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Sensory neuron regulation of gastrointestinal inflammation and bacterial host defence

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Sensory neurons in the gastrointestinal tract have multifaceted roles in maintaining homeostasis, detecting danger and initiating protective responses. The gastrointestinal tract is innervated by three types of sensory neurons: dorsal root ganglia, nodose/jugular ganglia and intrinsic primary afferent neurons. Here, we examine how these distinct sensory neurons and their signal transducers participate in regulating gastrointestinal inflammation and host defence. Sensory neurons are equipped with molecular sensors that enable neuronal detection of diverse environmental signals including thermal and mechanical stimuli, inflammatory mediators and tissue damage. Emerging evidence shows that sensory neurons participate in host–microbe interactions. Sensory neurons are able to detect pathogenic and commensal bacteria through specific metabolites, cell-wall components, and toxins. Here, we review recent work on the mechanisms of bacterial detection by distinct subtypes of gut-innervating sensory neurons. Upon activation, sensory neurons communicate to the immune system to modulate tissue inflammation through antidromic signalling and efferent neural circuits. We discuss how this neuro-immune regulation is orchestrated through transient receptor potential ion channels and sensory neuropeptides including substance P, calcitonin gene-related peptide, vasoactive intestinal peptide and pituitary adenylate cyclase-activating polypeptide. Recent studies also highlight a role for sensory neurons in regulating host defence against enteric bacterial pathogens including *Salmonella typhimurium, Citrobacter rodentium* and enterotoxigenic *Escherichia coli*. Understanding how sensory neurons respond to gastrointestinal flora and communicate with immune cells to regulate host defence enhances our knowledge of host physiology and may form the basis for new approaches to treat gastrointestinal diseases.

Keywords: gastrointestinal inflammation, host defence, neuro-immunology, pain, sensory neuron, vagus nerve.

Introduction

The peripheral sensory nervous system plays a critical role in regulating host physiology by monitoring the physical and chemical environment, relaying information to the central nervous system and initiating reflexes to maintain homeostasis. These features are important in coordinating gastrointestinal functions, such as nutrient absorption, gut motility, blood flow, and secretion. In addition to these well-established roles, sensory neurons play a crucial role in detecting danger and initiating protective responses. Nociceptive sensory neurons express molecular transducers at their nerve terminals to detect noxious and tissue-damaging stimuli including heat, cold and reactive chemicals. Similar to the surveillance capabilities of immune cells, sensory neurons also directly detect bacterial and fungal pathogens. The response kinetics of neurons is orders of magnitude faster than immune cells (typically milliseconds compared to hours). Therefore, the early response of the nervous system coordinates host defences to eliminate threats and mediate tissue repair processes. A growing body of evidence shows that sensory neurons communicate bidirectionally

with immune cells via signalling mediators to modulate inflammatory responses in ways that may be helpful or detrimental to the host [1, 2].

Here, we will review the role of gut-innervating sensory neurons in regulating gastrointestinal inflammation and bacterial host defence. We will examine how sensory neurons and their molecular transducers and signalling mediators modulate gut immune activation. We will also discuss mechanisms of bacterial detection by sensory neurons, and how these neurons contribute to host defence against enteric bacterial pathogens. Understanding how sensory neurons shape host defence has profound implications for our knowledge of host physiology and may augment our ability to treat gastrointestinal diseases such as irritable bowel syndrome (IBS), inflammatory bowel disease (IBD), gastrointestinal cancers, and microbial infections.

Multiple sensory nervous systems innervate the gastrointestinal tract

The human gut has an estimated 200–600 million neuronal cell bodies and is also the most densely innervated peripheral organ of the body [3]. It contains nerve endings that originate from both extrinsic and intrinsic sources (Fig. 1). Extrinsic innervation comprises spinal and vagal sensory afferents whose cell bodies are housed in the dorsal root ganglia (DRGs) and nodose/jugular ganglia, respectively. The endings of spinal afferents terminate in the dorsal horn of the spinal cord, whilst vagal afferents project to the nucleus of the solitary tract in the brainstem [4]. Both types of extrinsic afferents innervate the muscular and mucosal layers within the gut. However, vagal innervation is densest in the proximal small intestine and decreases, but is still present, in the colon [5]. The small intestine is mainly, but not exclusively innervated by thoracolumbar DRGs, while the large intestine is preferentially innervated by lumbosacral DRGs [6, 7]. These anatomically distinct neural systems can be further categorized by their neurochemical expression (e.g. ion channels, neuropeptides, transcription factors), conduction velocities and information transmitted [8]. Spinal afferents transmit noxious stimuli and convey visceral sensations, including pain, thermal and mechanical sensation [9]. Vagal afferents are involved in homeostatic physiological processes (e.g. secretion, motility, nutrient sensation), nonpainful visceral sensations (e.g. satiety, nausea) and vomiting [10]. Subsets of vagal afferents encode different gastrointestinal inputs. For example, it was recently found that GPR65-expressing neurons innervating the intestinal villi detect nutrients and control gut motility, whereas GLP1R-expressing neurons innervating the stomach and duodenum detect intestinal stretch [11].

Within the gut, intrinsic enteric neurons are spatially arranged into two continuous ganglionated networks encircling the digestive tube and extending the length of the tract. The denser myenteric plexus lies between the longitudinal and circular muscular layers, and the sparser submucosal plexus lies within the submucosa [12]. The nerve processes of the myenteric and submucosal plexuses are interconnected, extend into all layers of the gut (muscularis externa, submucosa, epithelia) and also innervate structural elements including Peyer's patches and the vasculature. The sensory portion of the enteric nervous system, called intrinsic primary afferent neurons (IPANs), forms complete reflex circuits with enteric interneurons and motor neurons, and together it influences all aspects of digestive function [3]. Unlike the extrinsic afferents, IPANs do not convey visceral sensations from the intestine to the brain [13].

Sensory neurons regulate gut inflammation

The concept that neurons may contribute to regulating inflammatory processes was first proposed in 1874 by Goltz, who observed that stimulation of the sciatic nerve induced vasodilation [14]. In 1901, Bayliss identified sensory afferents from DRGs as the main cellular mediators [15]. At the time, it was proposed that stimulating sensory fibres that terminate near arterioles in the skin caused cutaneous vasodilation through antidromic axon reflexes. Investigation over subsequent years helped strengthen the notion of dual transmission by sensory neurons, namely afferent signals are sent from the periphery to the spinal cord, whilst efferent impulses propagate antidromically from neural axons back into nerve terminals resulting in the local release of vasoactive mediators in peripheral tissues. Studies by Jancso and others [16–18] showed that this 'neurogenic inflammation' could be elicited by chemical irritants that activate sensory neurons. The components of the neurogenic inflammatory response include increased vasodilation, plasma extravasation and leucocyte recruitment [19]. Of note, capsaicin, the active ingredient of chilli peppers that induces a burning sensation, was found to activate neurons transiently, followed by a long-lasting desensitization [20]. Repeated

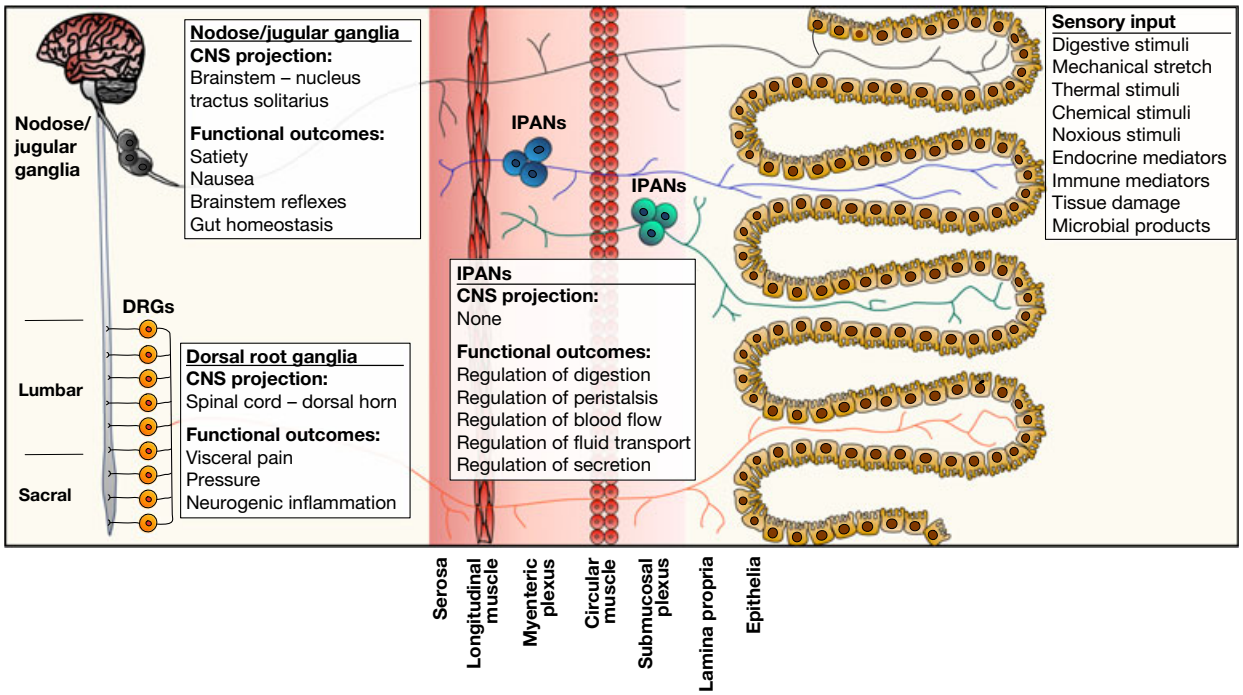

Fig. 1 *Distinct types of sensory neurons innervate the gastrointestinal tract and mediate different functional outcomes. Three sensory neuron types innervate the gastrointestinal tract and are able to respond to various environmental and internal stimuli. The sensory neurons of the enteric nervous system, termed intrinsic primary afferent neurons (IPANs), have cell bodies in the myenteric and submucosal plexus layers of the gut. They form complete reflex circuits with enteric interneurons and motor neurons to regulate many aspects of digestive and gastrointestinal functions such as gut motility, blood flow, fluid transport, and secretion. The gut receives extrinsic innervation by sensory afferents from the dorsal root ganglia (DRGs) that mediate visceral pain, pressure, and neurogenic inflammation. The gut also receives extrinsic innervation by sensory afferents from the nodose/jugular ganglia, mediating satiety, nausea, gut homeostasis, and inflammatory reflex circuits. Therefore, sensory information from the gut is differentially processed by distinct neuronal types, resulting in different gastrointestinal sensations and functional outcomes. CNS, central nervous system.*

applications of capsaicin prevented neurogenic inflammatory responses and also depleted capsaicin-sensitive nerve fibres in tissues, thus giving rise to the concepts of sensory sensitization and denervation respectively [20, 21]. The discovery that capsaicin selectively activates a large, well-defined subset of DRG neurons was an important milestone in allowing future studies to characterize the role of these neurons in inflammation [16].

Activation of vagal sensory afferents also elicits a regulatory feedback loop via an efferent arm, termed the cholinergic anti-inflammatory pathway, which induces release of neural mediators that regulate T cell and innate immune responses in the periphery. The mechanism of this potent anti-inflammatory reflex was discovered and characterized by Tracey and others [22–24]. In this reflex, inflammatory signals sensed by vagal afferents activate a neural reflex via the brainstem and vagal autonomic fibres to inhibit excessive inflammation during sepsis and other inflammatory conditions. In the spleen, vagal efferents trigger the release of acetylcholine from T cells that acts through nicotinic receptors on macrophages to suppress pro-inflammatory cytokine release [25]. Given that the focus of this review is on direct sensory neuron crosstalk with immune cells and microbes in the gut, the related cholinergic anti-inflammatory pathway is beyond our scope. For comprehensive discussions, readers are referred to in-depth reviews by Pavlov *et al.* [25], Olofsson *et al.* [26] and Tracey [27].

Role of sensory neurons in regulating gut inflammation

Studies depleting peripheral sensory fibres through physical denervation or chemical ablation

Table 1 *Sensory neuron regulation of gastrointestinal inflammation*

		References
Effect of sensory neuron inhibition		
Physical denervation	Increased pro-inflammatory cytokines in DSS-induced colitis	[29, 30]
	Exacerbation of DSS-induced colitis	[30–32]
Chemical ablation	Attenuation of TNBS- and DSS-induced colitis	[37–40, 44, 57]
	Exacerbation of TNBS- and DSS-induced colitis	[41, 42, 57, 169]
Role of ion channels		
TRPV1	Mediation of mechanical and thermal hyperalgesia	[56]
	Anti-ulcerative due to CGRP release	[42, 57]
	Antagonists improved TNBS- and DSS-induced colitis	[40, 52–54]
	Knockout mice protected from DSS-induced colitis	[55]
TRPA1	Visceral hyperalgesia	[63–65]
	Agonists are protective in murine colitis	[67]
	Knockout mice protected against TNBS- and DSS-induced colitis	[37]
	Knockout mice developed severe colitis	[66]
TRPM8	Agonist attenuated TNBS-induced colitis	[72]
	Knockout mice similar to wild-type mice in DSS-induced colitis	[72]
	Knockout mice hypersusceptible to DSS-induced colitis	[73]
Role of neuropeptides		
SP	SP induced pro-inflammatory cytokines	[37, 38, 82, 83, 170]
	SP promoted tissue recovery and survival	[86, 87]
	Antagonist improved disease score	[82]
	Antagonists exacerbated or had no difference in colitis	[84, 85]
	Knockout mice less susceptible to colitis	[37, 38, 82, 83, 170]
	Visceral hyperalgesia	[56]
CGRP	CGRP attenuated TNBS- and DSS-induced colitis	[44, 73, 94]
	Antagonists exacerbated TNBS-induced colitis	[38, 85, 94]
	Knockout mice developed severe TNBS-induced colitis	[37, 38]
	Receptor-knockout mice hypersusceptible to DSS-induced colitis	[73]
VIP	VIP administration improved inflammatory pathology	[105, 107, 108, 158, 171]
	VIP did not improve colitis at high dose	[108]
	VIP influences immune cell functions	[99, 102, 105, 172]
	Knockout mice less susceptible to DSS- and TNBS-induced colitis	[106, 109]
	Knockout mice developed more severe DNBS-induced colitis	[107]
	Differential outcomes for VPAC1−/− and VPAC2−/− in DSS-induced colitis	[111]
PACAP	Knockout mice developed severe DSS-induced colitis	[120, 121]

TRPV1, transient receptor potential vanilloid 1; TRPA1, transient receptor potential ankyrin-repeat 1; TRPM8, transient receptor potential melastatin 8; SP, substance P; CGRP, calcitonin gene-related peptide; VIP, vasoactive intestinal peptide; PACAP, pituitary adenylate cyclase-activating polypeptide; DSS, dextran sulphate sodium; TNBS, trinitrobenzene sulphonic acid; DNBS, dinitrobenzene sulphonic acid; VPAC, vasoactive intestinal peptide receptor.

have revealed a key role for nociceptive sensory neurons in gut inflammation (Table 1). This role has mainly been examined in animal models of colitis that involve colonic inflammation induced by reactive haptens, dinitrobenzene or trinitrobenzene sulphonic acid (TNBS) or by feeding of dextran

sulphate sodium (DSS) to injure intestinal epithelial cells and trigger inflammation through dissemination of enteric bacteria [28]. It remains to be determined whether neurons also play a role in T-cell transfer or transgenic preclinical models of colitis.

Physical denervation

Physical transection of the vagus nerve was once commonly used to prevent ulcers from excessive gastric acid; however, the development of modern drugs has decreased the use of vagotomies in humans. Regardless, recent studies in vagotomized animals lend support to the idea that the vagus nerve confers protection against gastrointestinal inflammation and colitis [29–32]. Vagotomy increased expression of NF-κB, a master transcription factor controlling the expression of many pro-inflammatory genes [29], and led to accelerated progression of inflammation in DSS-induced colitis, as indicated by higher colonic pro-inflammatory cytokine levels (TNF-α, IL-1β, IL-6, IFN-γ) and macroscopic and histopathological scores [30, 31]. Although vagotomy had deleterious effects on acute experimental colitis, these effects diminished with longer rest intervals between surgical vagotomy and DSS treatment suggesting that other compensatory anti-inflammatory processes may occur post-vagotomy (e.g. regulatory T-cell induction and IL-10 secretion) [31–33]. These findings suggest an anti-inflammatory role for vagal neurons, although it is not clear whether this is mediated by vagal sensory or autonomic fibres, or both.

Chemical ablation

Repeated or high-dose treatment of animals with capsaicin, or the ultra-potent vanilloid analog resiniferatoxin, has been used to chemically desensitize or ablate nociceptive sensory neurons *in vivo* [1]. These ligands selectively activate heat-sensitive transient receptor potential vanilloid 1 (TRPV1) ion channels [34] leading to calcium-dependent mitochondrial damage, osmotic dysregulation and eventually neuronal cell death [35, 36]. Several studies showed that chemical ablation of TRPV1+ neurons significantly attenuated colitis induced by TNBS and DSS, as indicated by reduced weight loss, colonic histological damage, and neutrophil inflammation measured by myeloperoxidase activity [37–40]. By contrast, other studies showed that sensory denervation by capsaicin treatment exacerbated the severity of colonic damage upon DSS and TNBS treatment [41–44]. These discrepant results may in part be due to factors such as DSS dosages and time-points of denervation. For example, in one study, increased colonic damage was found at low doses of DSS in sensory-denervated rats, but equivalent damage was observed at higher doses [41]. In another study, sensory denervation resulted in increased mucosal damage at early time-points, but not later when recovery processes may be occurring. Therefore, sensory neurons may participate in both inflammatory and reparative processes [42]. Interpreting denervation studies is also complicated by the fact that sensory neurons release several neuropeptides and other immunomodulatory mediators, which may interact with each other and have opposing effects [45].

Role of ion channels in regulating gut inflammation

Sensory neurons respond to diverse chemical and physical stimuli and are equipped with molecular sensors to transduce these environmental signals. Transient receptor potential (TRP) ion channels are a class of receptors involved in detection of thermal and chemical stimuli, including acids and irritants. There are five families of TRP channels and over 30 different members have been identified in mammals [1]. Recent work using transgenic and pharmacological strategies to target TRP channels has revealed their important roles in gastrointestinal inflammation and host defence (Table 1).

Transient receptor potential vanilloid 1 (TRPV1)

The TRPV1 ion channel was initially identified as the major receptor for capsaicin [34] and subsequently found to mediate noxious heat sensitivity and inflammatory pain. In the nodose ganglia, TRPV1 is expressed by about 40–70% of sensory neurons [46, 47], whilst 65–95% of DRG neurons express it depending on the lumbar–sacral level [6, 7, 48]. Its presence in enteric neurons is controversial with some studies finding TRPV1 immunoreactivity, and others failing to do so [49]. Additionally, there have been some reports of TRPV1 expression in non-neural cell types, including intestinal epithelial cells and smooth muscle cells; however, the existence of several splice variants and false-positive antisera may contribute to disparate findings [1]. Nevertheless, TRPV1 participates in regulating mucosal blood flow, bicarbonate secretion to counter excess acid, and mucus secretion to enhance epithelial barrier integrity [10, 50, 51].

During gastrointestinal inflammation, both sensitization and upregulation of TRPV1 channels contributed to heightened sensitivity to mechanical and thermal pain [49]. Patients with IBD reported increased abdominal pain, including 'burning' sensations, and correspondingly have increased tissue expression of TRPV1+ immunoreactive fibres [1]. In murine studies of experimental colitis, TRPV1 antagonists reduced colon shrinkage, histological scores and weight loss in TNBS and DSS models [40, 52–54]. TRPV1−/− mice showed decreased susceptibility to DSS treatment compared to wild-type mice [55], although conflicting results have been reported [56]. The protective effects observed during TRPV1 loss-of-function suggested that TRPV1 plays a role in augmenting inflammation. However, administration of capsaicin ameliorated DSS- and TNBS-induced colitis [41, 57]. Goso *et al.* suggested that downstream effects of TRPV1 channel activation, such as release of calcitonin gene-related peptide (CGRP), may contribute to anti-ulcerative effects [57]. Conclusions from work using potent TRPV1 agonists should be interpreted with caution as these ligands may also desensitize neurons.

Transient receptor potential ankyrin-repeat 1 (TRPA1)

The TRPA1 ion channel recognizes damaging reactive chemicals. Electrophilic compounds, such as mustard oils and allicin, induce gating of TRPA1 by covalent modification of cytoplasmic cysteine residues [58, 59]. In nodose ganglia and DRGs, TRPA1 is expressed in a smaller proportion (20–30%) of sensory neurons than TRPV1, although it mostly overlaps with TRPV1 in unmyelinated, small-diameter C-fibres [46, 58]. TRPA1+ neurons in the gastrointestinal tract often express neuropeptides [e.g. substance P (SP), CGRP], and TRPA1 ligands induce neuropeptide release upon activation [37, 60], implying that TRPA1 participates in neurogenic inflammation [59]. In addition to extrinsic afferents, TRPA1 transcripts and proteins are expressed by enteric neurons [61], as well as non-neuronal enterochromaffin cells in the gut [62].

Evidence suggests that TRPA1 has a role in inducing or maintaining hyperalgesia and pain sensation during colitis. Intracolonic administration of TNBS induced inward currents in a TRPA1-dependent manner, sensitized colonic sensory neurons, and elicited increased nocifensive visceromotor responses to colorectal distension [63, 64]. These responses were absent in TRPA1−/− mice or inhibited in antagonist-treated mice [63–65]. Aside from pain, the role of TRPA1 in mediating colitis-associated inflammation is less clear. Functional evidence from clinical studies is lacking, although one study found upregulation of TRPA1 mRNA and immunoreactivity in inflamed colons of patients with IBD [66]. Studies of TRPA1−/− mice showed mixed results in that colitis inflammation decreased or was similar to that of wild-type mice after TNBS or DSS treatment [37, 64]. By contrast, other studies showed that TRPA1 was upregulated in murine colitis and its activation exerted protective effects [66, 67]. Together, these findings suggest that TRPA1 is robustly activated by a wide variety of inflammatory compounds and may play a role in mediating pain and inflammation in the gastrointestinal tract.

Transient receptor potential melastatin 8 (TRPM8)

The TRPM8 ion channel is activated by cold temperatures or agents such as menthol and icilin, which elicit a cooling sensation [68]. Peppermint oil, containing a high menthol content, reduced calcium influx and caused long-lasting relaxation of the gastrointestinal smooth muscles [61]. About 10% of DRG neurons respond to cold temperatures or icillin [69, 70]. Expression in nodose ganglia has not been definitively confirmed; however, it is expressed in trigeminal ganglia, particularly in tongue-innervating regions [46, 71]. About a third of TRPM8-expressing and cold-sensitive neurons overlap with TRPV1-expressing neurons [69]; however, its coexpression with TRPA1 is less clear [46, 69].

Recent studies indicate that TRPM8 has an anti-inflammatory role in colitis, in part due to neuropeptide release. TRPM8 expression is upregulated in human- and murine-inflamed colon samples [72]. Systemic activation of TRPM8, using the selective agonist icilin, attenuated the severity of TNBS-induced colitis, as measured by decreased histological damage scores, bowel thickness, myeloperoxidase activity, and pro-inflammatory cytokines. In the same study, Ramachandran *et al.* showed no difference in DSS-induced inflammation in TRPM8−/− mice, compared to wild-type mice, although colonic levels of CGRP were increased [72]. To confirm a link between TRPM8 and neuropeptide release, the authors showed that icilin blocked calcium currents and CGRP secretion from colon tissues *ex vivo*. Later findings confirmed that the anti-inflammatory effects of TRPM8 were linked to induction of CGRP release

[73] and, furthermore, showed that TRPM8−/− mice were hypersusceptible to DSS-induced colitis. The number of CD11c+ dendritic cells (DCs) found in close proximity to CGRP+ colonic nerve fibres was increased following DSS treatment in TRPM8−/− mice relative to wild-type animals. Additionally, absence of CGRP signalling as demonstrated by mice deficient in RAMP1, the main receptor for CGRP, resulted in increased susceptibility to colitis, whilst RAMP1−/− DCs displayed a hyperinflammatory phenotype. Finally, treatment of TRPM8−/− mice with CGRP ameliorated excessive DC activation and colitis. These lines of evidence indicate that TRPM8+ nerve fibres promote an anti-inflammatory tissue environment through local release of CGRP and influence on mucosal innate immune cells.

Role of neuropeptides in regulating gut inflammation

Activation of sensory neurons induces calcium-dependent release of dense core vesicles containing neuropeptides from nerve terminals. Neuropeptides are a family of peptides that have local neuroendocrine and signaling function. In some cases, larger precursor peptides are processed into multiple neuropeptides that exert effects through one or more neuropeptide receptors expressed by neurons, immune cells, and stromal cells in the gut [74, 75].

Substance P (SP)

Substance P, a member of the tachykinin neuropeptide family, was discovered in *in vitro* extracts as the ability to stimulate intestinal contractility and decrease blood pressure [76]. The *Tac1* gene encodes preprotachykinin-1, which can be further processed into four alternatively spliced tachykinin neuropeptides: SP, neurokinin A, neurokinin K, and neurokinin gamma [77]. Their physiological receptors include NK_1R, NK_2R, and NK_3R (encoded by *Tacr1, Tacr2*, and *Tacr3,* respectively), which are G protein-coupled receptors expressed by neuronal and non-neuronal cell types. In the gut, the major sources of SP are intrinsic enteric neurons, whilst extrinsic neurons contribute to a lesser extent [42, 78–80]. Tachykinin immunoreactivity was observed in neuronal bodies of the myenteric and submucosal plexuses, as well as nerve processes innervating smooth muscle, submucosal arteries, and mucosa. NK_1R is expressed by enteric neurons, interstitial cells of Cajal, and epithelial cells. NK_2R is expressed by muscle and epithelial cells, and expression is higher in the ileum relative to the duodenum and colon. Finally, NK_3R is predominantly expressed by enteric neurons and mediates neuro-neuronal transmission. On immune cells, NK_1R and NK_2R have been localized to lamina propria T lymphocytes, macrophages, and mast cells; and their expression increases during inflammation [78]. Assessment in patients with IBD has led to inconsistent results, with some studies showing increased SP content in tissues, and others showing no alterations or a decrease in SP [81].

Substance P induces immune cells to secrete pro-inflammatory cytokines (e.g. IL-1β, IL-6, IL-8, TNF-α) via an NF-κB-dependent pathway in target cells [78]. Studies point to a deleterious role for SP and NK_1R activation in DSS- and TNBS-induced colitis. *Tac1*−/− and NK_1R antagonist-treated mice showed decreased weight loss, myeloid peroxidase activity, histopathological scores, and pro-inflammatory cytokine production compared to wild-type mice [37, 38, 82, 83]. A small number of studies showed no difference or more severe colitis after blockade of NK_1R signalling [84, 85]. Although SP enhances tissue inflammation, it may also contribute to tissue recovery. Stimulation of NK_1R in colonic fibroblasts increased collagen synthesis and fibrogenesis [86] and promoted the survival of colonocytes via anti-apoptotic Akt signalling mechanisms [87]. Thus, although NK_1R antagonists may have potential anti-inflammatory benefits in IBD, they may also counteract tissue repair processes [77].

Calcitonin gene-related peptide (CGRP)

Calcitonin gene-related peptide is a sensory neuropeptide with highly potent, long-lasting vasodilatory activity in the femtomolar range and is 10- to 1000-fold more potent than other classic vasodilators (e.g. acetylcholine) [88]. There are two isoforms of this neuropeptide, CGRPα and CGRPβ, which differ by only three amino acids in humans, and are encoded by the genes *Calca* and *Calcb,* respectively. CGRP immunoreactivity is found in all layers of the gut and concentrated around submucosal blood vessels. CGRP is largely coexpressed with SP; however, in contrast to SP fibres, CGRP fibres innervate Peyer's patches [89]. Additionally, capsaicin-induced denervation and co-labelling experiments indicated that the majority (50–80%) of CGRP expression in small and large intestines is from extrinsic and DRG origins [90]. Enteric neurons represent a minor contribution to the total CGRP intestinal expression, and these neurons

preferentially express the CGRPβ isoform [90, 91]. The receptor for CGRP consists of a heterodimer between calcitonin receptor-like receptor (CLR) and receptor activity-modifying protein 1 (RAMP1). CGRP also promiscuously binds other heterodimers, for example CLR/RAMP3 complexes that are the high-affinity receptors for adrenomedullin. CGRP is a neuropeptide that exemplifies neuroimmune bidirectional crosstalk in that many innate and adaptive immune cells modify their function in response to CGRP, whilst some immune cells (e.g. monocytes, T cells, B cells) release CGRP or regulate sensory neuron expression of CGRP [92]. CGRP plays important roles in lymphocyte maturation, proliferation, migration, antigen presentation, and cytokine production (for an extensive review, see Ref. [93]).

During colonic inflammation, CGRP immunoreactivity and release increase in distal portions of the colon [38]. Exogenous administration of CGRP showed anti-inflammatory effects, leading to amelioration of ulcerative lesions, and attenuation of colonic weight increase [44, 94]. Blockade of CGRP signalling by specific antagonists in mice increased macroscopic damage, ulcers, and myeloperoxidase activity [45, 85, 94]. CGRP−/− mice developed severe TNBS-induced colitis, although this was later attributed to a combination of the deleterious effects of SP and the lack of CGRP-mediated tissue protection [45]; the authors argued that CGRP counters the pro-inflammatory effects of SP, but is not required for colonic protection in wild-type mice [45]. CGRP/SP double-knockout mice were equally protected against TNBS-induced colitis compared to SP−/− mice, suggesting that CGRP does not provide additional protection if tissue-damaging effects of SP are absent. Currently, the cellular and molecular mechanisms of CGRP regulation of gastrointestinal inflammation have not been finely mapped.

Vasoactive intestinal peptide (VIP)

Vasoactive intestinal peptide (VIP) was discovered as a vasodilatory polypeptide in porcine intestine [95]. VIP acts on smooth muscle and intestinal epithelial cells to influence gut motility, fluid absorption, and electrolyte and mucus secretion [96–98]. It exerts immunological effects through action on its main receptors, VPAC1 and VPAC2 (encoded by the genes *Vipr1* and *Vipr2,* respectively). The receptors are expressed on several innate and adaptive immune cell types, including T cells, macrophages, DCs, neutrophils, and innate lymphoid cells [99–102]. VIP+ nerves are found in all layers of the gut and also innervate Peyer's patches [103]. In TNBS-induced colitis, the VIP content in nerve fibres decreased in the submucosa and increased in the mucosa [104].

The role of VIP in gastrointestinal inflammation is unclear due to contrasting results. Several studies showed that VIP administration conferred protection in mouse models of colitis with improvement of survival, weight loss, diarrhea, and tissue histopathology [105–107]. The authors of these studies proposed different mechanisms by which VIP exerted its beneficial effects, including promoting anti-inflammatory cytokines (IL-10, IL-4, IL-13), decreasing pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), and ameliorating bacteria-induced disruption of intestinal epithelial tight junctions. VIP also restored homeostatic immune cell trafficking to mesenteric lymph nodes and Peyer's patches [105]. However, protection disappeared when VIP was administered at higher doses [108]. In contrast to studies in which a protective role of exogenous VIP was demonstrated, VIP−/− mice appeared to be resistant to DSS- and TNBS-induced colitis, exhibiting modest weight loss, lower levels of pro-inflammatory cytokines, and no difference in histopathology scores compared to wild-type mice [106, 109]. The discrepancy may in part be due to the fact that VIP binds to two receptors, VPAC1 and VPAC2, which have distinct affinities for VIP and are expressed at varying levels on different immune cell types. VPAC1 is expressed on resting T cells and macrophages. On the other hand, VPAC2 expression is constitutively low on T cells, but is upregulated upon activation, whereas VPAC1 is downregulated [110]. The relative importance of these two receptors was investigated *in vivo*. VPAC1−/− mice were resistant to DSS-induced colitis, whereas colitis in VPAC2−/− mice appeared rapidly with greater weight loss and pro-inflammatory cytokine production, and more severe histopathology compared to wild-type mice [111]. The intriguing findings of this study highlight the need to utilize receptor-specific agonists or antagonists to target distinct neuropeptide signalling pathways during gastrointestinal inflammation.

Pituitary adenylate cyclase-activating polypeptide (PACAP)

The neuropeptide PACAP was originally extracted from ovine hypothalamus and found to potently stimulate adenylate cyclase to produce cyclic AMP in rat pituitary cultures [112]. The *Adcyap1* gene

encodes a protein that is cleaved into two peptides, 27 and 38 amino acids long, that are both biologically active. PACAP has 70% amino acid homology to VIP, and biochemical studies indicate that it binds promiscuously to receptors for VIP (VPAC1 and VPAC2), as well as its receptor PAC1 (encoded by *Adcyap1r1*). A higher proportion (70%) of nodose/jugular neurons express PACAP compared to DRG neurons (30%) [113, 114]. In enteric neurons, PACAP immunoreactivity is denser in myenteric neurons than in submucosal neurons [115]. PACAP is highly coexpressed with VIP and SP, and CGRP to a lesser extent, in nodose ganglia [114]. Similar to VIP, PACAP induces relaxation of smooth muscles and decreases gut motility [116]. PACAP also acts on intestinal epithelial cells and enterochromaffin cells to induce gastrointestinal secretion of histamine, bicarbonate, and chloride [116–118]. Unlike VPAC receptors, PAC1 may be more limited in the immune system and is mainly expressed by macrophages and neutrophils [100, 119].

Relative to VIP, less is known about the effects of PACAP on gastrointestinal inflammation. PACAP−/− mice developed more severe DSS-induced colitis with increased mortality, colonic pathology, colon length reduction, and pro-inflammatory cytokine production (IL-1β, IL-6, IFN-γ) [120, 121]. Exogenous administration of PACAP to PACAP−/− mice at an early time-point during DSS treatment improved survival and colitis disease [121]. Given the complexity of VIP and PACAP signalling with multiple receptors, the specific roles for PACAP or PAC1 have not been fully defined in the gastrointestinal tract.

Sensory neuron regulation of bacterial host defence

The human gastrointestinal tract is inhabited by trillions of microbes that vastly outnumber our own cells [122]. Commensal microbes not only produce metabolites and vitamins that are utilized by mammalian hosts, but they also enhance protection of epithelial barriers, regulate gastrointestinal functions, and modulate cognitive processes through the gut–brain axis [123]. Whilst intestinal epithelial cells and immune cells have well-recognized roles in gastrointestinal surveillance and initiation of protective responses during breach of the intestinal barrier, recent work indicates that sensory neurons also participate in these processes [124]. Sensory neurons may thus play an underappreciated role in bacterial detection and mediation of host defence in the gastrointestinal tract.

Sensory neuron detection of bacterial products

Recent studies have shown that sensory neurons respond directly to several bacterial products, including cell-wall components, toxins, and metabolites (Fig. 2). Bacterial activation of DRG neurons, vagal afferent neurons or IPANs could have various physiological outcomes on regulating pain, satiety, neuroendocrine control, and gut motility.

Bacterial detection by DRG afferent neurons

Bacterial infections of the gastrointestinal tract are often accompanied by pain, an unpleasant sensation mediated by nociceptive DRG neurons innervating the spinal cord. It was generally thought that inflammatory mediators produced by immune cells during bacterial infection induced nociceptor activation and pain; however, it has recently been shown that bacterial pathogens can directly induce these effects.

Citrobacter rodentium is a Gram-negative murine bacterial pathogen that causes similar gastrointestinal infections as enterotoxigenic *Escherichia coli* (ETEC) in humans. *C. rodentium* infection increased the hyperexcitability of mouse colonic DRG neurons and resulted in enhanced pain to colorectal distension [125]. The ability of individual bacterial products to activate colon-innervating DRG neurons was further investigated [126]. Bacterial lipopolysaccharide (LPS) enhanced the excitability and firing rate of DRG neurons and increased their production of pro-inflammatory TNF-α and IL-1β transcripts and cytokines [126].

Bacteria may also inhibit neural activity and pain. Oral administration of Lactobacillus strains reduced the firing rate of lumbar DRG neurons and reduced nociceptive muscle responses to colorectal distension as measured by electromyography [127, 128]. It was also found that oral gavage of *Lactobacillus acidophilus* induced the expression of μ-opioid and cannabinoid receptors in intestinal epithelial cells and produced analgesic effects [129]. These findings demonstrate that sensory neurons change their membrane properties in response to bacterial products, although the specific molecular mechanisms involved are not yet fully defined.

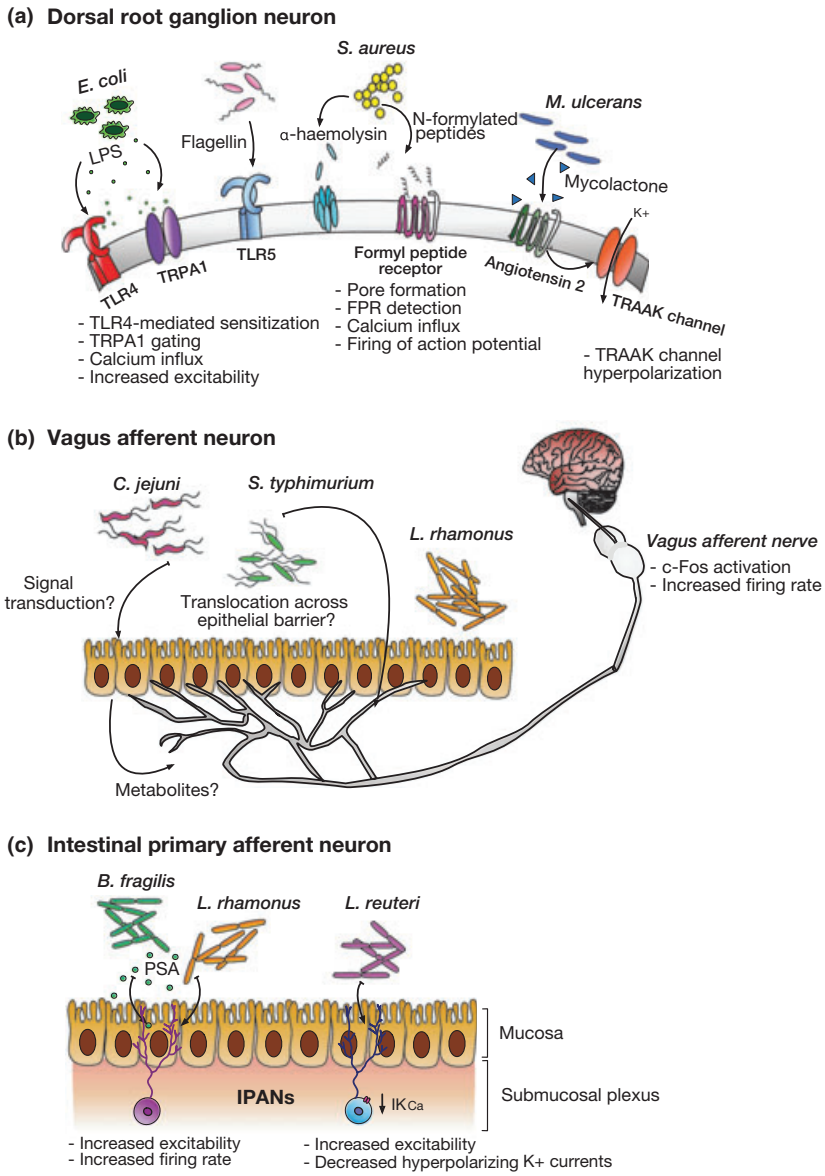

Fig. 2 *Sensory neuron detection of bacterial products. Sensory neurons of the dorsal root ganglia (a) and jugular/nodose ganglia (b) and intrinsic primary afferent neurons of the enteric nervous system (c) have been found to respond directly to pathogenic and commensal bacteria. Bacterial cell-wall components, metabolites and toxins from different commensal and pathogenic bacteria interact with neuronal receptors or ion channels to induce changes in ion flux, signalling, and neuronal excitability. TLR4, Toll-like receptor 4; TRPA1, transient receptor potential ankyrin-like 1; TLR5, Toll-like receptor 5; FPR, formyl peptide receptor; TRAAK, TWIK-related arachidonic acid-activated K+ channel; IPAN, intrinsic primary afferent neuron; PSA, polysaccharide A; IKCa, calcium-activated potassium current.*

Toll-like receptors (TLRs) are expressed by DRG neurons [130], and TLR ligands induce neuronal activation and pain [131]. *E. coli*-derived LPS sensitized TRPV1 channels and enhanced inward calcium currents via a TLR4-dependent mechanism in trigeminal neurons [132]. Additionally, the lipid A moiety of LPS was found to directly gate TRPA1 channels independently of TLR4 and induced neuronal excitability, calcium influx, and neurogenic inflammation [133]. In *Staphylococcus aureus* infections, binding of bacterial N-formylated peptides to formyl peptide receptor 1 and pores formed by α-haemolysin induced calcium flux, depolarization, and firing of action potentials in DRG neurons [134]. This neuronal activation was independent of tissue swelling and innate immune components. Conversely, mycolactone, a metabolite from *Mycobacterium ulcerans*, the causative agent of painless skin ulcers, signalled through angiotensin-2 receptors to activate TRAAK-mediated potassium channels, hyperpolarized neurons, and produced analgesia [135]. Although these recent studies indicate that DRG neurons respond to bacterial products directly by

increasing or decreasing their activity, the functional outcomes on host defence remain mostly underexplored.

Bacterial detection by vagal afferent neurons

Vagal sensory afferent fibres are an important neural link between the gut and brain and may serve as an early signalling pathway of infection by gastrointestinal pathogens [123]. Oral inoculation of enteric pathogens *Campylobacter jejuni* or *Salmonella typhimurium* led to activation of neurons in the vagal sensory ganglia and the nucleus solitary tract in the brain as measured by c-Fos expression [136]. Other studies demonstrated that these effects were mediated by capsaicin-sensitive vagal afferents because vagotomy partially prevented c-Fos induction in the hypothalamus [137].

Within the gut, vagal sensory nerve fibres extend up into the villi and are found in close proximity to intestinal epithelial cells. It is still unclear whether enteric pathogens breach the intestinal epithelial barrier to activate underlying vagal processes directly, or whether epithelial cells transduce bacterial signals and secrete paracrine mediators that subsequently alter vagal activity [138]. Direct application of LPS has been shown to elicit currents in nodose neurons in culture [139]. Moreover, it was demonstrated that *Lactobacillus rhamonus* increased the intrinsic firing rate of vagal afferents to gut distension within minutes of exposure of the bacterium to the gut lumen [138]. Using CSFE-labelled bacteria, the authors of the study found no evidence of bacterial translocation across the epithelial layer into deeper mucosal and submucosal layers. Thus, the mechanisms of neuronal activation by bacteria *in vivo* have yet to be determined.

Bacterial detection by IPANs

Intrinsic primary afferent neurons are the sensory arm of the enteric nervous system. IPANs express innate immune receptors, including TLR2, TLR3, TLR4 and TLR7, that enable detection of pathogen-associated molecular patterns [124]. Luminal application of *L. rhamosus* and *Bacteroides fragilis* to the epithelia produced responses in myenteric IPANs within seconds and facilitated excitability within minutes [140]. The short latency of orthodromic action potentials suggested that IPAN responses were direct sensory action potentials mediated by neural processes extending into the mucosal epithelia. IPANs also received synaptic inputs from local IPAN circuits as secondary excitatory postsynaptic potentials were also recorded. It was found that the capsular polysaccharide A from *B. fragilis* was a critical mediator of activation of sensory neuronal responses [140]. It is likely that other commensal or pathogenic bacterial strains could signal to IPANs via specific molecules or metabolites.

Bacteria can also induce distinct effects on different intestinal sensory subtypes. Although *Lactobacillus reuteri* decreased firing in extrinsic DRG neurons to mediate analgesia [127], the same bacterium increased excitability in a subset of colonic myenteric IPANs [141]. IPANs were more excitable due to decreased hyperpolarizing potassium currents mediated through IK_{Ca} channels [141]. Another study showed that IK_{Ca} channel antagonists decreased muscle contraction and colon motility [142]. These lines of evidence suggest that *L. reuteri*-mediated enhancement of IPAN excitability may reduce colonic motility and that lower muscle tension may be a contributing factor in probiotic alleviation of visceral pain [141]. Activation of enteric neurons also resulted in neural modulation of the inflammatory response as exemplified by enhanced TNF-α production in LPS-treated myenteric plexus preparations and primary enteric cultures [143].

Sensory neuron regulation of gastrointestinal bacterial infections

The ability of neurons to directly respond to bacteria may be a beneficial mechanism for mammalian hosts to combat pathogens. Indeed, increasing evidence indicates a role for sensory neurons in regulating effective host defence against bacterial pathogens during gastrointestinal infections. Here, we focus on the specific enteric pathogens *Salmonella*, *C. rodentium*, and ETEC (Table 2). Much remains to be determined regarding the role of neuronal sensing in successful host defence against bacterial pathogens.

Salmonella

It has been shown that *S. typhimurium* translocates across the intestinal lumen by inducing uptake by M cells in Peyer's patches, which are highly innervated by peptidergic sensory neurons, as well as sympathetic and cholinergic neurons [144]. Neuronal input may regulate barrier defences against the invasiveness of *Salmonella*. Total pharmacological blockade of neural activity increased epithelial uptake of *S. choleraesuis* in porcine jejunum [145].

Table 2 *Neuropeptide modulation of gastrointestinal bacterial host defence*

Neuropeptides	Bacterial pathogen	References
Substance P	*Salmonella typhimurium*	
	Expression increased in gut-associated tissues	[146, 147]
	Influenced immune cell functions	[146, 148–150]
	Receptor knockout protected from infection	[150]
	Antagonists decreased survival	[146]
	Citrobacter rodentium	
	Expression not changed upon infection	[162]
	Enterotoxigenic Escherichia coli	
	Enhanced LPS-induced faecal output	[168]
VIP	*Salmonella typhimurium*	
	Protected mice from LPS-induced endotoxemia	[154]
	Inhibited *S. typhimurium* clearance by immune cells	[156, 157, 173, 174]
	Inhibited pro-inflammatory cytokine production	[157]
	Citrobacter rodentium	
	Expression increased upon infection	[158]
	Prevented epithelial tight junction redistribution	[158]
	Enterotoxigenic Escherichia coli	
	Protected against infection	[163]
	Reduced pro-inflammatory cytokines	[163]
	Prevented toxin-induced fluid secretion from epithelial cells	[164]
PACAP	*Salmonella typhimurium*	
	Influenced immune cell functions	[119, 156, 157, 173]
	Protected mice from LPS-induced endotoxemia	[154]
	Receptor inhibited leucocyte recruitment	[119]

VIP, vasoactive intestinal peptide; PACAP, pituitary adenylate cyclase-activating polypeptide; LPS, lipopolysaccharide.

Sensory neuron-derived neuropeptides and their signalling receptors may play an important role in regulating innate and adaptive immune responses during *Salmonella* infections. Expression of *Tac1* (tachykinin precursor for SP) and SP receptor transcripts increased within hours of oral infection in Peyer's patches, mesenteric lymph nodes, and spleen of infected animals [146, 147]. Macrophages cocultured with *Salmonella* upregulated SP receptors and exhibited increased binding of SP *in vitro* [146]. SP polarized immune cells to adopt a more pro-inflammatory phenotype, including induction of IL-1, IL-6, and TNF-α expressions by monocytes [148], and IL-12 expression by macrophages [149]. Mice treated with an SP antagonist succumbed more rapidly to *Salmonella* infection and showed lower IL-12 and IFN-γ expressions in mucosal tissues [146]. These studies suggest that SP mediates protection against infection by enhancing innate immune cell function, in part through SP receptor upregulation and production of IL-12 and IFN-γ. By contrast, another study found improved survival in receptor-deficient $NK_1R-/-$ mice when challenged with *Salmonella* compared to wild-type mice [150]. SP may also suppress adaptive immune cell function. Immunization of $NK_1R-/-$ mice with a *Salmonella*-adjuvant vaccine showed increased mucosal and systemic immunoglobulin IgA responses. Secretory IgA responses may have been boosted in $NK_1R-/-$ mice by CD4 T helper cells secreting IL-5 and IL-6 in Peyer's patches [150]. Whilst this contradicts earlier studies showing that SP enhances IgA secretion [151–153], the authors argued that there are compartmentalized effects due to SP+ fibres differentially innervating B-cell and T-cell zones in lymphoid tissues [150]. Taken together, these results indicate that SP influences both innate and adaptive responses during *Salmonella* infection.

Vasoactive intestinal peptide and PACAP protect mice from endotoxic shock induced by lethal injections of *Salmonella*-derived LPS though inhibition of TNF-α and IL-6 productions [154]. PAC1, the receptor for PACAP, was shown to inhibit LPS-induced neutrophil recruitment, as measured by myeloperoxidase activity in the liver and intestine [119]. VIP also downregulated pro-inflammatory cytokines (TNF-α, IL-1, IL-12, IFN-γ) and upregulated anti-inflammatory cytokines (IL-10, TGFβ) [154, 155]. Although VIP-mediated immunosuppression was beneficial during excessive inflammation induced by lethal endotoxemia, it may be detrimental to host clearance of pathogens. VIP inhibited IFNγ-mediated production of reactive oxygen species and prevented killing of *Salmonella* by cultured macrophages [156]. VIP also inhibited TNF-α and IL-1β productions in *S. typhimurium*-infected macrophages [157].

Citrobacter rodentium

Citrobacter rodentium is a noninvasive, attaching and effacing Gram-negative murine pathogen that adheres to and disrupts colonic epithelial cells, thereby inducing mucosal inflammation and colitis [158]. Animal models of *C. rodentium* infection have been used to model human inflammatory bowel disorders. Neurobehavioural changes occur following *C. rodentium* infection, including changes in anxiety parameters in open-field tests, and increase in risk assessment behaviours, which were correlated with increased vagal sensory activation as measured by c-Fos expression [159]. Vagal afferents likely serve as an early neural pathway transmitting gastrointestinal signals from the gut to the brain. Modification of behaviour may serve a protective role in allowing the host to avoid danger and recuperate [159]. Although plasma levels of IFN-γ, TNF-α, and IL-12 did not differ post-infection, it is possible that gastrointestinal release of inflammatory mediators by immune or epithelial cells activated cytokine receptors on vagal sensory neurons [160, 161]. It is also possible that vagal sensory neurons are able to directly detect *C. rodentium* and signal to brain circuitry that regulates anxiety-like behaviour.

Sensory neuropeptides may also play a role in host defence against *C. rodentium*. VIP immunoreactivity in inflamed colon tissues was increased upon *C. rodentium* infection in one study [158]. However, another study showed that immunoreactivity of several neuropeptides (VIP, SP, CGRP) in gastrointestinal tissues of *C. rodentium*-infected mice was not different compared with sham-infected controls [162]. Exogenous administration of VIP did not impact bacterial attachment to epithelial cells; however, VIP prevented *C. rodentium*-induced redistribution of tight junction proteins (e.g. ZO-1, occludin) in a myosin light-chain kinase-dependent manner [158]. Body weight loss, colonic epithelial damage, and paracellular permeability were also reduced, indicating that VIP ameliorates *C. rodentium*-induced gastrointestinal inflammation.

ETEC

Enterotoxigenic *Escherichia coli* is a major bacterial pathogen that causes diarrhoea in humans. ETEC produces high levels of enterotoxins that stimulate excessive fluid secretion by intestinal epithelial cells. Recent studies indicate that sensory neuropeptides may be utilized as an alternative to antibiotics in treating diarrhoeal diseases. Exogenously administered VIP to newly weaned piglets infected with ETEC reduced the incidence of diarrhoea and improved growth rates [163]. VIP treatment also reversed ETEC-induced increases in inflammatory mediators including IL-2, IL-12, IFN-γ, and TNF-α [163]. Supporting the hypothesis that VIP may be protective against enterotoxin-induced diarrhoeal disease, one study showed that heat-labile and heat-stable enterotoxins from *E. coli* induced increases in jejunal fluid secretion that were dose-dependently reversed by VIP treatment [164]. Others have shown that vagotomy and intraluminal capsaicin administration inhibit ETEC-induced fluid secretion, suggesting that TRPV1-expressing vagal sensory neurons play a role in regulating epithelial function [165, 166]. Systemic administration of LPS derived from *E. coli* increased nitric oxide synthase enzymatic activity in the small intestine and accelerated intestinal transit [167]. LPS-induced increase in fecal output was found to be mediated by capsaicin-sensitive vagal neurons and could be blocked by treatment with SP receptor antagonists or nitric oxide synthase inhibitors [168]. Therefore, sensory neurons may play an important role in regulating inflammatory responses, gut motility, and intestinal secretions during bacterial infection.

Summary and future directions

The studies highlighted above show the complex roles that sensory neurons and their mediators play during gastrointestinal inflammation and bacterial host defence. On one hand, sensory neurons detect bacterial products and

inflammatory inputs and transmit information through neural circuits to the central nervous system; on the other hand, they can also potently drive or suppress innate and adaptive immune responses in the gastrointestinal tract through neurogenic mechanisms such as the release of neuropeptides. Because many distinct subsets of peptidergic and nonpeptidergic neurons innervate the gastrointestinal tract with distinct sensory modalities and anatomical distributions, the role of the sensory nervous system in gastrointestinal host defence will be an important field of future study. It is possible that distinct species of commensal and pathogenic bacteria stimulate or modulate neuronal excitability of different sensory subtypes, thus regulating pain, gut motility, and inflammation. These bacteria–neuron molecular interactions remain to be defined. Furthermore, evidence indicates differential roles for distinct TRP channels and neuropeptides expressed by sensory neurons in regulating immune cell function. Therefore, defining how distinct subtypes of sensory neurons communicate with innate and adaptive branches of the immune system may be critical in allowing us to understand their roles in homeostasis, inflammation, and host defence. Eventually, targeting these specific neuron–microbe and neuron–immune interactions may lead to new treatments for gastrointestinal diseases including IBS/IBD and bacterial infections.

Conflict of interest statement

No conflicts of interest to declare.

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