a quantitative immunohistochemical study. *Dig Dis Sci* **47**, 495-502 (2002).
41. Straub, R.H. et al. Anti-inflammatory role of sympathetic nerves in chronic intestinal inflammation. *Gut* **57**, 911-21 (2008).
42. Dvorak, A.M., Osage, J.E., Monahan, R.A. & Dickersin, G.R. Crohn's disease: transmission electron microscopic studies. III. Target tissues. Proliferation of and injury to smooth muscle and the autonomic nervous system. *Hum Pathol* **11**, 620-34 (1980).
43. Oehmichen, M. & Reifferscheid, P. Intramural ganglion cell degeneration in inflammatory bowel disease. *Digestion* **15**, 482-96 (1977).
44. Roberts, P.J., Morgan, K., Miller, R., Hunter, J.O. & Middleton, S.J. Neuronal COX-2 expression in human myenteric plexus in active inflammatory bowel disease. *Gut* **48**, 468-72 (2001).

45. Nadorra, R., Landing, B.H. & Wells, T.R. Intestinal plexuses in Crohn's disease and ulcerative colitis in children: pathologic and microdissection studies. *Pediatr Pathol* **6**, 267-87 (1986).
46. Strobach, R.S., Ross, A.H., Markin, R.S., Zetterman, R.K. & Linder, J. Neural patterns in inflammatory bowel disease: an immunohistochemical survey. *Mod Pathol* **3**, 488-93 (1990).
47. Neunlist, M. et al. Changes in chemical coding of myenteric neurones in ulcerative colitis. *Gut* **52**, 84-90 (2003).
48. Gulbransen, B.D. et al. Activation of neuronal P2X7 receptor-pannexin-1 mediates death of enteric neurons during colitis. *Nat Med* **18**, 600-4 (2012).
49. Bassotti, G. et al. Enteric neuroglial apoptosis in inflammatory bowel diseases. *J Crohns Colitis* **3**, 264-70 (2009).
50. Geboes, K. et al. Major histocompatibility class II expression on the small intestinal nervous system in Crohn's disease. *Gastroenterology* **103**, 439-47 (1992).
51. Koretz, K., Momburg, F., Otto, H.F. & Moller, P. Sequential induction of MHC antigens on autochthonous cells of ileum affected by Crohn's disease. *Am J Pathol* **129**, 493-502 (1987).
52. Ohlsson, B., Sundkvist, G. & Lindgren, S. Subclinical sympathetic neuropathy appears early in the course of Crohn's disease. *BMC Gastroenterol* **7**, 33 (2007).
53. Ferrante, M. et al. The value of myenteric plexitis to predict early postoperative Crohn's disease recurrence. *Gastroenterology* **130**, 1595-606 (2006).
54. Sokol, H. et al. Plexitis as a predictive factor of early postoperative clinical recurrence in Crohn's disease. *Gut* **58**, 1218-25 (2009).
55. Rao, S.S., Read, N.W., Davison, P.A., Bannister, J.J. & Holdsworth, C.D. Anorectal sensitivity and responses to rectal distention in patients with ulcerative colitis. *Gastroenterology* **93**, 1270-5 (1987).
56. Farthing, M.J. & Lennard-jones, J.E. Sensibility of the rectum to distension and the anorectal distension reflex in ulcerative colitis. *Gut* **19**, 64-9 (1978).
57. Drewes, A.M. et al. Pain and mechanical properties of the rectum in patients with active ulcerative colitis. *Inflamm Bowel Dis* **12**, 294-303 (2006).
58. Buchmann, P., Kolb, E. & Alexander-Williams, J. Pathogenesis of urgency in defaecation in Crohn's disease. *Digestion* **22**, 310-6 (1981).
59. Docherty, M.J., Jones, R.C., 3rd & Wallace, M.S. Managing pain in inflammatory bowel disease. *Gastroenterol Hepatol (N Y)* **7**, 592-601 (2011).
60. Schirbel, A. et al. Impact of pain on health-related quality of life in patients with inflammatory bowel disease. *World J Gastroenterol* **16**, 3168-77 (2010).
61. Greenley, R.N., Kunz, J.H., Schurman, J.V. & Swanson, E. Abdominal pain and health related quality of life in pediatric inflammatory bowel disease. *J Pediatr Psychol* **38**, 63-71 (2013).
62. Wagtmans, M.J., Verspaget, H.W., Lamers, C.B. & van Hogezand, R.A. Crohn's disease in the elderly: a comparison with young adults. *J Clin Gastroenterol* **27**, 129-33 (1998).
63. Ibeakanma, C. & Vanner, S. TNFalpha is a key mediator of the pronociceptive effects of mucosal supernatant from human ulcerative colitis on colonic DRG neurons. *Gut* **59**, 612-21 (2010).
64. Farrokhyar, F., Marshall, J.K., Easterbrook, B. & Irvine, E.J. Functional gastrointestinal disorders and mood disorders in patients with inactive inflammatory bowel disease: prevalence and impact on health. *Inflamm Bowel Dis* **12**, 38-46 (2006).
65. Minderhoud, I.M., Oldenburg, B., Wismeijer, J.A., van Berge Henegouwen, G.P. & Smout, A.J. IBS-like symptoms in patients with inflammatory bowel disease in remission; relationships with quality of life and coping behavior. *Dig Dis Sci* **49**, 469-74 (2004).

66. Loening-Baucke, V., Metcalf, A.M. & Shirazi, S. Anorectal manometry in active and quiescent ulcerative colitis. *Am J Gastroenterol* **84**, 892-7 (1989).
67. Mayer, E.A. et al. Differences in brain responses to visceral pain between patients with irritable bowel syndrome and ulcerative colitis. *Pain* **115**, 398-409 (2005).
68. Furlan, R. et al. Sympathetic overactivity in active ulcerative colitis: effects of clonidine. *Am J Physiol Regul Integr Comp Physiol* **290**, R224-32 (2006).
69. Ganguli, S.C. et al. A comparison of autonomic function in patients with inflammatory bowel disease and in healthy controls. *Neurogastroenterol Motil* **19**, 961-7 (2007).
70. Straub, R.H. et al. Association of autonomic nervous hyperreflexia and systemic inflammation in patients with Crohn's disease and ulcerative colitis. *J Neuroimmunol* **80**, 149-57 (1997).
71. Lechin, F. et al. Treatment of ulcerative colitis with clonidine. *J Clin Pharmacol* **25**, 219-26 (1985).
72. Mouzas, I.A. et al. Autonomic imbalance during the day in patients with inflammatory bowel disease in remission. Evidence from spectral analysis of heart rate variability over 24 hours. *Dig Liver Dis* **34**, 775-80 (2002).
73. Sharma, P., Makharia, G.K., Ahuja, V., Dwivedi, S.N. & Deepak, K.K. Autonomic dysfunctions in patients with inflammatory bowel disease in clinical remission. *Dig Dis Sci* **54**, 853-61 (2009).
74. Lovell, R.M. & Ford, A.C. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. *Clin Gastroenterol Hepatol* **10**, 712-721 e4 (2012).
75. Camilleri, M. Peripheral mechanisms in irritable bowel syndrome. *N Engl J Med* **367**, 1626-35 (2012).
76. Berman, S. et al. Evidence for alterations in central noradrenergic signaling in irritable bowel syndrome. *Neuroimage* **63**, 1854-63 (2012).
77. Bercik, P., Verdu, E.F. & Collins, S.M. Is irritable bowel syndrome a low-grade inflammatory bowel disease? *Gastroenterol Clin North Am* **34**, 235-45, vi-vii (2005).
78. Spiller, R.C. Inflammation as a basis for functional GI disorders. *Best Pract Res Clin Gastroenterol* **18**, 641-61 (2004).
79. Peters, G.A. & Bargen, J.A. The irritable bowel syndrome. *Gastroenterology* **3**, 399-402 (1944).
80. Barratt, S.M. et al. Prodromal irritable bowel syndrome may be responsible for delays in diagnosis in patients presenting with unrecognized Crohn's disease and celiac disease, but not ulcerative colitis. *Dig Dis Sci* **56**, 3270-5 (2011).
81. Burgmann, T. et al. The Manitoba Inflammatory Bowel Disease Cohort Study: prolonged symptoms before diagnosis--how much is irritable bowel syndrome? *Clin Gastroenterol Hepatol* **4**, 614-20 (2006).
82. Ansari, R. et al. Ulcerative colitis and irritable bowel syndrome: relationships with quality of life. *Eur J Gastroenterol Hepatol* **20**, 46-50 (2008).
83. Olbe, L. Concept of Crohn's disease being conditioned by four main components, and irritable bowel syndrome being an incomplete Crohn's disease. *Scand J Gastroenterol* **43**, 234-41 (2008).
84. Turner, J.R. Intestinal mucosal barrier function in health and disease. *Nat Rev Immunol* **9**, 799-809 (2009).
85. Bertiaux-Vandaele, N. et al. The expression and the cellular distribution of the tight junction proteins are altered in irritable bowel syndrome patients with differences according to the disease subtype. *Am J Gastroenterol* **106**, 2165-73 (2011).
86. Dunlop, S.P. et al. Abnormal intestinal permeability in subgroups of diarrhea-predominant irritable bowel syndromes. *Am J Gastroenterol* **101**, 1288-94 (2006).
87. Hughes, P.A. et al. Immune activation in irritable bowel syndrome: can neuroimmune interactions explain symptoms? *Am J Gastroenterol* **108**, 1066-74 (2013).

88. Donovan, J. & Grundy, D. Endocannabinoid modulation of jejunal afferent responses to LPS. *Neurogastroenterol Motil* **24**, 956-e465 (2012).
89. Ochoa-Cortes, F. et al. Bacterial cell products signal to mouse colonic nociceptive dorsal root ganglia neurons. *Am J Physiol Gastrointest Liver Physiol* **299**, G723-32 (2010).
90. Wang, B. et al. Lipopolysaccharide-induced changes in mesenteric afferent sensitivity of rat jejunum in vitro: role of prostaglandins. *Am J Physiol Gastrointest Liver Physiol* **289**, G254-60 (2005).
91. Liu, C.Y., Mueller, M.H., Rogler, G., Grundy, D. & Kreis, M.E. Differential afferent sensitivity to mucosal lipopolysaccharide from Salmonella typhimurium and Escherichia coli in the rat jejunum. *Neurogastroenterol Motil* **21**, 1335-e129 (2009).
92. Barbara, G. et al. Postinfectious irritable bowel syndrome. *J Pediatr Gastroenterol Nutr* **48 Suppl 2**, S95-7 (2009).
93. Spiller, R. & Garsed, K. Postinfectious irritable bowel syndrome. *Gastroenterology* **136**, 1979-88 (2009).
94. Thabane, M. & Marshall, J.K. Post-infectious irritable bowel syndrome. *World J Gastroenterol* **15**, 3591-6 (2009).
95. Barbara, G., De Giorgio, R., Stanghellini, V., Cremon, C. & Corinaldesi, R. A role for inflammation in irritable bowel syndrome? *Gut* **51 Suppl 1**, i41-4 (2002).
96. Barbara, G. et al. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. *Gastroenterology* **126**, 693-702 (2004).
97. Hughes, P.A. et al. Sensory neuro-immune interactions differ between Irritable Bowel Syndrome subtypes. *Gut* (2012).
98. Liebregts, T. et al. Small bowel homing T cells are associated with symptoms and delayed gastric emptying in functional dyspepsia. *Am J Gastroenterol* **106**, 1089-98 (2011).
99. Liebregts, T. et al. Immune activation in patients with irritable bowel syndrome. *Gastroenterology* **132**, 913-20 (2007).
100. Ohman, L. et al. T-cell activation in patients with irritable bowel syndrome. *Am J Gastroenterol* **104**, 1205-12 (2009).
101. Lindberg, G. et al. Full-thickness biopsy findings in chronic intestinal pseudo-obstruction and enteric dysmotility. *Gut* **58**, 1084-90 (2009).
102. Ohlsson, B., Ekblad, E., Veress, B., Montgomery, A. & Janciauskiene, S. Antibodies against gonadotropin-releasing hormone (GnRH) and destruction of enteric neurons in 3 patients suffering from gastrointestinal dysfunction. *BMC Gastroenterol* **10**, 48 (2010).
103. Gwee, K.A. et al. Increased rectal mucosal expression of interleukin 1beta in recently acquired post-infectious irritable bowel syndrome. *Gut* **52**, 523-6 (2003).
104. Chadwick, V.S. et al. Activation of the mucosal immune system in irritable bowel syndrome. *Gastroenterology* **122**, 1778-83 (2002).
105. Barbara, G. et al. Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. *Gastroenterology* **132**, 26-37 (2007).
106. Buhner, S. et al. Activation of human enteric neurons by supernatants of colonic biopsy specimens from patients with irritable bowel syndrome. *Gastroenterology* **137**, 1425-34 (2009).
107. Gonsalkorale, W.M., Perrey, C., Pravica, V., Whorwell, P.J. & Hutchinson, I.V. Interleukin 10 genotypes in irritable bowel syndrome: evidence for an inflammatory component? *Gut* **52**, 91-3 (2003).
108. Shea-Donohue, T., Notari, L., Sun, R. & Zhao, A. Mechanisms of smooth muscle responses to inflammation. *Neurogastroenterol Motil* **24**, 802-11 (2012).
109. Crosthwaite, A.I., Huizinga, J.D. & Fox, J.A. Jejunal circular muscle motility is decreased in nematode-infected rat. *Gastroenterology* **98**, 59-65 (1990).

110. Grossi, L., McHugh, K. & Collins, S.M. On the specificity of altered muscle function in experimental colitis in rats. *Gastroenterology* **104**, 1049-56 (1993).
111. Jodal, M., Wingren, U., Jansson, M., Heidemann, M. & Lundgren, O. Nerve involvement in fluid transport in the inflamed rat jejunum. *Gut* **34**, 1526-30 (1993).
112. Vermillion, D.L. & Collins, S.M. Increased responsiveness of jejunal longitudinal muscle in Trichinella-infected rats. *Am J Physiol* **254**, G124-9 (1988).
113. Hosseini, J.M., Goldhill, J.M., Bossone, C., Pineiro-Carrero, V. & Shea-Donohue, T. Progressive alterations in circular smooth muscle contractility in TNBS-induced colitis in rats. *Neurogastroenterol Motil* **11**, 347-56 (1999).
114. Martinolle, J.P., Garcia-Villar, R., Fioramonti, J. & Bueno, L. Altered contractility of circular and longitudinal muscle in TNBS-inflamed guinea pig ileum. *Am J Physiol* **272**, G1258-67 (1997).
115. Moreels, T.G. et al. Effect of 2,4,6-trinitrobenzenesulphonic acid (TNBS)-induced ileitis on the motor function of non-inflamed rat gastric fundus. *Neurogastroenterol Motil* **13**, 339-52 (2001).
116. Strong, D.S. et al. Purinergic neuromuscular transmission is selectively attenuated in ulcerated regions of inflamed guinea pig distal colon. *J Physiol* **588**, 847-59 (2010).
117. Perdue, M.H., Marshall, J. & Masson, S. Ion transport abnormalities in inflamed rat jejunum. Involvement of mast cells and nerves. *Gastroenterology* **98**, 561-7 (1990).
118. Masson, S.D. et al. Nippostrongylus brasiliensis infection evokes neuronal abnormalities and alterations in neurally regulated electrolyte transport in rat jejunum. *Parasitology* **113 (Pt 2)**, 173-82 (1996).
119. Sanchez de Medina, F. et al. Disturbances of colonic ion secretion in inflammation: role of the enteric nervous system and cAMP. *Pflugers Arch* **444**, 378-88 (2002).
120. MacNaughton, W.K., Lowe, S.S. & Cushing, K. Role of nitric oxide in inflammation-induced suppression of secretion in a mouse model of acute colitis. *Am J Physiol* **275**, G1353-60 (1998).
121. Perez-Navarro, R., Ballester, I., Zarzuelo, A. & Sanchez de Medina, F. Disturbances in epithelial ionic secretion in different experimental models of colitis. *Life Sci* **76**, 1489-501 (2005).
122. Motomura, Y. et al. Mechanisms underlying gut dysfunction in a murine model of chronic parasitic infection. *Am J Physiol Gastrointest Liver Physiol* **299**, G1354-60 (2010).
123. Bercik, P. et al. Visceral hyperalgesia and intestinal dysmotility in a mouse model of postinfective gut dysfunction. *Gastroenterology* **127**, 179-87 (2004).
124. Krauter, E.M. et al. Changes in colonic motility and the electrophysiological properties of myenteric neurons persist following recovery from trinitrobenzene sulfonic acid colitis in the guinea pig. *Neurogastroenterol Motil* **19**, 990-1000 (2007).
125. Lomax, A.E., O'Hara, J.R., Hyland, N.P., Mawe, G.M. & Sharkey, K.A. Persistent alterations to enteric neural signaling in the guinea pig colon following the resolution of colitis. *Am J Physiol Gastrointest Liver Physiol* **292**, G482-91 (2007).
126. Van Nassauw, L., Bogers, J., Van Marck, E. & Timmermans, J.P. Role of reactive nitrogen species in neuronal cell damage during intestinal schistosomiasis. *Cell Tissue Res* **303**, 329-36 (2001).
127. Demedts, I. et al. Neural mechanisms of early postinflammatory dysmotility in rat small intestine. *Neurogastroenterol Motil* **18**, 1102-11 (2006).
128. Bradley, J.S., Jr., Parr, E.J. & Sharkey, K.A. Effects of inflammation on cell proliferation in the myenteric plexus of the guinea-pig ileum. *Cell Tissue Res* **289**, 455-61 (1997).
129. Poli, E., Lazzaretti, M., Grandi, D., Pozzoli, C. & Coruzzi, G. Morphological and functional alterations of the myenteric plexus in rats with TNBS-induced colitis. *Neurochem Res* **26**, 1085-93 (2001).

130. Sarnelli, G. et al. Myenteric neuronal loss in rats with experimental colitis: role of tissue transglutaminase-induced apoptosis. *Dig Liver Dis* **41**, 185-93 (2009).
131. Linden, D.R. et al. Indiscriminate loss of myenteric neurones in the TNBS-inflamed guinea-pig distal colon. *Neurogastroenterol Motil* **17**, 751-60 (2005).
132. Boyer, L. et al. Myenteric plexus injury and apoptosis in experimental colitis. *Auton Neurosci* **117**, 41-53 (2005).
133. Linden, D.R. Colitis is associated with a loss of intestinofugal neurons. *Am J Physiol Gastrointest Liver Physiol* **303**, G1096-104 (2012).
134. Margolis, K.G. et al. Enteric neuronal density contributes to the severity of intestinal inflammation. *Gastroenterology* **141**, 588-98, 598 e1-2 (2011).
135. Mya, G.H., Ng, W.F., Cheng, W. & Saing, H. Colonic hyperganglionosis presenting as neonatal enterocolitis and multiple strictures. *J Pediatr Surg* **29**, 1628-30 (1994).
136. Linden, D.R., Sharkey, K.A. & Mawe, G.M. Enhanced excitability of myenteric AH neurones in the inflamed guinea-pig distal colon. *J Physiol* **547**, 589-601 (2003).
137. Chen, Z. et al. Cyclic AMP signaling contributes to neural plasticity and hyperexcitability in AH sensory neurons following intestinal Trichinella spiralis-induced inflammation. *Int J Parasitol* **37**, 743-61 (2007).
138. Linden, D.R., Sharkey, K.A., Ho, W. & Mawe, G.M. Cyclooxygenase-2 contributes to dysmotility and enhanced excitability of myenteric AH neurones in the inflamed guinea pig distal colon. *J Physiol* **557**, 191-205 (2004).
139. Krauter, E.M., Linden, D.R., Sharkey, K.A. & Mawe, G.M. Synaptic plasticity in myenteric neurons of the guinea-pig distal colon: presynaptic mechanisms of inflammation-induced synaptic facilitation. *J Physiol* **581**, 787-800 (2007).
140. Lomax, A.E., Mawe, G.M. & Sharkey, K.A. Synaptic facilitation and enhanced neuronal excitability in the submucosal plexus during experimental colitis in guinea-pig. *J Physiol* **564**, 863-75 (2005).
141. Blandizzi, C. et al. Altered prejunctional modulation of intestinal cholinergic and noradrenergic pathways by alpha2-adrenoceptors in the presence of experimental colitis. *Br J Pharmacol* **139**, 309-20 (2003).
142. Collins, S.M., Blennerhassett, P.A., Blennerhassett, M.G. & Vermillion, D.L. Impaired acetylcholine release from the myenteric plexus of Trichinella-infected rats. *Am J Physiol* **257**, G898-903 (1989).
143. Hoffman, J.M., McKnight, N.D., Sharkey, K.A. & Mawe, G.M. The relationship between inflammation-induced neuronal excitability and disrupted motor activity in the guinea pig distal colon. *Neurogastroenterol Motil* **23**, 673-e279 (2011).
144. Nurgali, K. et al. Morphological and functional changes in guinea-pig neurons projecting to the ileal mucosa at early stages after inflammatory damage. *J Physiol* **589**, 325-39 (2011).
145. O'Hara, J.R., Lomax, A.E., Mawe, G.M. & Sharkey, K.A. Ileitis alters neuronal and enteroendocrine signalling in guinea pig distal colon. *Gut* **56**, 186-94 (2007).
146. Hons, I.M., Burda, J.E., Grider, J.R., Mawe, G.M. & Sharkey, K.A. Alterations to enteric neural signaling underlie secretory abnormalities of the ileum in experimental colitis in the guinea pig. *Am J Physiol Gastrointest Liver Physiol* **296**, G717-26 (2009).
147. Linden, D.R. Enhanced excitability of guinea pig ileum myenteric AH neurons during and following recovery from chemical colitis. *Neurosci Lett* **545**, 91-5 (2013).
148. Jacobson, K., McHugh, K. & Collins, S.M. The mechanism of altered neural function in a rat model of acute colitis. *Gastroenterology* **112**, 156-62 (1997).
149. Motagally, M.A., Neshat, S. & Lomax, A.E. Inhibition of sympathetic N-type voltage-gated Ca2+ current underlies the reduction in norepinephrine release during colitis. *Am J Physiol Gastrointest Liver Physiol* **296**, G1077-84 (2009).

150. Birch, D., Knight, G.E., Boulos, P.B. & Burnstock, G. Analysis of innervation of human mesenteric vessels in non-inflamed and inflamed bowel--a confocal and functional study. *Neurogastroenterol Motil* **20**, 660-70 (2008).
151. Lomax, A.E., O'Reilly, M., Neshat, S. & Vanner, S.J. Sympathetic vasoconstrictor regulation of mouse colonic submucosal arterioles is altered in experimental colitis. *J Physiol* **583**, 719-30 (2007).
152. Mathison, R. & Davison, J.S. Capsaicin sensitive nerves in the jejunum of Nippostrongylus brasiliensis-sensitized rats participate in a cardiovascular depressor reflex. *Naunyn Schmiedebergs Arch Pharmacol* **348**, 638-42 (1993).
153. Mathison, R. & Davison, J.S. Regulation of jejunal arterioles by capsaicin-sensitive nerves in Nippostrongylus brasiliensis-sensitized rats. *J Pharmacol Exp Ther* **273**, 337-43 (1995).
154. Neshat, S. et al. Loss of purinergic vascular regulation in the colon during colitis is associated with upregulation of CD39. *American Journal of Physiology - Gastrointestinal and Liver Physiology* **296**, G399-G405 (2009).
155. Linden, D.R. Enhanced excitability of guinea pig inferior mesenteric ganglion neurons during and following recovery from chemical colitis. *Am J Physiol Gastrointest Liver Physiol* **303**, G1067-75 (2012).
156. Dong, X.X., Thacker, M., Pontell, L., Furness, J.B. & Nurgali, K. Effects of intestinal inflammation on specific subgroups of guinea-pig celiac ganglion neurons. *Neurosci Lett* **444**, 231-5 (2008).
157. Castex, N. et al. Brain Fos expression and intestinal motor alterations during nematode-induced inflammation in the rat. *Am J Physiol* **274**, G210-6 (1998).
158. Holzer, P. et al. Increase in gastric acid-induced afferent input to the brainstem in mice with gastritis. *Neuroscience* **145**, 1108-19 (2007).
159. Wultsch, T. et al. Deletion of the acid-sensing ion channel ASIC3 prevents gastritis-induced acid hyperresponsiveness of the stomach-brainstem axis. *Pain* **134**, 245-53 (2008).
160. Ghia, J.E., Blennerhassett, P. & Collins, S.M. Vagus nerve integrity and experimental colitis. *Am J Physiol Gastrointest Liver Physiol* **293**, G560-7 (2007).
161. Ghia, J.E., Blennerhassett, P., Kumar-Ondiveeran, H., Verdu, E.F. & Collins, S.M. The vagus nerve: a tonic inhibitory influence associated with inflammatory bowel disease in a murine model. *Gastroenterology* **131**, 1122-30 (2006).
162. Matteoli, G. & Boeckxstaens, G.E. The vagal innervation of the gut and immune homeostasis. *Gut* **62**, 1214-22 (2013).
163. Page, A.J., O'Donnell, T.A. & Blackshaw, L.A. P2X purinoceptor-induced sensitization of ferret vagal mechanoreceptors in oesophageal inflammation. *J Physiol* **523 Pt 2**, 403-11 (2000).
164. Yu, S. & Ouyang, A. Effect of synthetic cationic protein on mechanoexcitability of vagal afferent nerve subtypes in guinea pig esophagus. *Am J Physiol Gastrointest Liver Physiol* **301**, G1052-8 (2011).
165. Yu, S., Gao, G., Peterson, B.Z. & Ouyang, A. TRPA1 in mast cell activation-induced long-lasting mechanical hypersensitivity of vagal afferent C-fibers in guinea pig esophagus. *Am J Physiol Gastrointest Liver Physiol* **297**, G34-42 (2009).
166. Kang, Y.M., Bielefeldt, K. & Gebhart, G.F. Sensitization of mechanosensitive gastric vagal afferent fibers in the rat by thermal and chemical stimuli and gastric ulcers. *J Neurophysiol* **91**, 1981-9 (2004).
167. Hughes, P.A. et al. Post-inflammatory colonic afferent sensitisation: different subtypes, different pathways and different time courses. *Gut* **58**, 1333-41 (2009).

168. De Schepper, H.U. et al. TRPV1 receptors on unmyelinated C-fibres mediate colitis-induced sensitization of pelvic afferent nerve fibres in rats. *J Physiol* **586**, 5247-58 (2008).
169. Castro, J. et al. Linaclotide Inhibits Colonic Nociceptors and Relieves Abdominal Pain via Guanylate Cyclase-C and Extracellular Cyclic Guanosine 3′,5′-Monophosphate. *Gastroenterology* (2013).
170. Jones, R.C., 3rd, Xu, L. & Gebhart, G.F. The mechanosensitivity of mouse colon afferent fibers and their sensitization by inflammatory mediators require transient receptor potential vanilloid 1 and acid-sensing ion channel 3. *J Neurosci* **25**, 10981-9 (2005).
171. Brierley, S.M. et al. The ion channel TRPA1 is required for normal mechanosensation and is modulated by algesic stimuli. *Gastroenterology* **137**, 2084-2095 e3 (2009).
172. Cattaruzza, F. et al. Transient receptor potential ankyrin-1 has a major role in mediating visceral pain in mice. *Am J Physiol Gastrointest Liver Physiol* **298**, G81-91 (2010).
173. Brierley, S.M. et al. Selective role for TRPV4 ion channels in visceral sensory pathways. *Gastroenterology* **134**, 2059-69 (2008).
174. Sipe, W.E. et al. Transient receptor potential vanilloid 4 mediates protease activated receptor 2-induced sensitization of colonic afferent nerves and visceral hyperalgesia. *Am J Physiol Gastrointest Liver Physiol* **294**, G1288-98 (2008).
175. Jones, R.C., 3rd et al. Short-term sensitization of colon mechanoreceptors is associated with long-term hypersensitivity to colon distention in the mouse. *Gastroenterology* **133**, 184-94 (2007).
176. Miranda, A. et al. The role of transient receptor potential vanilloid 1 in mechanical and chemical visceral hyperalgesia following experimental colitis. *Neuroscience* **148**, 1021-32 (2007).
177. Brierley, S.M., Jones, R.C., 3rd, Xu, L., Gebhart, G.F. & Blackshaw, L.A. Activation of splanchnic and pelvic colonic afferents by bradykinin in mice. *Neurogastroenterol Motil* **17**, 854-62 (2005).
178. Coldwell, J.R., Phillis, B.D., Sutherland, K., Howarth, G.S. & Blackshaw, L.A. Increased responsiveness of rat colonic splanchnic afferents to 5-HT after inflammation and recovery. *J Physiol* **579**, 203-13 (2007).
179. Cenac, N. et al. Potentiation of TRPV4 signalling by histamine and serotonin: an important mechanism for visceral hypersensitivity. *Gut* **59**, 481-8 (2010).
180. Cattaruzza, F. et al. Cathepsin S is activated during colitis and causes visceral hyperalgesia by a PAR2-dependent mechanism in mice. *Gastroenterology* **141**, 1864-74 e1-3 (2011).
181. Engel, M.A. et al. TRPA1 and substance P mediate colitis in mice. *Gastroenterology* **141**, 1346-58 (2011).
182. Vergnolle, N. et al. A role for transient receptor potential vanilloid 4 in tonicity-induced neurogenic inflammation. *Br J Pharmacol* **159**, 1161-73 (2010).
183. D'Aldebert, E. et al. Transient receptor potential vanilloid 4 activated inflammatory signals by intestinal epithelial cells and colitis in mice. *Gastroenterology* **140**, 275-85 (2011).
184. Brierley, S.M. et al. Differential chemosensory function and receptor expression of splanchnic and pelvic colonic afferents in mice. *J Physiol* **567**, 267-81 (2005).
185. Feng, B. et al. Long-term sensitization of mechanosensitive and -insensitive afferents in mice with persistent colorectal hypersensitivity. *Am J Physiol Gastrointest Liver Physiol* **302**, G676-83 (2012).
186. Bielefeldt, K., Ozaki, N. & Gebhart, G.F. Experimental ulcers alter voltage-sensitive sodium currents in rat gastric sensory neurons. *Gastroenterology* **122**, 394-405 (2002).

187. Dang, K., Bielefeldt, K. & Gebhart, G.F. Gastric ulcers reduce A-type potassium currents in rat gastric sensory ganglion neurons. *Am J Physiol Gastrointest Liver Physiol* **286**, G573-9 (2004).
188. Hillsley, K. et al. Dissecting the role of sodium currents in visceral sensory neurons in a model of chronic hyperexcitability using Nav1.8 and Nav1.9 null mice. *J Physiol* **576**, 257-67 (2006).
189. Stewart, T., Beyak, M.J. & Vanner, S. Ileitis modulates potassium and sodium currents in guinea pig dorsal root ganglia sensory neurons. *J Physiol* **552**, 797-807 (2003).
190. Beyak, M.J., Ramji, N., Krol, K.M., Kawaja, M.D. & Vanner, S.J. Two TTX-resistant Na+ currents in mouse colonic dorsal root ganglia neurons and their role in colitis-induced hyperexcitability. *Am J Physiol Gastrointest Liver Physiol* **287**, G845-55 (2004).
191. Ibeakanma, C. et al. Citrobacter rodentium colitis evokes post-infectious hyperexcitability of mouse nociceptive colonic dorsal root ganglion neurons. *J Physiol* **587**, 3505-21 (2009).
192. King, D.E., Macleod, R.J. & Vanner, S.J. Trinitrobenzenesulphonic acid colitis alters Na 1.8 channel expression in mouse dorsal root ganglia neurons. *Neurogastroenterol Motil* **21**, 880-e64 (2009).
193. Ozaki, N., Bielefeldt, K., Sengupta, J.N. & Gebhart, G.F. Models of gastric hyperalgesia in the rat. *Am J Physiol Gastrointest Liver Physiol* **283**, G666-76 (2002).
194. Traub, R.J. Evidence for thoracolumbar spinal cord processing of inflammatory, but not acute colonic pain. *Neuroreport* **11**, 2113-6 (2000).
195. Traub, R.J. & Murphy, A. Colonic inflammation induces fos expression in the thoracolumbar spinal cord increasing activity in the spinoparabrachial pathway. *Pain* **95**, 93-102 (2002).
196. Aerssens, J. et al. Alterations in the brain-gut axis underlying visceral chemosensitivity in Nippostrongylus brasiliensis-infected mice. *Gastroenterology* **132**, 1375-87 (2007).
197. Rong, W., Keating, C., Sun, B., Dong, L. & Grundy, D. Purinergic contribution to small intestinal afferent hypersensitivity in a murine model of postinfectious bowel disease. *Neurogastroenterol Motil* **21**, 665-71, e32 (2009).
198. Keating, C. et al. Afferent hypersensitivity in a mouse model of post-inflammatory gut dysfunction: role of altered serotonin metabolism. *J Physiol* **586**, 4517-30 (2008).
199. Keating, C., Pelegrin, P., Martinez, C.M. & Grundy, D. P2X7 receptor-dependent intestinal afferent hypersensitivity in a mouse model of postinfectious irritable bowel syndrome. *J Immunol* **187**, 1467-74 (2011).
200. Traub, R.J. et al. A rat model of chronic postinflammatory visceral pain induced by deoxycholic acid. *Gastroenterology* **135**, 2075-83 (2008).
201. Adam, B. et al. Severity of mucosal inflammation as a predictor for alterations of visceral sensory function in a rat model. *Pain* **123**, 179-86 (2006).
202. Marshall, J.K. et al. Eight year prognosis of postinfectious irritable bowel syndrome following waterborne bacterial dysentery. *Gut* **59**, 605-11 (2010).
203. Gschossmann, J.M. et al. Long-term effects of transient chemically induced colitis on the visceromotor response to mechanical colorectal distension. *Dig Dis Sci* **49**, 96-101 (2004).
204. Lamb, K., Zhong, F., Gebhart, G.F. & Bielefeldt, K. Experimental colitis in mice and sensitization of converging visceral and somatic afferent pathways. *Am J Physiol Gastrointest Liver Physiol* **290**, G451-7 (2006).
205. Harrington, A.M. et al. Sprouting of colonic afferent central terminals and increased spinal mitogen-activated protein kinase expression in a mouse model of chronic visceral hypersensitivity. *J Comp Neurol* **520**, 2241-55 (2012).
206. Hughes, P.A. et al. Increased kappa-opioid receptor expression and function during chronic visceral hypersensitivity. *Gut* (2013).

207. de Araujo, A.D. et al. Selenoether oxytocin analogues have analgesic properties in a mouse model of chronic abdominal pain. *Nat Commun* **5**, 3165 (2014).
208. Larsson, M.H., Rapp, L. & Lindstrom, E. Effect of DSS-induced colitis on visceral sensitivity to colorectal distension in mice. *Neurogastroenterol Motil* **18**, 144-52 (2006).
209. Verma-Gandhu, M. et al. Visceral pain perception is determined by the duration of colitis and associated neuropeptide expression in the mouse. *Gut* **56**, 358-64 (2007).
210. Valdez-Morales, E. et al. Release of endogenous opioids during a chronic IBD model suppresses the excitability of colonic DRG neurons. *Neurogastroenterol Motil* **25**, 39-46 e4 (2013).
211. Eijkelkamp, N. et al. Increased visceral sensitivity to capsaicin after DSS-induced colitis in mice: spinal cord c-Fos expression and behavior. *Am J Physiol Gastrointest Liver Physiol* **293**, G749-57 (2007).
212. Matsumoto, K. et al. Experimental colitis alters expression of 5-HT receptors and transient receptor potential vanilloid 1 leading to visceral hypersensitivity in mice. *Lab Invest* **92**, 769-82 (2012).
213. Shinoda, M., La, J.H., Bielefeldt, K. & Gebhart, G.F. Altered purinergic signaling in colorectal dorsal root ganglion neurons contributes to colorectal hypersensitivity. *J Neurophysiol* **104**, 3113-23 (2010).
214. Ibeakanma, C. et al. Brain-gut interactions increase peripheral nociceptive signaling in mice with postinfectious irritable bowel syndrome. *Gastroenterology* **141**, 2098-2108 e5 (2011).
215. Liebregts, T. et al. Psychological stress and the severity of post-inflammatory visceral hyperalgesia. *Eur J Pain* **11**, 216-22 (2007).
216. Yang, C.Q., Wei, Y.Y., Zhong, C.J. & Duan, L.P. Essential role of mast cells in the visceral hyperalgesia induced by T. spiralis infection and stress in rats. *Mediators Inflamm* **2012**, 294070 (2012).
217. Yang, C.Q. et al. Vesicular glutamate transporter-3 contributes to visceral hyperalgesia induced by Trichinella spiralis infection in rats. *Dig Dis Sci* **57**, 865-72 (2012).
218. Larsson, M.H., Miketa, A. & Martinez, V. Lack of interaction between psychological stress and DSS-induced colitis affecting colonic sensitivity during colorectal distension in mice. *Stress* **12**, 434-44 (2009).
219. Winston, J., Shenoy, M., Medley, D., Naniwadekar, A. & Pasricha, P.J. The vanilloid receptor initiates and maintains colonic hypersensitivity induced by neonatal colon irritation in rats. *Gastroenterology* **132**, 615-27 (2007).
220. Christianson, J.A., Bielefeldt, K., Malin, S.A. & Davis, B.M. Neonatal colon insult alters growth factor expression and TRPA1 responses in adult mice. *Pain* **151**, 540-9 (2010).
221. Xu, G.Y., Shenoy, M., Winston, J.H., Mittal, S. & Pasricha, P.J. P2X receptor-mediated visceral hyperalgesia in a rat model of chronic visceral hypersensitivity. *Gut* **57**, 1230-7 (2008).
222. Liu, L.S. et al. A rat model of chronic gastric sensorimotor dysfunction resulting from transient neonatal gastric irritation. *Gastroenterology* **134**, 2070-9 (2008).
223. Liu, L.S., Shenoy, M. & Pasricha, P.J. The analgesic effects of the GABAB receptor agonist, baclofen, in a rodent model of functional dyspepsia. *Neurogastroenterol Motil* **23**, 356-61, e160-1 (2011).
224. Winston, J.H. & Sarna, S.K. Developmental origins of functional dyspepsia-like gastric hypersensitivity in rats. *Gastroenterology* **144**, 570-579 e3 (2013).
225. Larson, S.J., Collins, S.M. & Weingarten, H.P. Dissociation of temperature changes and anorexia after experimental colitis and LPS administration in rats. *Am J Physiol* **271**, R967-72 (1996).

226. McHugh, K.J., Weingarten, H.P., Keenan, C., Wallace, J.L. & Collins, S.M. On the suppression of food intake in experimental models of colitis in the rat. *Am J Physiol* **264**, R871-6 (1993).
227. Lyte, M., Li, W., Opitz, N., Gaykema, R.P. & Goehler, L.E. Induction of anxiety-like behavior in mice during the initial stages of infection with the agent of murine colonic hyperplasia Citrobacter rodentium. *Physiol Behav* **89**, 350-7 (2006).
228. Bercik, P. et al. Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. *Gastroenterology* **139**, 2102-2112 e1 (2010).
229. Gareau, M.G. et al. Bacterial infection causes stress-induced memory dysfunction in mice. *Gut* **60**, 307-17 (2011).
230. Bercik, P. et al. Role of gut-brain axis in persistent abnormal feeding behavior in mice following eradication of Helicobacter pylori infection. *Am J Physiol Regul Integr Comp Physiol* **296**, R587-94 (2009).
231. McHugh, K.J., Collins, S.M. & Weingarten, H.P. Central interleukin-1 receptors contribute to suppression of feeding after acute colitis in the rat. *Am J Physiol* **266**, R1659-63 (1994).
232. De Schepper, H.U. et al. TRPV1 receptor signaling mediates afferent nerve sensitization during colitis-induced motility disorders in rats. *Am J Physiol Gastrointest Liver Physiol* **294**, G245-53 (2008).
233. Karanjia, R., Spreadbury, I., Bautista-Cruz, F., Tsang, M.E. & Vanner, S. Activation of protease-activated receptor-4 inhibits the intrinsic excitability of colonic dorsal root ganglia neurons. *Neurogastroenterol Motil* **21**, 1218-21 (2009).
234. Hong, S. et al. Reciprocal changes in vanilloid (TRPV1) and endocannabinoid (CB1) receptors contribute to visceral hyperalgesia in the water avoidance stressed rat. *Gut* **58**, 202-10 (2009).
235. Harrington, A.M. et al. A novel role for TRPM8 in visceral afferent function. *Pain* **152**, 1459-68 (2011).