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Review article

Gut–brain communication: types of sensory nerves and mechanisms of activation

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Abstract

Understanding the locations of extrinsic sensory nerve endings in the gastrointestinal tract and their mechanisms of activation is essential to advancing our understanding of how communication along the gut–brain axis affects health and disease. The gastrointestinal tract detects diverse stimuli (chemical, mechanical and thermal signals) via two major types of primary afferent (sensory) nerves: vagal and spinal afferents. Viscerofugal neurons represent a third pathway that has been indirectly implicated in gut–brain signalling. These spinal and vagal afferents transmit sensory signals to the brain through distinct pathways, and although the origins of their nerve cell bodies are known, their nerve endings remain poorly understood. New evidence indicates that single dorsal root ganglia neurons can give rise to multiple different morphological types of endings within different gut layers, and that Piezo2 channels have a major role in detecting mechanosensory stimuli by gut-projecting spinal afferents. Morphological studies suggest that substances released from enteroendocrine cells can activate the terminals of vagal and spinal afferent endings within the mucosa through a paracrine mechanism. Here, we review the distinct spinal and vagal afferent types alongside viscerofugal pathways revealed by advances in neurogenetic techniques and high-resolution anterograde tracing, linking them to their physiological role in gut–brain communication.

Sections

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Key points

- Compared with the upper gastrointestinal tract (stomach and oesophagus), the distal colon and rectum exhibit the highest density and greatest morphological diversity of spinal afferent endings; however, little is known about those nerve endings in the distal small intestine, caecum and proximal colon.
- Single multi-ending spinal afferents in the colon have been identified, giving rise to multiple morphological types of sensory endings across different gut layers.
- A major class of spinal afferent endings, identified as intraganglionic varicose endings, has been found within myenteric ganglia of both the stomach and colon; spinal intraganglionic varicose endings might belong to multiple genetically distinct classes of afferent fibres.
- Distinct populations of vagal intraganglionic laminar endings and mucosal afferents have been implicated in mediating satiety, oesophageal peristalsis and the motivational aspects of feeding behaviour.
- Enteric viscerofugal neurons have been implicated in peripheral reflexes, including the 'ileal brake', which can affect feeding behaviour.
- Enterochromaffin cells communicate with spinal and vagal afferent endings in the mucosa via paracrine signalling mechanisms.

Introduction

In vertebrate animals, two distinct sensory pathways have evolved to provide extensive extrinsic innervation of the gastrointestinal tract and other internal organs — the spinal and vagal afferent nerves. These nerves are capable of detecting various sensory modalities within the gut and follow distinct anatomical pathways into the central nervous system (CNS)[1–4]. Although spinal afferent neurons first send sensory signals into the spinal cord and then brain, vagal afferent neurons send their sensory signals directly into the brainstem, bypassing the spinal cord (Fig. 1). Nerve cell bodies of spinal and vagal afferents lie in the peripheral nervous system, within dorsal root ganglia (DRG) and nodose/jugular ganglia, respectively. Both pathways are formed by pseudounipolar neurons whose axons bifurcate, projecting at one end into the CNS and into the gut at the other. Together, these spinal and vagal pathways account for the great majority of afferent gut–brain communication. Although this Review focuses on the fundamental anatomical and functional characteristics of the sensory endings in the gut arising from these two afferent pathways, it is noteworthy that gut–brain interactions, often in combination with the gut microbial environment, have garnered substantial attention owing to their roles in cognitive–emotional functions such as anxiety, depression, motivation and memory, as well as neurodegenerative conditions and ageing[5–8]. This aspect underscores the broader importance of gut–brain pathways beyond gut-related functions and sensation. Additionally, a third neuronal pathway, formed by enteric viscerofugal neurons (VFNs), has been implicated in gut–brain signalling and is described separately later.

The gut is a nexus of enteric, sympathetic and parasympathetic neurons, in addition to spinal and vagal afferent nerves, presenting a major challenge to discrimination and characterization of individual sensory neuronal populations. Thus, the locations and characteristics of the nerve cell bodies of vagal and spinal afferents that supply the gut are well characterized compared with their peripheral axons and terminals, particularly spinal afferents. As molecular profiling and classification of spinal and vagal afferents has moved ahead rapidly[9–13], a lack of robust and high-resolution afferent-selective labelling techniques has hampered the study of their terminals, leading to a conspicuous weakness in our understanding of how and where sensory transduction take place in the gut wall — a critical component of interoception and gut–brain communication.

Previous studies applied non-selective neuronal tracing from peripheral nerves to the gut following ex vivo electrophysiological recording from the same nerves. This powerful approach enabled putative afferent nerve endings to be systematically correlated with electrophysiologically mapped mechanotransduction sites, including vagal and spinal intraganglionic laminar endings (IGLEs)[14–16], and spinal vascular afferents[17]. However, non-selective labelling of both afferent and efferent axons presented an ongoing challenge to identification of only sensory endings, particularly in densely innervated areas. To achieve selective spinal afferent labelling, anterograde tracing from DRG was required. This had been demonstrated in larger animals[18–20] and was well established for vagal afferent tracing from rat and mouse nodose ganglia[21,22]. Thus, anterograde tracing from DRG using dextran biotin[22,23] was developed as a survival surgery for the first selective, high-resolution labelling of spinal afferent endings in mice[24,25] and now also in rats[26]. An unexpectedly complex array of ending types were revealed in mice[25,27]. Those identified include endings previously correlated with electrophysiological mapping (IGLEs and vascular afferents), as well as multiple 'new' types whose functional correlates are unknown. It is perhaps no coincidence that the previously unknown types of afferent endings are located in densely innervated regions (for example, myenteric plexus, submucosal plexus and circular muscle), with structures that are otherwise indistinguishable from intrinsic and extrinsic efferent nerves. Anterograde tracing has also shown the occurrence of multiple different morphological types of sensory endings arising from single-parent axons[26,28,29], supporting similar observations in vagal afferents[30,31], and whose functional implications remain unknown. Tracing from different spinal segments (for example, thoracic compared with lumbosacral DRG) also uncovered major differences in diversity of spinal afferent endings across different gut regions (for example, stomach versus distal colon) and visceral organs (for example, uterus where only three distinct types of endings were identified[32]).

Transgenic reporter mice have been useful for both central and peripheral neuroscience by enabling live visualization of genetically defined neuronal populations. Reporter mice enable bulk labelling of nerve populations in peripheral organs, such as the gut. This process can help identify targets of entire neurochemical classes of neurons[33], but high density labelling obscures fine morphological detail of nerve endings and makes it challenging to follow the trajectory of a single axon and identify the endings that arise from single primary afferent neurons. Many neurochemical markers also lack specificity, for example, calcitonin gene-related peptide (CGRP) is expressed in vagal and spinal afferents and some enteric neurons, making uncertain the origin of labelled axons. Despite these challenges, the morphologically distinct vagal IGLEs[34], and vagal mucosal endings, have been successfully identified by bulk labelling in transgenic reporter mice, whose origins are demonstrated by vagotomy[35]. Further advances have been driven

by refinements including intersectional genetic labelling, viral transduction of sensory ganglia and sparse labelling through controlled induction of recombinase[36–39].

Identification of sensory nerve terminals is a major focus of this Review, but this aspect represents one of many ways to describe and classify sensory neurons. Sensory nerve terminals correlate with specific molecular profiles, sensory and physiological functions. The process of determining these correlations represents a more incomplete and larger task that is currently underway to understand gut–brain communication. However, extensive studies combining novel molecular profiling, neuroanatomical and neurophysiological approaches have recently made important advances towards defining these correlations among vagal afferents[40]. At present, vagal IGLEs are perhaps the most comprehensively understood type of sensory nerve ending in the gut. They have been extensively mapped along the gut and reconciled with their electrophysiological functional class with detailed studies of their mechanotransduction mechanisms[14,15,41]. Molecular profiling reveals multiple IGLE subclasses and molecular targets, whereas neurogenetic manipulations suggest that IGLE populations control satiety and regulate oesophageal peristalsis[39,40,42]. Comparably little is understood of the other sensory nerve endings in the gut. Indeed, although gut spinal[9] and vagal afferents[13] have been classified into multiple groups based on molecular profiling, none among the spinal afferents is conclusively linked to a nerve ending type, highlighting a major knowledge gap in gut–brain pathways. Here, we survey the morphological types of extrinsic sensory neurons along the gut–brain axis, linking them where possible to their molecular characteristics and physiological functions. The VFNs are then also described, representing a unique enteric neuronal population now implicated in gut–brain interactions.

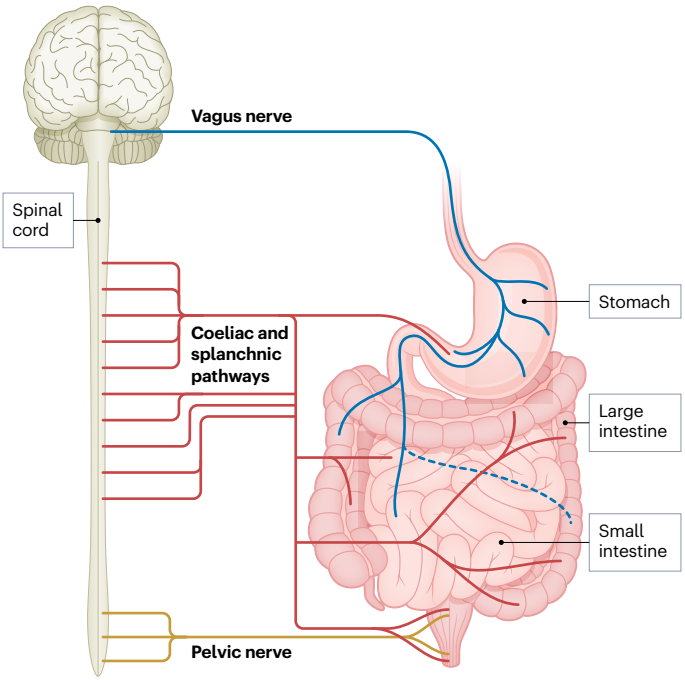


Fig. 1 | Major sensory pathways linking gut to brain. Schematic diagram showing the major sensory pathways linking gut to brain, including the vagal (blue), thoracolumbar spinal (red) and lumbosacral spinal (yellow) sensory pathways.

Spinal afferent nerve endings

Intraganglionic varicose endings

Spinal intraganglionic varicose endings (IGVEs) comprise varicose axons that traverse myenteric ganglia, often branching into multiple varicose axon terminal arbors that weave around myenteric neurons[25]. They represented ~17% of L6–S1 colonic spinal endings[25] and 43% of T8–T12 gastric spinal afferent endings in the mouse[27] (Fig. 2). T7–T11 DRG in rat supply similar 'ganglionic-type' endings in stomach[26]. Physiological importance of varicosities in IGVEs is currently not clear, although they might represent sites of increased sensory ion channel expression and/or release sites for CGRP or other substances to fulfil an efferent-type function. A similar type of afferent neurons in myenteric plexus are 'internodal' endings, comprising varicose axons such as IGVEs but are restricted to internodal strands without branching[25,27]. A population of IGVEs expresses the mechanosensor, Piezo2 (ref. 43), and these IGVEs seem to occur along the whole mouse gut from stomach to distal colon[43].

IGVEs belong to multiple afferent types. First, most IGVEs contained CGRP (66% of colonic lumbosacral IGVEs and >90% of gastric T8–T12 IGVEs)[25,27]. Secondly, single-parent axons possess multiple combinations of afferent structures, which include IGVEs, with or without intramuscular endings, and submucosal and mucosal endings[28,29] (discussed further later). Finally, at least four genetically and physiologically distinct spinal afferent populations in distal colon have IGVEs, and a fifth seems to have the internodal endings[37]. Properties of the five genetically defined spinal afferent populations identified in the extensive study by Wolfson et al. are summarized in Table 1. They are characterized by their main genetic handles to tyrosine receptor kinase B ($TrkB^+$), tyrosine hydroxylase (TH^+), bone morphogenetic protein receptor type 1b ($Bmpr1b^+$), somatostatin receptor 2 ($Sstr2^+$) and adrenoceptor alpha 2a ($Adra2a^+$).

Although it remains to be demonstrated by anterograde tracing, the sparse genetic labelling approach used by Wolfson et al.[37] showed that each genetically defined population had IGVEs, except Sstr2 afferents, which had internodal endings (Table 1). TrkB afferents had extensive IGVEs, engulfing more myenteric cell bodies than IGVEs of other classes. Fully compatible with immunolabelling[25,44], two of four IGVE afferent classes co-expressed the CGRPα encoding gene, Calca, as did Sstr2 afferents with internodal endings, and TH afferents lacked TrpV1 (Table 1).

All the genetically defined populations tested by Wolfson et al. showed sensitivity to colonic distension. Although three of five afferent populations gave rise to endings in other layers (for example, labelling TH^+ afferents revealed both IGVEs and intramuscular endings, see Table 1), both $TrkB^+$ and $Bmpr1b^+$ afferents gave rise to IGVEs alone, strongly suggesting that IGVEs are distension-sensitive, probably mediated by their Piezo2 expression[43]. Nevertheless, the distension response profiles of different afferents that use IGVEs were distinct[37], compatible with the idea of variability or uncoupling of sensory modality with nerve ending morphology[40].

Interestingly, $Bmpr1b^+$ afferents are a population that lack TrpV1, the pain-associated and heat-sensitive vanilloid channel, and yet this population of afferents mediated the largest contribution to colonic pain-evoked behaviours (through Piezo2)[37]. This finding suggests that TrpV1 is not a complete marker of nociceptive endings in mouse, but also reinforces the importance of Piezo2 for mechanically evoked pain signalling from the colon when considering previous data showing

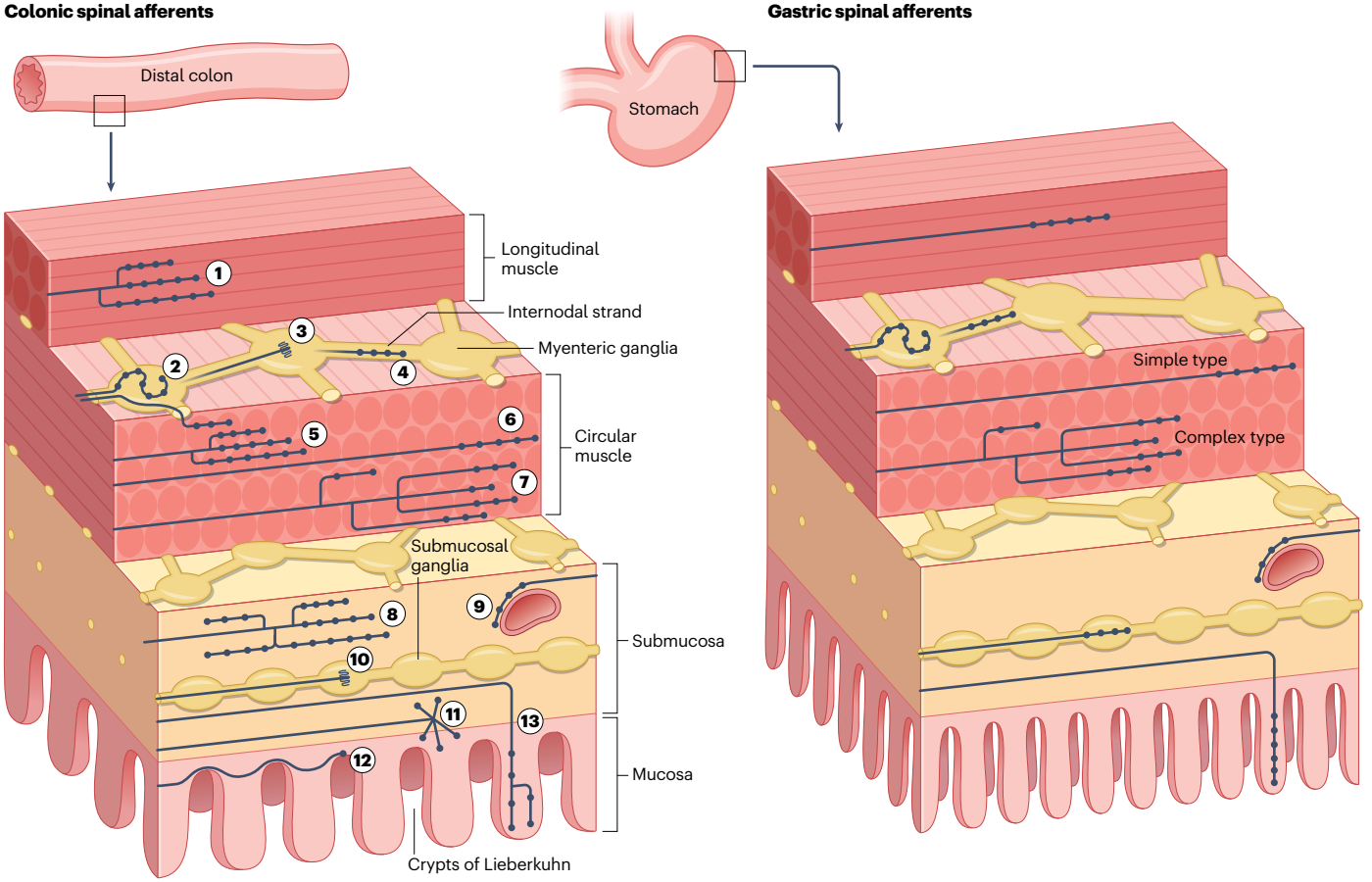


Fig. 2 | Mouse colonic and gastric spinal afferents. Schematic diagram summarizing the types of mouse colonic and gastric spinal afferents identified by anterograde and their distribution across tissue layers within the gut wall. Numbers denote the morphological subtypes of spinal afferents identified in the colon. Longitudinal intramuscular endings (rare) (1); intraganglionic varicose endings (2); intraganglionic laminar endings (rare in mouse colorectum) (3); internodal endings (4); branching intramuscular endings (5); simple intramuscular endings (6); complex intramuscular endings (7); branching submucosal endings (8); vascular afferent endings (9); submucosal intraganglionic laminar endings (rare) (10); complex submucosal endings (11); simple mucosal crypt endings (12); mucosal villus endings (13).

major contributions to mechanically evoked pain signalling from TrpV1$^+$ afferents that express Piezo2, but not Piezo1 (refs. 45,46). See Table 2 for a summary of studies that have identified IGVEs or IGVE-like endings.

Rectal intraganglionic laminar endings

Morphologically, intraganglionic lamellar endings in rectum (rIGLEs) are highly distinct, and their functional correlate has been identified in electrophysiological recordings[16]. They have highly arborized, flattened endings corresponding to low-threshold, tension-sensitive mechanoreceptors that were presumed to be of spinal origin[47]. Confirming this aspect, rIGLEs were traced from L6–S1 DRG in mice[25]. They are primarily located in myenteric ganglia, although rare rIGLEs occur in submucous ganglia[25]. Rectal IGLEs lack CGRP[25,47], but about 30% contain glutamate transporter VGluT1 or VGluT2 (ref. 47). Rectal IGLEs are less common than IGVEs in mouse, representing ~5% of identified spinal afferent endings[25]. This finding suggests that, in myenteric ganglia, IGVEs preferentially arise from spinal afferents, whereas IGLEs preferentially originate from vagal afferents in the upper gastrointestinal tract (discussed subsequently), where none seems to have a spinal origin in mouse[27] or rat[26]. See Table 2 for a summary of studies that have identified rIGLEs.

Intramuscular endings

About 25% of lumbosacral colonic spinal afferent endings and T8–12 gastric spinal afferent endings were intramuscular[25,27]. Along the gut, the density of intramuscular innervation from spinal and vagal afferent sources is similar in the stomach, but preferentially arises from spinal sources in small intestines and colon, where it is over twice the density of vagal innervation[48]. The varicose nerve ending structures of spinal intramuscular afferents were virtually always located in circular muscle, but small numbers (<5%) occur in longitudinal muscle[25,27]. In the colon, intramuscular endings were divided into three morphological types: simple, branching and complex[25], of which more than 90% were branching or complex. Gastric endings comprised only two of these: simple and complex, with the great majority simple (~88%). Thus, the colonic lumbosacral endings seem to favour more complex structures than T8–T12 gastric endings. The rat gastric intramuscular innervation

by spinal afferents shows a similar preponderance of circular muscle innervation over longitudinal muscle[26]. Though not quantified, the longitudinal innervation in rat appears more extensive than in mouse and the intramuscular endings generally seem to have more complex branching patterns[26]. Those afferents, as well as colonic branching endings in mouse, have drawn comparisons with vagal intramuscular arrays (IMAs), although differences in terminal morphology and orientation were noted[26]. There is a paucity of functional correlation studies with intramuscular afferents, and it is unknown whether the different branching patterns of intramuscular afferents confer unique functional capabilities or reflect differing capacities to detect and integrate sensory input. In one study, IMA-like endings in the guinea pig internal anal sphincter were reported to correlate with low-threshold slowly adapting mechanoreceptors[49]. In addition, Wolfson et al. identified TH$^+$ colonic spinal afferents had IMA-like endings or IGVEs and this neuronal class behaved similar to low-threshold slowly adapting mechanoreceptors. However, it is unclear whether TH$^+$ afferents detect stretch from IMAs, IGVEs or both[37]. See Table 2 for a summary of studies that have identified spinal afferent intramuscular endings.

Vascular afferents

The fine varicose nerve endings of the gut vasculature are well characterized, correlating with mid-high threshold mechanoreceptors[17], and likely represent the so-called serosal and mesenteric functional afferent classifications that have been described in electrophysiological studies[4]. In mice, vascular afferents represented ~9% of T8–12 gastric endings and ~5% of lumbosacral colorectal endings[25,27]. Vascular afferents innervate mesenteric arteries, following them through to submucosal arterioles. Thus, individual afferents can transduce sensory stimuli from locations inside and outside the gut wall[17] and are sensitive to gut distension and strong contractions[17], as well as distortion of the mesenteric vasculature[50]. Vascular afferents express CGRP, conferring an effector role as a vasodilator[51], which can be activated by gut distension[52]. Vascular afferents might also act on gut enteric nervous system motor circuits[50], suggesting they co-innervate enteric ganglia. Thus, some IGVE-like endings might be collaterals of vascular afferents. However, co-innervation was not observed in rat stomach[53] and this question remains unresolved. See elsewhere for further review of vascular afferents[4] and see Table 2 for a summary of studies that have identified spinal vascular afferents.

Submucosal endings

Although not present in the stomach[27], a substantial proportion (~32%) of lumbosacral spinal afferent endings innervate the mouse colonic submucosa[25] (Table 2). About one-third of these are 'simple' submucosal afferents, comprising bare axons, conspicuous by their absence of prominent varicosities, that weave around colonic crypts and lack specialized terminals (for example, see Fig. 10 in ref. 25). The tendency to weave around and encircle crypts resembles a much simplified type of vagal mucosal crypt afferents (discussed subsequently)[54]. Most simple

Table 1 | Summary of the spinal afferent characteristics described by Wolfson et al.[37]

Characteristics	Key feature	Genetic subtype				
		TrkB	TH	Bmpr1b	Sstr2	Adra2a
Expression profile	Calca	–	–	+	+	+
	TrpV1	–	–	–	+	+
	Piezo2	+	+	+	Low	–
	TrpA1	–	–	–	–	+
	NEFH	+	–	+	–	–
Neuroanatomy	IGVE	++++	+	+	IN	+
	Intramuscular	–	+	–	–	–
	SMP	–	–	–	+	+
	Mucosa	–	–	–	+	–
	NCB size	Large	Small	Large	Small	Small
Physiology[a]	Threshold	Low	Low	High	High	ND
	Adaptation	Rapid	Slow	Slow	Slow	ND
	Profile across noxious range	Saturating	Saturating	Encode	Encode	ND
	Peak firing (Hz)	~70	~300	~500	~100	ND
	Fibre type	Aδ	C	Aδ	C	ND
	AP waveform	Narrow	Wide	Narrow	Wide	ND
Optogenetic activation[b]	Pain response magnitude	None	Low	High	Mod	ND
	Evoked pain behaviours	None	PD	Voc/PD/mov	Voc/PD	ND
Putative role	–	Physiological	Physiological	Pain	Pain	ND

+and – denote expression and lack of expression, respectively. ++++ denotes the more extensive ramification and myenteric nerve cell bodies engulfed by TrkB IGVEs compared with the other genetic subtypes described by Wolfson et al.[37]. –, not applicable; Adra2a, adrenoceptor alpha 2a; AP, action potential; Bmpr1b, bone morphogenetic protein receptor type 1B; IGVE, intraganglionic varicose ending; IN, internodal; mov, inhibition of movement; NCB, neuron cell body; ND, no data; NEFH, neurofilament heavy polypeptide; PD, pupil dilation; SMP, submucosal plexus; Sstr2, somatostatin receptor 2TH, tyrosine hydroxylase; TrkB, tyrosine receptor kinase B; Voc, vocalization; [a]Distention response profile and electrophysiology. [b]Upon optogenetic activation.

Table 2 | Identified spinal sensory nerve endings

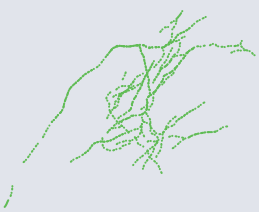
Tissue layer	Ending type	Morphology	Putative functions	Gastrointestinal location or other organs	Method (model species)	DRG	Gene/protein markers	Ref.
Myenteric plexus	IGVE		Pain[37], regulating gut motility[43]	Distal colon, rectum	DRG tracing (Ms)	L6–S1	CGRP$^+$(66%)	25
				Distal colon	SGL (Ms)	–	TrkB, TH, Bmpr1b, Adra2a	37
				Stomach	DRG tracing (Ms)	T8–T12	CGRP$^+$(>90%)	27
				Stomach, duodenum, jejunum, ileum, proximal colon, distal colon	IVT (Ms)	–	Piezo2	43
				Stomach	DRG tracing (rat)	T7–T11	–	26
	Internodal		Pain[37]	Distal colon, rectum	DRG tracing (Ms)	L6–S1	CGRP$^+$(100%)	25
				Distal colon	SGL (Ms)	–	Sstr2	37
	rIGLE		Low-threshold mechanoreceptors[16] speculated role in defecation and sensation of fullness[4,16]	Distal colon, rectum	DRG tracing (Ms)	L6–S1	CGRP (100%, myenteric)	25
				Rectum	BT+electrophysiology (GP)	–	–	16
				Rectum	BT (GP)		VGluT1 (~30%), VGluT2 (~30%)	47
Circular muscle	Intramuscular		–	Distal colon, rectum	DRG tracing (Ms)	L6–S1	CGRP$^+$ varying by morphology	25
				Stomach	DRG tracing (Ms)	T8–T12	CGRP$^+$(100%)	27
				Distal colon	SGL (Ms)	–	TH	37
				Stomach	DRG tracing (rat)	T7–T11	–	26
				IAS	BT+electrophysiology (GP)	–	–	49
Blood vessels	Vascular		Medium–high threshold mechanoreceptors with speculated role in pain[4,17]	Distal colon, rectum	DRG tracing (Ms)	L6–S1	CGRP$^+$(100%)	25
				Stomach	DRG tracing (Ms)	T8–T12	CGRP$^+$(100%)	27
				Stomach	DRG tracing (Rat)	T7–T11		26
				Ileum, distal colon, mesentery, bladder	BT+electrophysiology (GP)	–	–	17
Submucosa	Submucosal		–	Distal colon, rectum	DRG tracing (Ms)	L6–S1	CGRP$^+$ varying by morphology	25

Table 2 (continued) | Identified spinal sensory nerve endings

Tissue layer	Ending type	Morphology	Putative functions	Gastrointestinal location or other organs	Method (model species)	DRG	Gene/protein markers	Ref.
Mucosa	Mucosal		Speculated role detecting luminal content and mucosal shear, contributing to sense of defecatory urge[165]	Distal colon, rectum	DRG tracing (Ms)	L6–S1	CGRP$^+$(93%)	25
				Stomach	DRG tracing (Ms)	T8–T12	CGRP$^+$(100%)	27
				Duodenum, jejunum, Ileum, caecum, distal colon	IGL (Ms)	–	NaV1.8$^+$/Wnt1$^+$	48

A full version of this table is available as Supplementary Table 1. Morphology of nerve endings based on authors' observations of own data. – indicates a lack of testing or specific reporting. Adra2a, adrenoceptor alpha 2a; Bmpr1b, bone morphogenetic protein receptor type 1B; BT, bulk tracing; CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglia; GP, guinea pig; IAS, internal anal sphincter; IGL, intersectional genetic labelling; IGVE, intraganglionic varicose ending; IVT, intrathecal viral transduction; Ms, mouse; Piezo2, piezo-type mechanosensitive ion channel component 2; rIGLE, intraganglionic lamellar ending in rectum; SGL, sparse genetic labelling; Sstr2, somatostatin receptor 2; TH, tyrosine hydroxylase; TrKB, tyrosine receptor kinase B; VGluT1, vesicular glutamate transporter 1; VGluT2, vesicular glutamate transporter 2.

submucosal afferents contain CGRP[25] and TRPV1 (ref. 36). The remaining two-thirds of submucosal afferents lack CGRP and are more highly ramifying, termed 'branching' and 'complex' submucosal afferents. Despite their abundance, functional studies of identified submucosal endings are lacking. Submucosal endings were also identified among the Sstr2$^+$ and Adra2a$^+$ spinal afferent populations studied by Wolfson et al. (Table 1). Sstr2$^+$ afferents showed high threshold stretch sensitivity, but whether this feature is mediated by submucosal endings or other types of Sstr2$^+$ endings (Table 1) remains to be shown. Notably, both Sstr2 and Adra2a were expressed across multiple classes defined by Hockley et al., possibly explaining the occurrence of multiple ending types within those populations.

Mucosal endings

Mucosal endings in the stomach and colon comprised 16% and 11% of spinal afferent endings (from L6–S1 and T8–12 DRG, respectively), with the vast majority containing CGRP. Mucosal afferents do not reach the gut lumen but terminate adjacent to the mucosal epithelial cell border[25,27]. Microbial, inflammatory and nutritional signals in the gut lumen can be transmitted through this neuroepithelial interface, discussed together with vagal mucosal afferents later.

In the stomach, spinal afferents comprised less than half the number of mucosal endings supplied by vagal afferents[48]. Different genetic reporter approaches show general concurrence that spinal afferents provide the minority of small intestinal mucosal innervation (approximately <25% of the total afferent supply) relative to vagal afferents[35,48]. Detailed analysis by Serlin and Fox[35] further discriminated villus and crypt innervation, finding the supply of both types of spinal mucosal afferents is largely constant along the small intestine, contrasting with an overt and decreasing proximo-distal gradient of vagal mucosal afferents, which was most pronounced among the villus afferents. A strong reversal of the preponderance of vagal mucosal afferents occurs in the colon, where most mucosal afferents are spinal and vagal afferents supply ~10% of the level of spinal innervation[48]. See Table 2 for a summary of studies that have identified spinal afferent mucosal endings.

Multi-ending afferents

Earlier vagal afferent tracing from nodose ganglia to rat stomach revealed single afferents possessing both intraganglionic and intramuscular ending structures[22,30]. The functional implications of vagal multi-ending afferents remain unknown[55]. A similar situation has become apparent among spinal afferents, after sparse anterograde labelling generated by small volume tracer injections in DRG made single afferent tracing possible[28,29].

To date, three different types of multi-ending spinal afferents have been observed arising from lumbosacral DRG to the mouse colon[28,29]. These include myenteric-muscular afferents, comprising both intramuscular endings and myenteric IGVEs[28] (Fig. 3). Notably, Ma et al. also report a 'mixed-type' spinal afferent in the rat stomach, including an IGVE-like ganglionic component and branching intramuscular endings.

There are also CGRP$^+$ myenteric–muscular–submucosal afferents that have IGVEs, combined with intramuscular and complex-type submucosal endings[29]. A third type of spinal afferent is myenteric–submucosal–mucosal neurons that lack CGRP (Fig. 4), which feature IGVEs combined with branching-type submucosal endings and simple-type mucosal endings[29].

The occurrence of single spinal afferent neurons with multiple different morphological types of endings is likely underappreciated owing to the inherent difficulty of axon tracing, and the current data are unlikely to represent the full array of afferent types. We speculate that the assortment of endings possessed by an afferent might contribute to modularity of sensory capabilities and explain the difference between well-known functional classes identified in electrophysiological studies (for example, see ref. 56) such as muscular units, which are sensitive to stretch, and muscular-mucosal units, which are sensitive to both stretch and mucosal stoking. An additional and non-mutually exclusive possibility is that multiple endings enable local effector functions.

Vagal afferent nerve endings

Features

Intraganglionic laminar endings. Vagal afferent neurons provide a rich sensory innervation to the upper gut, including the oesophagus, stomach, small intestine and to a lesser extent, parts of the colon[57]. Vagal IGLEs are the most distinguishable afferent structures in the gut and one of the most extensively characterized (Table 3). They comprise flattened leaf-like endings that ramify within the myenteric ganglia. Vagal IGLEs were first identified in dog oesophagus[58] and then more comprehensively characterized in monkeys and cats[59,60], rats[21], guinea pigs[14] and mice[22,61]. They are distributed along most of the gastrointestinal tract, from oesophagus to distal colon. Within and

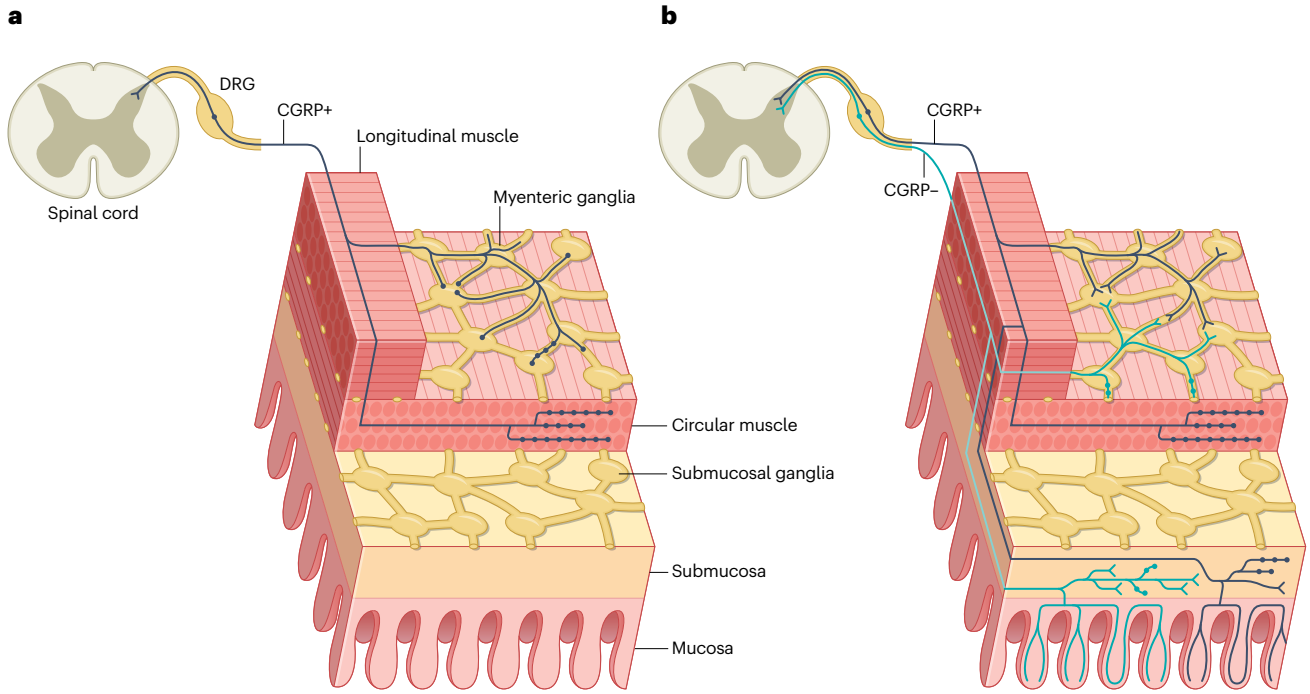


Fig. 3 | Myenteric–mucosal and multi-ending spinal afferents. Schematic diagram of a multi-ending myenteric–mucosal spinal afferent neuron (part **a**) and myenteric–muscular–submucosal and myenteric–submucosal–mucosal spinal afferents (part **b**). CGRP, calcitonin gene-related peptide; DRG, dorsal root ganglia. Part **a** adapted from ref. 28, Springer Nature Limited. Part **b** adapted with permission from ref. 29, Wiley.

across gut organs, vagal IGLE density generally follows a proximodistal gradient favouring higher densities in the oesophagus, stomach and upper small intestine[57,62,63].

Different gut regions are preferentially populated by genetically distinct vagal IGLEs[40]. In mice, most oesophageal IGLEs are Prox2$^+$/Glp1r$^-$, gastric IGLEs Prox2$^+$/Glp1r$^+$ and intestinal IGLEs Prox2$^-$/Oxtr$^+$ (refs. 38,39,42). Piezo2 was more common in oesophageal and gastric IGLEs than those of intestine and colon, suggesting that similar nerve ending structures might use different mechanosensors in different gut regions[40]. Trophic factor interactions also seem to be regionally specific, as survival and abundance of intestinal but not gastric IGLEs were enhanced by neurotrophin-4 (NT-4)[64] and suppressed by brain-derived neurotrophic factor[65], whereas oesophageal IGLEs also show neurotrophin-3 dependence[66]. In rats, most intestinal but not oesophageal or gastric IGLEs are capsaicin-sensitive[67], compatible with regional Trpv1 mRNA expression patterns identified in mouse IGLEs[40].

Oesophageal and gastric IGLEs in guinea pig were the first gut afferent structures identified by neuronal tracing following transduction site mapping, and they corresponded to low-threshold, slowly adapting tension receptors[14,41] as hypothesized[21]. They are functionally similar in mice, but a small subset might be rapidly adapting[42]. The genes expressed by putative IGLEs include several mechanotransduction-associated ion channels, such as Piezo2, Asic1, Asic2 and Kcnk2 (refs. 42,68).

Compatible with observed abnormalities of low-threshold tension receptors in oesophageal dysmotility[69], it was demonstrated that ablation of oesophageal and gastric IGLEs leads to severely disrupted oesophageal transit[42]. Although disrupted oesophageal transit could represent a downstream, indirect consequence of vagal IGLE ablation, the finding is consistent with their long-suspected role in regulating peristalsis[70,71]. Similarly, other gastric IGLE populations are hypothesized to regulate gastric motility[42].

The intestinal vagal IGLEs are implicated in satiation, as meal sizes are decreased or increased by modulating the abundance of intestinal IGLEs through NT-4 over-expression (more IGLEs[72]) and under-expression (fewer IGLEs[65]), respectively. Accordingly, short-term feeding was potently inhibited by acute chemogenetic or optogenetic stimulation of Prox2$^-$/Oxtr$^+$ IGLEs, whereas activation of the predominantly gastric Glp1r$^+$ IGLEs also evoked a major but less pronounced feeding inhibition[39,48,73]. Importantly, long-term feeding behaviour is altered to compensate for IGLE-associated changes in meal sizes[65,72], suggesting the role of IGLEs in satiation is principally short term and separate from mechanisms underlying long-term maintenance of food intake and body weight. Indeed, this process might be the case for the entire vagal innervation of the gut as vagotomy or deafferentation in mice had little effect on long-term food intake, despite alterations in meal structure[74]. Paradoxically, vagotomy in rodents and humans can cause decreases, rather than increases in food intake[75,76]; an effect possibly mediated by hyperexcitability of central vagal circuits after vagotomy, referred to as 'phantom satiation'[77].

Intramuscular arrays. Vagal IMAs are intramuscular endings featuring multiple linked and parallel varicose nerve fibres that run together with longitudinal muscle or circular muscle[22,30,57]. IMAs associate with intramuscular interstitial cells of Cajal, with which they run in parallel, form appositions and require for development[78,79]. Some IMAs issue

extensive collateral endings into myenteric ganglia, forming apparent contacts with enteric neurons[31]. The distribution of IMAs along the gut is focused, occurring most prominently in the lower oesophageal sphincter, gastric fundus, distal antrum, pylorus and restricted locations in duodenum and colon[22,31,57] (Table 3). The IMA-dense stomach regions are noted as more likely to show dissociation between gut wall tension and length[31], forming in part the basis of speculation that vagal IMAs are length receptors that complement tension-sensitive IGLEs. In line with this finding, Zhao et al.[40] obtained the first functional data from putative IMAs, finding genetically identified IMAs showed sustained activation to gut distension and express Piezo2. Neurogenetic manipulation of IMAs to probe their broader physiological role remains limited[39].

Specialized antral IMAs form web-like endings adjacent to the gastric sling muscles[31]. Described as a honeycombed network of lamellar neurites located between the serosa and longitudinal muscle, they were initially considered a distinct type of vagal ending[80,81] but subsequently identified as a morphological variation of longitudinal IMAs that correlates with location along the antrum[31]. No functional data from identified web-like endings are yet available.

Mucosal endings. Vagal mucosal innervation is not as well characterized as the endings in the muscularis externa. Two general types, vagal villus and crypt endings, have been described in the rodent small intestine[35,54,82]. Villus endings occur within the lamina propria of villi and, similar to spinal mucosal endings, can reach the basal lamina without protruding between epithelial cells to the lumen[54]. Among vagal villus endings, three morphological subtypes were identified in the small intestine, which were classified as simple, branched and spiral-type endings[83]. Additionally, 'light bulb-like' and 'umbrella-like' endings in villi were also described in mouse duodenum, as well as rare endings that bridge conjoined villi[35]. Mucosal vagal afferent endings are rare in the colon compared with those supplied by spinal afferents[48]. They show the simple-type morphology, but also complex, and lamellar[84]. Vagal crypt endings in small intestine issue multiple collateral axons from the base of a mucosal crypt that spiral up to encircle the crypt neck and those of several adjacent crypts[54]. In gastric antrum, distinct vagal endings form 'bushy' terminal arbors of varicose endings along the epithelial walls of the gastric glands[54]. Viral anterograde labelling from the nodose/jugular complex in mice revealed oesophageal mucosal afferents comprising varicose, longitudinally oriented and highly branching axons located beneath the epithelium[85]. Networks of longitudinally oriented vagal mucosal afferents also occur in rats, densely innervating the uppermost oesophagus and, to a lesser extent, the lowermost oesophagus, with relatively sparse innervation of the oesophageal body[21,86,87]. The upper oesophagus might have special importance for protective reflexes and its large mucosal afferent supply arises from not just nodose but also the jugular-petrosal ganglia, which have a distinct embryonic origin common with DRG neurons[87].

Vagal mucosal afferents have been genetically identified along the gut[35,40,48] (Table 3). Gpr65$^+$ vagal afferents target gastric, intestinal and colonic mucosa, most densely innervating villi of duodenum and jejunum[35,38,48] and they are distension-insensitive[38]. Despite apparent sensitivity to intraluminal food[38], acute activation or inactivation of Gpr65$^+$ vagal afferents does not affect food intake[39,48], but can alter hepatic glucose production, suggesting a gluocoregulatory role[48]. The Gpr65$^+$ vagal afferents might not represent a single type, nor the only vagal mucosal afferents[39,40,48]. SSt$^+$ and Calca$^+$ vagal afferents also express Gpr65$^+$ but comprise non-overlapping populations that target gastric mucosal villi in distinct regions: the pyloric antrum and lesser curvature region of the corpus, respectively[39]. Another population of vagal intestinal afferents that are distinct from Gpr65$^+$ afferents expresses both Vip and Uts2b and innervates the intestinal mucosal villi[39]. Unlike Gpr65$^+$ afferents, these mucosal afferents were activated by duodenal stretch[40] and therefore might represent the 'muscular–mucosal' or 'tension–mucosal' functional class of vagal afferents described in electrophysiological studies[88,89]. Despite functional differences, the Gpr65$^+$ and Vip/Uts2b$^+$ mucosal afferents were described as morphologically indistinguishable, suggesting an uncoupling of nerve ending morphological class from sensory modality[40]. As with Gpr65$^+$ vagal afferents, activation of Vip/Uts2b vagal afferents did not acutely alter food intake[39], but they might act a generalist gut nutrient sensor (responding to dietary fat, sugar and amino acids) that acts in parallel with Trpa1-expressing vagal afferents that respond specifically to dietary fat, both of which were required for development of fat-containing food preference; a process independent from taste perception of fat[90]. However, this finding requires further clarification and confirmation of the specific afferent types involved, as later studies have suggested vagal afferent pathways that underlie motivated behaviours are nutrient-specific[91].

Extent of vagal afferent innervation along the gastrointestinal tract. Anterograde labelling from nodose ganglia has consistently revealed a weak or absent vagal afferent innervation in the terminal gastrointestinal tract – distal colon and rectum. Early studies of Wang and Powley[57] and Berthoud et al.[92] demonstrated that there is a gradient in vagal afferent endings in the gastrointestinal tract with many more endings rostrally (in oesophagus and stomach) and a progressive decline towards the colon (see Figs. 13 and 19 in ref. 57 as examples). In rats, Berthoud et al.[92] performed anterograde labelling from nodose ganglia and found IGLEs in the ascending and transverse colon but did not report any IGLEs in the descending (distal colon). These findings are similar to those reported by Spencer et al.[84] in mouse colon, in

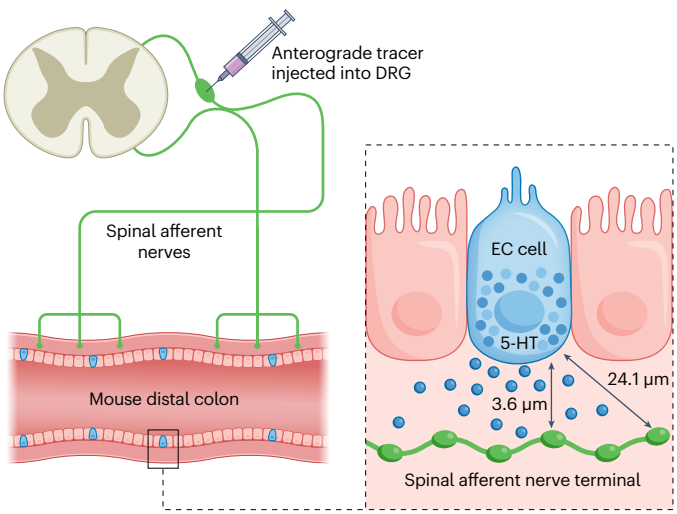


Fig. 4 | Mucosal paracrine signalling at nerve terminals. Depiction of mucosal paracrine signalling, showing an epithelial cell and basolateral mucosal nerve terminals. At present, the bulk of evidence indicates the primary mode of transmission from enteroendocrine cells to afferent neurons, which is paracrine rather than synaptic. DRG, dorsal root ganglia; EC, enterochromaffin; 5-HT, 5-hydroxytryptamine.

Table 3 | Identified vagal sensory nerve endings

Tissue layer	Ending type	Morphology	Putative functions	Gastrointestinal location or other region	Method (species)	Gene/protein markers	Ref.
Myenteric plexus	IGLE		Tension-sensitive mechanoreceptors[14], intestinal IGLEs might be polymodal[40]; implicated in short-term satiation (intestinal and gastric)[39] and regulation of oesophageal peristalsis[42]	Oesophagus, cardia	NG TI (rat)	–	21
				Fundus	NG TI (rat)	–	30
				Pylorus	NG TI (rat)	–	217
				Fundus	NG TI (rat)	–	218
				Pylorus	NG TI (rat)	–	82
				Fundus, corpus, antrus, duodenum, jejunum, ileum, carcum proximal colon, mid colon	NG TI (rat)	–	92
				Oesophagus, fundus, corpus	NG TI (rat)	–	67
				Fundus, corpus, antrum, duodenum, caecum	NG TI (rat)	–	219
				Oesophagus, fundus, corpus	NG TI (rat)	–	63
				LES, fundus, corpus, antrum, pylorus, duodenum	NG TI (Ms)	–	22
				LES, cardia, fundus, corpus, antrum, pylorus, duodenum, jejunum, ileum, caecum (ileocaecal junction), proximal colon, mid colon, distal colon	NG TI (rat)	–	57
				Fundus, corpus, antrum, duodenum, ileum	NG TI (Ms)	–	64
				Fundus, corpus, antrum	NG TI (Ms)	–	78
				Fundus, corpus, antrum	NG TI (Ms)	–	79
				Oesophagus	NG TI (Ms)	–	66
				Oesophagus	NG TI (Ms)	–	62
				LES, cardia	NG TI (rat)	–	80
				Antrum	NG TI (rat)	–	81
				Cardia, duodenum	NG VT (Ms)	$Glp1r^+$	38
				Fundus, corpus, antrum	NG VT (Ms)	$Glp1r^+$	39
				Duodenum, jejunum, ileum, proximal colon, mid colon, distal colon, rectum	NG VT (Ms)	$Oxtr^+$	39
				Oesophagus	NG + JG VT (Ms)	–	85
				Oesophagus, LES, fundus, corpus, antrum, pylorus, duodenum, mid colon	NG VT (Ms)	$VGluT2^+$	40
				Cardia	NG VT (Ms)	$Glp1r^+$	40
				Cardia, duodenum, mid colon	NG VT (Ms)	$Agtr1a^+$	40
				Cardia	NG VT (Ms)	$Piezo2^+$	40
				Oesophagus	NG VT (Ms)	Nts^+	40
				Mid colon	NG VT (Ms)	$Trpv1^+$	40
				Cardia, duodenum	NG VT (Ms)	$Drd2^+$	40
				Oesophagus	IGL (Ms)	$Prox2^+/Glp1r^-$	42
				Fundus	IGL (Ms)	$Prox2^+/Glp1r^+$	42
				Antrum	IGL (Ms)	$Runx3^+/Glp1r^+$	42
				Oesophagus	BT+electrophysiology (GP)	–	14
				Cardia	BT+electrophysiology (GP)	–	41
				Oesophagus	NG VT (GP)	–	220

Table 3 (continued) | Identified vagal sensory nerve endings

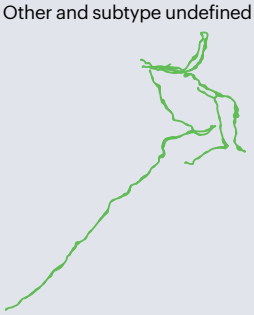
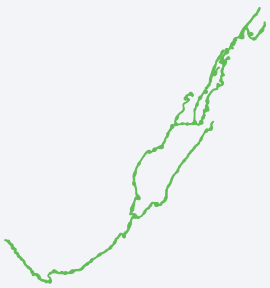
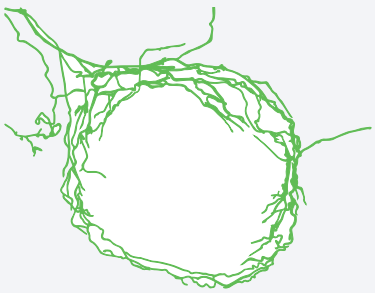
Tissue layer	Ending type	Morphology	Putative functions	Gastrointestinal location or other region	Method (species)	Gene/protein markers	Ref.
Circular and longitudinal muscle	IMA		Stretch receptors[40], broader function unresolved, anatomical distributions in stomach and around sphincters suggest potential role controlling local muscle activities[31,57,71]	Fundus	NG TI (rat)	–	30
				Oesophagus, fundus, corpus	NG TI (rat)	–	67
				Fundus, pylorus	NG TI (rat)	–	219
				LES, fundus	NG TI (rat)	–	63
				Fundus	NG TI (rat)	–	221
				LES, cardia, fundus, corpus, antrum, pylorus, duodenum	NG TI (Ms)	–	22
				LES, cardia, fundus, corpus, antrum, pylorus, duodenum, jejunum, ileum, caecum, proximal colon, mid colon, distal colon	NG TI (rat)	–	57
				Fundus, corpus, antrum	NG TI (Ms)	–	78
				Fundus, corpus, antrum	NG TI (Ms)	–	79
				Antrum	NG TI (rat)	–	81
				LES, cardia, antrum, pylorus	NG TI (rat)	–	80
				Pylorus	NG TI (rat)	–	222
				Fundus, corpus, antrum	NG TI (rat)	–	31
				LES, cardia, antrum	NG VT (Ms)	VGluT2$^+$	39
				Oesophagus	NG + JG VT (Ms)	–	85
				LES, fundus, corpus, antrum, pylorus, mid colon	NG VT (Ms)	VGluT2$^+$	40
				LES, fundus, corpus, antrum, pylorus, mid colon	NG VT (Ms)	VGluT2$^+$α	40
				Oesophagus	NG VT (Ms)	VGluT2$^+$β	40
				Oesophagus	NG VT (Ms)	Piezo2$^+$	40
				Cardia	NG VT (Ms)	Vip$^+$	40
				Oesophagus, cardia, mid colon	NG VT (Ms)	Trpv1$^+$	40
				LES	NG VT (Ms)	P2ry1$^+$	40
				LES	NG VT (Ms)	Calb2$^+$α	40
				Oesophagus, mid colon	NG VT (Ms)	Drd2$^+$	40
Mucosa	Other and subtype undefined		–	Oesophagus	NG TI (rat)	–	21
				Pylorus	NG TI (rat)	–	217
				Duodenum, jejunum, ileum	NG TI (rat)	–	114
				Jejunum	NG TI (rat)	–	223
				Oesophagus	NG + JG VT (Ms)	–	85
				Oesophagus, LES, fundus, corpus, antrum, pylorus, duodenum, mid colon	NG VT (Ms)	VGluT2$^+$	40
				Oesophagus, cardia, duodenum	NG VT (Ms)	Gpr65$^+$	40
				Antrum	NG VT (Ms)	Sst$^+$	40
				Oesophagus, LES, mid colon	NG VT (Ms)	Trpv1$^+$	40
				Cardia	NG VT (Ms)	Glp1r$^+$	40
				Oesophagus, cardia	NG VT (Ms)	Agtr1a$^+$	40
				Duodenum	NG VT (Ms)	Vip$^+$	40
				Oesophagus, cardia	NG VT (Ms)	P2ry1$^+$	40
				Oesophagus, cardia, mid colon	NG VT (Ms)	Drd2$^+$	40

Table 3 (continued) | Identified vagal sensory nerve endings

Tissue layer	Ending type	Morphology	Putative functions	Gastrointestinal location or other region	Method (species)	Gene/protein markers	Ref.
Mucosa (continued)	Villus		Gpr65$^+$ subtype — nutrient[38] or osmolarity sensors[90] involved in acute regulation of gastric motility[38]	Duodenum	NG TI (rat)	–	82
				Proximal colon, mid colon, distal colon	NG TI (rat)	–	57
				Duodenum	NG TI (rat and Ms)	–	54
				Duodenum, jejunum, ileum	TR±vagotomy (Ms)	NaV1.8$^+$	35
				Proximal colon, mid colon	NG TI (Ms)	–	84
				Corpus, antrum, duodenum, jejunum, ileum	NG VT (Ms)	VGluT2$^+$	39
				Duodenum, jejunum, ileum	NG VT (Ms)	Gpr65$^+$	38
				Corpus, antrum, duodenum, jejunum, ileum	NG VT (Ms)	Gpr65$^+$	39
				Duodenum, jejunum, ileum	NG VT (Ms)	Vip$^+$/Uts2b$^+$	39
				Duodenum, jejunum, ileum	NG VT (Ms)	Glp1r$^+$	39
				Duodenum	NG VT (Ms)	Sst$^+$	39
				Fundus, corpus, antrum, duodenum, jejunum, ileum, mid colon	IGL (Ms)	NaV1.8$^+$/ Gpr65$^+$	48
	Crypt		–	Duodenum	NG TI (rat)	–	82
				Duodenum	NG TI (rat and Ms)	–	54
				Duodenum, jejunum, ileum, mid colon, rectum	NG VT (Ms)	VGluT2$^+$	39
				Duodenum, jejunum, ileum	TR±vagotomy (Ms)	NaV1.8$^+$	35

A full version of this table is available as Supplementary Table 2; regions with higher and lower densities (when reported) are indicated on Supplementary Table 2. Morphology of nerve endings based on authors' observations of own data unless stated otherwise. Zhao et al.[40] report % area innervated by IMAs and mucosal endings rather than density. α, 'cIMAs' described as 'irregular muscular endings with circular parent neurites'; β, 'oesophageal IMAs'. – indicates a lack of testing or specific reporting. Agtr1a, angiotensin II receptor type 1; BT, bulk tracing; Calb2, calbindin 2; Drd2, dopamine D2 receptor; GL, genetic labelling; Glp1r, glucagon-like peptide 1 receptor; GP, guinea pig; Gpr65, G protein-coupled receptor 65; IGL, intersectional genetic labelling; IGLE, intraganglionic laminar ending; IMA, intramuscular array; JG, jugular ganglion; LES, lower oesophageal sphincter; Ms, mouse; NaV1.8, voltage-gated sodium ion channel 1.8; NG, nodose ganglion; Nts, neurotensin; Oxtr, oxytocin receptor; P2ry1, purinergic receptor P2Y1; Piezo2, piezo-type mechanosensitive ion channel component 2; Prox2, prospero homeobox 2; Runx3, runt-related transcription factor 3; Sst, somatostatin; TI, tracer injection; Trpv1, transient receptor potential cation channel subfamily V member 1; Uts2b, urotensin 2B; VGluT2, vesicular glutamate transporter 2; Vip, vasoactive intestinal polypeptide; VT, viral tracing. IMA ending type morphology adapted with permission from ref. 224, Elsevier. Mucosal villus morphology adapted with permission from ref. 225, Elsevier. Mucosal crypt morphology adapted with permission from ref. 54, Wiley.

which anterograde tracing studies from nodose ganglia were found to generate sparse labelling in the proximal and sometimes mid colon, but never the distal 30 mm of large bowel.

Interestingly, three independent laboratories recently suggested that vagal afferents provide a rich sensory innervation to the distal colon of mice[93–95]. Technical issues could underlie the different observations in antegrade and retrograde tracing studies. Both techniques are subject to limitations. Anterograde tracing requires that sufficient time is allowed for tracer to reach nerve terminals while tissue is fixed before clearance of the tracer[21] or, in ex vivo preparations, signs of nerve degeneration[96]. The assumption with retrograde tracing studies is that the injected tracer does not leak out of the gut from the injection site and, second the tracer does not spread within the gut wall, remaining spatially restricted at the injection site. Our studies[97] have shown this aspect is not true for cholera toxin-B (CTB), suggesting extreme caution when interpreting the results of injecting even small volumes of CTB into visceral organs. For example, a recent retrograde tracing study reported that even single injections of minute quantities of CTB into

the distal colon caused extensive nonspecific labelling of large numbers of neurons in nodose ganglia[97]. Notwithstanding, distal colon vagal afferents were identified by retrograde tracing with efforts to control for potential spread of tracer[94]. Although colonic distension could activate neurons in the nucleus of the tractus solitarius (NTS)[95], it is unclear whether this finding is due to direct distal colonic innervation by vagal afferents and/or secondary activation of other NTS-projecting cranial nerves (for example, 5: trigeminal, 7: facial and 9: glossopharyngeal), as colon distension in conscious mice induces multiple behavioural changes including pupil dilation, mobility, writhing, facial grimace and vocalization[37].

Transgenic reporter mice might help resolve these discrepant observations of distal colonic vagal afferents associated with neuronal tracers and functional studies. Borgmann et al. noted: 'innervation density of PHOX2B vagal afferents decreased beyond the ileum' and 'significantly fewer endings in muscular layers and sparse innervation of crypts' were detected[48]. In fact, the spinal afferent 'innervation of colon crypts was ~10-fold more as compared with PHOX2B innervation', which labelled vagal afferents. Using the Wnt1-cre line to label spinal afferents, the authors noted: 'innervation of the stomach and small intestine was … sparser, as compared with vagal afferent innervation'[48]. These results would favour the long-held view that vagal innervation of the distal colon is sparse, but further studies are needed.

Spinal afferents encode noxious and non-noxious stimuli

Until relatively recently it had been taught that spinal afferent neurons functionally encode largely or exclusively painful sensations, with little emphasis on potential to encode non-nociceptive stimuli. This notion has changed substantially in recent. For example, compelling evidence shows spinal afferents have a role in regulating steady-state feeding responses[48], and signal the presence of ingested macronutrients from the upper small intestine and hepatic portal vein[98], including detection of glucose for regulating hypothalamic neurons that control food intake[99]. From an electrophysiological standpoint, direct recordings in mice[100] and guinea pig[16] have shown that a major population of spinal afferents responds to low thresholds of mechanical stimulation, which are activated well below the noxious range. Indeed, stimuli such as noxious distension that can recruit afferents with high-distension thresholds inevitably recruit all low-threshold afferents too, which can continue to encode higher firing intensities into the noxious range. Thus, it seems tenable that nociception, at least from the distal colon and rectum, likely arises from bulk activation and recruitment of different classes of visceral afferents with different morphological and neurochemical phenotypes[101], although the relative contributions from specific neuronal classes might not be equal[37,46]. In contrast to spinal pathways, it is worth noting that vagal afferents, whose function is traditionally assumed non-nociceptive, can also encode noxious signals from oesophagus and stomach[102]. They have been shown to arise from the neural crest-derived jugular ganglion[103], and are relatively insensitive to serotonin[104], but the identity of their peripheral nerve endings and genetic classification currently remains undefined.

Spinal afferent axons in the rectal nerves are potently activated by low levels of mechanical distension and contractions of the muscle layers[16,56,105]. Various ion channels are expressed on spinal afferent endings that likely contribute to mechanosensory transduction from the lower bowel. Piezo2 channels in gut-projecting DRG neurons have a major part in sensing mechanical stimuli and ablation of Piezo2 from spinal but not vagal afferents over a large spatial distribution of spinal segments, which potently influenced transit throughout the gastrointestinal tract[43]. This study also showed that humans lacking Piezo2 have major deficits in colonic output[43]. The authors raised important questions as to whether Piezo2 in sensory endings detects the luminal contents passing through the gut or the constant gut contractions triggered by luminal contents. In a study published in 2022, L6 and S1 DRG were bilaterally removed from mice and no change in faecal pellet output was observed, but mechanical pain from the rectum was abolished[106]. This surgical procedure affects the terminal ~2 cm of sensory innervation in the colon and rectum[106], which is likely an area insufficient to affect whole gut transit.

EECs and extrinsic afferent endings

The vast majority of 5-hydroxytryptamine (5-HT) in the body is made in the gut mucosa[107], which is important because there is evidence associating endogenous 5-HT levels with various neuropsychiatric disorders such as anxiety and depression[108]. Hence, there is considerable interest in how 5-HT released from enteroendocrine cells (EECs) in the gut mucosa communicates with extrinsic primary afferent nerve endings in the gut wall. How peripherally released serotonin activates the sensory endings of spinal and vagal afferent endings in the periphery is particularly important because gut-released (peripheral) serotonin does not cross the blood–brain barrier but could contribute to anxiety-like behaviours[109].

It was proposed that some EECs might signal via synaptic contacts with mucosal afferent nerve endings[110,111]. However, although high tactile acuity in a cutaneous context has clear adaptive advantages for motor control by enabling rapid and accurate interaction with the environment, the physiological utility for the spatiotemporal precision of synaptic signalling from gut epithelial cells is unclear. Such synapses have also been questioned in the light of the relatively rapid migration and turnover of gut epithelial cells compared with the process of synapse formation[112]. There has been a paucity of key anatomical and quantitative evidence to support the idea that synapses are formed between EECs and extrinsic afferent nerve endings, in vivo[113]. Analysis of the spatial relationship between EECs and afferent nerve fibres does not rule out the existence of enteroendocrine synapses but suggests they are very rare. No close relationship was identified between rat vagal mucosal afferents and CCK-expressing EECs[114]. Moreover, nodose anterograde tracing in mice which showed vagal afferent nerve endings in the small intestine and colonic mucosa[83,84] never made close contacts with EECs, which is consistent with what is known about synaptic transmission (~10–20 nm distances). Likewise, single colonic mucosal afferents traced from DRG lacked close contacts with EECs[115]. Further studies found that apparent anatomical contacts between vagal afferents and intestinal or colonic GLP-1-secreting L-cells were 'exceptionally rare'[112] and that the mucosa lacked the common synaptic marker, PSD95 (ref. 112). Taken together, the distances between EECs and spinal or vagal afferent nerve endings in situ are typically many hundreds of times greater than those between presynpatic and postsynaptic membranes in vertebrates[116] (Fig. 5), and therefore most input from EECs to mucosal afferents is likely to be paracrine[117]. Indeed, it could be speculated that a paracrine mode of transmission is better suited than synaptic transmission to accommodate rapid cell dynamics in the mucosa and provides a divergent mode of communication to amplify signalling from sparse populations of enteroendocrine cells.

Visceral afferents and central brain function

The concept of interoception has been put forth to describe the process by which the body's internal state is detected, represented and

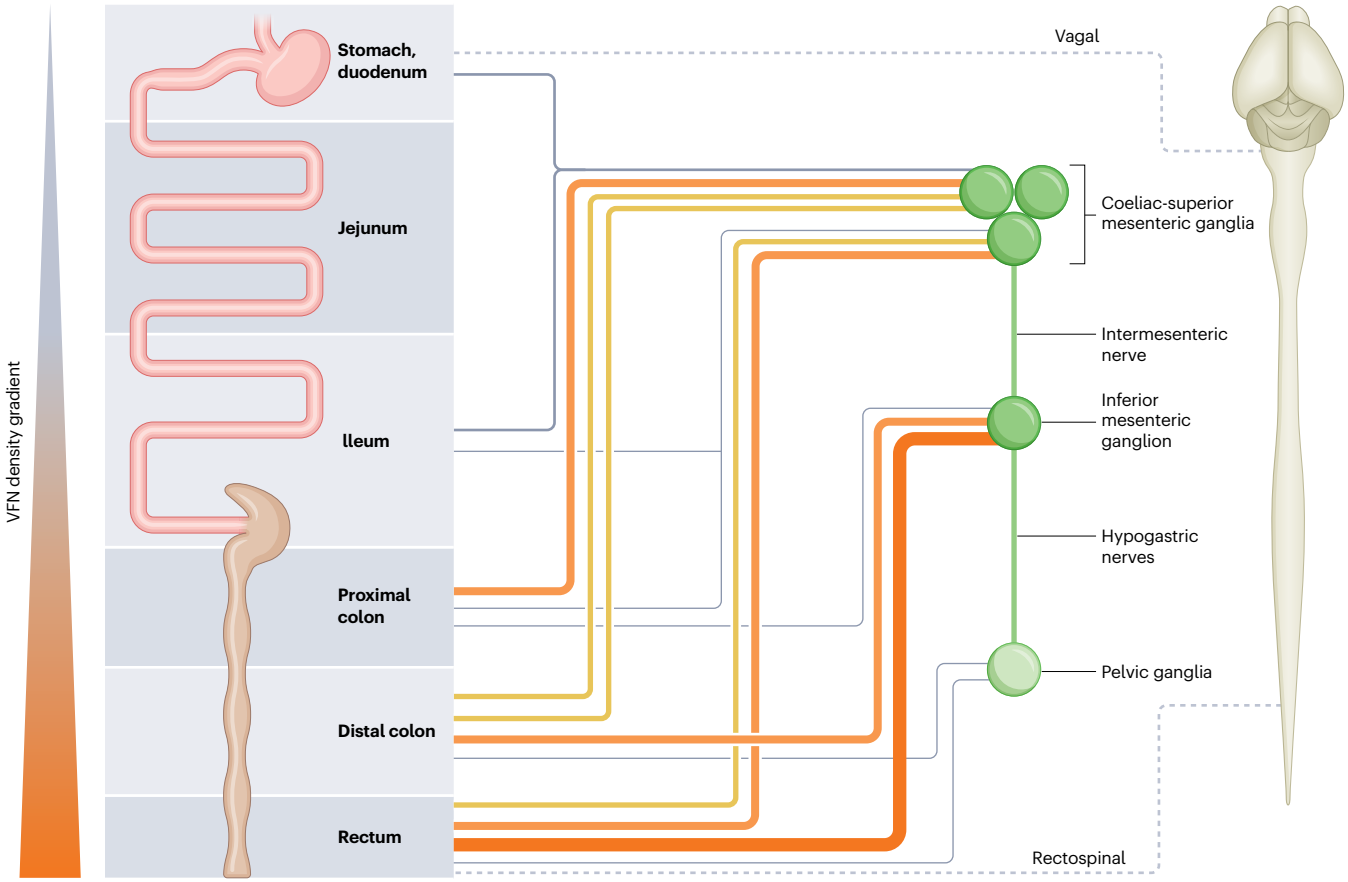


Fig. 5 | Gut viscerofugal pathways. Schematic diagram depicting the gut viscerofugal pathways. The size/colour of the links to prevertebral ganglia indicates the relative numbers of viscerofugal neurons in the pathway, as suggested by retrograde tracing studies in guinea pigs and rats[91,134,135]. The possibility of direct enteric to central nervous system (rectospinal and vagal) viscerofugal pathways has been suggested by a relatively small number of studies and is indicated by grey dotted lines, representing possible direct connections between the enteric and central nervous systems[92,93,96]. VFN, viscerofugal neuron.

integrated within the brain and its dysfunction is associated with a plethora of neurological and neuropsychological disorders[8]. Gut–brain communication, therefore, represents a critical component of interoception; although a full survey of the potential role of gut–brain axis in neurological and neuropsychiatric functions is beyond the scope of this Review, it is notable that major interest in gut–brain communication is driven by reports of its functional relevance extending to aspects of brain function as broad as emotion[118,119], motivation[120,121] and learning[122]. Gut vagal signalling has been implicated in energy restriction-induced[123,124] and microbially induced anxiolysis[125] in animals. Anxiety was reduced by vagal afferent lesion in rats[119] including chronic lesion of Cck receptor expressing vagal afferents[126], which are predominantly mucosal and intramuscular sensory neurons in the upper gastrointestinal tract[127]. By contrast, intestinal Vip/Uts2b$^+$ vagal mucosal afferents in mice co-express Cckar[13], raising the possibility that this genetic subclass contributes to modulation of anxiety. Depressive symptoms[128], and the action of antidepressants[129], have also been implicated with vagal afferent signalling and its interaction with the gut microbial environment. For more extensive review of gut–brain axis in development of psychiatric disorder and neurodegenerative disease, see elsewhere[108,130–132].

Outstanding questions

Mapping classification schemes and characterization of thoracolumbar endings. The advances in gene expression profiling, neuronal tracing and neurogenetic manipulation techniques add new ways of testing and classifying visceral afferents. While adding complexity, it can be unclear how classification schemes relate. One of the major classification schemes has been stimulus–response functional classification based on firing responses to stretch, mucosal stroking and von Frey hair probing, termed: mucosal; muscular/tension; muscular–mucosal/tension–mucosal; serosal/mesenteric; and mechanically insensitive/silent afferents[45,56,88,89,133–135]. How these afferent functional classes reconcile with new genetic classifications is currently unknown. Harnessing discriminating molecular targets of putative genetic classes identified by molecular profiling studies represents a potential pathway by which this issue could be resolved as neurogenetic manipulation techniques are amenable for combined use with established physiological recording techniques. Multimodal approaches could reveal shortcomings of classification schemes, as it had been thought that serosal and not mucosal afferents were nociceptive. Yet, neuroanatomical investigations highlight an absence of endings in the serosa, whereas some Sstr2$^+$ high-threshold afferent neurons have mucosal endings.

As noted, the most comprehensively profiled visceral afferents could be vagal IGLEs, particularly the gastric IGLEs, which were among the first to have their electrophysiological (tension-sensitive, muscular afferents) and morphological identities correlated[41]. The latest evidence links them to a genetic class, central projections and a physiological role in short-term satiety[39,40,48]. Our understanding of vagal afferents is ahead that of spinal afferents. Indeed, most peripheral endings of the thoracolumbar spinal afferent innervation of the gut remain to be characterized (particularly in mid gastrointestinal tract: small intestine, caecum and proximal colon). Characterizing both pathways is important because mechanical activation of thoracolumbar or lumbosacral afferents to the same gut region differentially activates spinal[136] and brainstem circuits[137], implying that the two pathways differentially contribute to autonomic and affective signalling among spinal afferents.

Moreover, how genetic classifications relate to anatomical–functional data of their peripheral endings is currently poorly understood among the spinal afferents versus vagal afferents. The molecular classification of DRG neurons[10–12] has been applied specifically to gut afferents, whereby Hockley et al. defined seven colonic spinal afferent classes; five that were shared between thoracolumbar and lumbosacral DRG and two populations specific to lumbosacral DRG[9]. Redundancy and shared expression of mechanosensory and chemosensory ion channels across multiple putative genetic classes led the authors to conclude that the prospect of predicting their relationship with other existing classification schemes based on molecular profile alone was limited. Nevertheless, two spinal afferent classes that expressed Nefh, termed 'mNFa' and 'mNFb', were speculated to be mucosal afferent subtypes based on expression of 5-HT receptors and peptone chemosensors[9]. Interestingly, their genetic profiles somewhat resemble the TrkB and Bmpr1b afferents, respectively, described by Wolfson et al.[37], which had IGVEs, whereas Sstr2$^+$ afferents had mucosal endings but low Nefh expression. Clearly, extensive multimodal investigations are necessary to complete the process of mapping molecular classifications to peripheral ending structures, central projections, electrophysiological properties and physiological functions.

Plasticity of sensory afferents. Vagal afferents show capacity for sensitization[138] and desensitization[139,140], including pregnancy and diet-driven changes in mechanosensitivity[141,142], as well as circadian variation in mechanosensitivity[143] and central processing[144]. In addition to central alterations[145,146], spinal afferents become chronically hypersensitive after a bout of inflammation, such as the ones that can occur in irritable bowel syndrome, which is characterized by visceral hypersensitivity[147]. Indeed, human irritable bowel syndrome is associated with increased extrinsic sensory and immune markers in the gut[148]. Likewise, human inflammatory bowel diseases show increased density of gut perivascular innervation[149], with upregulation of extrinsic sensory neurochemical markers, substance P and TRPV1 (refs. 150,151), as well as upregulation of substances capable of activating putatively nociceptive gut afferents[152]. Previously insensitive/silent afferents can acquire mechanosensitivity in response to inflammatory mediators[134] that can be released upon interaction with microbial products[153]. Moreover, the appearance of novel, previously unidentified genetically defined clusters of spinal afferent neurons following infection has been described[154]. Profound time-of-day variation in bladder spinal afferent excitability has been reported, probably reflecting physiological circadian clock gene oscillations[155]. Multiple gut functions oscillate over 24 h, as well as the relative abundances, metabolism and spatial organization of gut microbial populations[156,157]. It is likely that gut spinal afferents show functional oscillations across the day, but this aspect remains untested. Together, both long-term and short-term changes in physiological or pathophysiological status could underlie large differences in the potential for activation of primary afferent nerve endings and therefore interoception. The nature of these changes remains poorly understood, particularly in human gut.

Human extrinsic gut afferents. The first electrophysiological study of sensory nerve endings in human gut was reported by Sirotin in 1961 (ref. 158), who recorded from ex vivo stomach and small intestine. Fifty years later, several studies emerged that together identified the presence of all electrophysiological functional subclasses in human large intestine and distal small intestine[159–164], which are likely to be predominantly of spinal sensory origin and we have reviewed in detail[165]. Sirotin reported gastric and intestinal afferent responses to nutrient perfusion and this study is currently the most likely among human gut afferent studies to be based on vagal afferent recordings. Remarkably, even less is understood of the neuroanatomy of human extrinsic afferents. de Fontgalland et al.[151] first applied the rapid biotinamide tracing technique to colonic mesenteric paravascular nerve trunks, labelling branching varicose extrinsic nerve fibres along the vasculature. However, the afferent or efferent nature of these fibres is unknown. The same approach has been applied to human colonic nerves in combination with common immunohistochemical markers of sensory neurons[166], revealing ~4% of extrinsic axons contained substance P and 6% contained CGRP, whereas most axons (~34%) were presumptively sympathetic containing either TH or somatostatin. As yet, there are no detailed descriptions of the morphology of human extrinsic afferents, and even the most morphologically conspicuous afferents described in animal models, the IGLEs, are yet to be identified in human gut.

Viscerofugal neurons

Enteric VFNs are a special case in gut–brain communication. Being enteric neurons, their nerve cell bodies are located in the enteric ganglia within the gut wall. Unlike all other enteric neurons, their axons leave the gut. Extrinsic nerve trunks through which the spinal or vagal afferents and autonomic efferents project also contain axons of VFNs. Most viscerofugal axons terminate in the sympathetic prevertebral ganglia (PVG). However, there are reports of small populations of VFNs with projections as far as the spinal cord[167,168] and brainstem[169]. VFNs with prevertebral projections have been indirectly implicated in gut–brain satiety signalling through reports of a role in glucoregulation and through mediating the afferent arm of the so-called ileal brake[170]. In this section, we summarize what is known of this elusive neuronal class before describing advances in the past decade and questions that remain unresolved.

Discovery, distribution and targets

Viscerofugal neurons were implied by studies in the 1940s, showing that distension of a segment of gut acutely inhibited motility in a second gut segment, linked only through PVG[171,172]. These studies indicated that some enteric neurons left the gut wall and synapsed on prevertebral sympathetic neurons, which in turn projected back into the gut. Denervation and retrograde tracing studies later provided the structural evidence for VFNs.

The density of enteric viscerofugal nerve cell bodies with projections to the PVG has a proximodistal gradient along the gastrointestinal tract with the majority located in the large intestine. They are also preferentially positioned close to the mesenteric attachment, resulting in a circumferentially biased distribution around the gut. The exception to this finding is in the rectum, where the mesentery is more evenly distributed.

Retrograde tracing indicates that the great majority of VFNs project to prevertebral ganglia (Fig. 5). Small populations have been traced from pelvic ganglia[173] and the CNS. The latter includes VFNs in rectum[167,168] and colon[174,175], with projections via dorsal roots into the spinal cord, and VFNs that project along vagal pathways[14,41] from oesophagus, stomach and small intestine to the brainstem[169]. This aspect raises the possibility that VFNs directly connect enteric nervous system (ENS) and CNS.

Morphology and neurochemistry

Most enteric viscerofugal nerve cell bodies have shapes typical of uniaxonal neurons[176]. Thus, viscerofugal neurons resemble the most common neurons in the ENS. Although not identified in most studies, small proportions of multiaxonal VFNs have been reported[173,177,178].

Knowledge of VFN neurochemical profiles is based primarily on immunolabelling of retrogradely traced or lesioned VFNs in guinea pig models. Development of multiplexed immunolabelling in whole-mount tissue recently expanded the power of this approach[179]. It enabled testing of 14 neurochemical markers in the same VFNs in human colon, thereby in a single study making them arguably the best characterized VFN population to date. Single-cell RNA sequencing will readily surpass these data, but has yet to be applied to identified VFNs.

Among the markers tested, choline acetyltransferase is a consistently identified substance in guinea pig, rat and human VFNs, probably reflecting the importance of acetylcholine as a neurotransmitter in sympathetic ganglia. Vasoactive intestinal peptide (VIP) is a VFN marker in guinea pig, in which its role as a co-transmitter has been studied[180–183] and is useful for selective identification of VFN synapses in coeliac ganglia[184–186]. VIP also occurs in VFNs of the rat, dog and cat[187,188]. However, this prominent VFN marker is largely absent from pig[189,190] and human colonic VFNs[179]. In 2023, CART$^+$ VFNs in mouse ileum and colon were suggested to regulate blood glucose via projections to pancreas and liver-projecting sympathetic neurons[191]. As with VIP, human colonic VFNs lack CART[179]. Thus, it seems that neuropeptide transmitters in VFNs show cross-species variability, possibly indicating a less critical functional role or greater interchangeability. For extensive review of VFN neurochemistry, see elsewhere[192].

Sympathetic connections

VFN circuits are structured for regulating gut motility and secretion behaviour, as predicted by studies of intestinointestinal reflexes. The terminals of VFNs synapse with visceromotor sympathetic neurons within prevertebral ganglia, but not with vasomotor sympathetic neurons. The visceromotor sympathetic neurons receive numerous subthreshold cholinergic–nicotinic synaptic inputs from VFNs and relatively few suprathreshold inputs from preganglionic neurons. This finding suggests the importance of signal integration and the spatial and temporal summation of VFN inputs. Gut distension increases the frequency of nicotinic fast excitatory postsynaptic potentials in sympathetic neurons[193] and might also evoke slow excitatory postsynaptic potentials via VFN-released neuropeptides[182]. Visceromotor sympathetic axons densely innervate the entire gastrointestinal tract where they release noradrenaline, acting presynaptically to suppress cholinergic transmission among myenteric neurons[194] to inhibit motility and by a combination of presynaptic and postsynaptic mechanisms to inhibit enteric secretomotor neurons[195,196].

Gut volume sensors

Early studies of intestinointestinal reflexes which implied mechanical stimuli can activate VFNs. Ex vivo intracellular electrophysiological recordings from sympathetic neurons in guinea pig and mouse PVG preparations with the gut attached revealed that distension-evoked VFN firing was partly sensitive to blockade of synaptic transmission in the gut[180,197–199], implying distension activates VFNs directly by mechanotransduction, and synaptically via mechanosensitive enteric pathways that converge on VFNs. The analysis and manipulation of gut volume and pressure changes, linked to the frequency of VFN inputs, demonstrated sensitivity to gut volume in mouse and guinea pig[200,201]. This finding is compatible with the hypothesis that VFN circuits have a physiological role in regulating gut volume by permitting gut filling and by counteracting contractility triggered by intraluminal content[202].

Latest advances

In the past decade, a range of new observations driven in part by novel recording and neurogenetic techniques have led to new ideas as to the physiological role(s) of VFN–sympathetic circuits. These new findings are discussed here.

Identification of a potent physiological stimulus. The first single-unit recordings from identified VFNs in guinea pig colon gave further support for direct mechanosensitivity[203,204] to gut volume[205]. However, they also revealed strong activation just before gut contractions, that is, before changes in gut mechanical status. In addition, they revealed synchronization of burst firing behaviour among VFN assemblies mediated by nicotinic transmission. Together, this feature pointed to a major role for synaptic activation of VFNs that was associated with gut contractility, but was not dependent on its mechanical effects. A major advantage of recording motor patterns in mouse rather than guinea pig colon is that the neural activity during the major neurogenic motor pattern, the colonic motor complex (CMC), persists during paralysis of smooth muscle. Thus, we found that VFN firing is temporally synchronized and potently activated during the CMC, despite paralysis of smooth muscle mechanical activity[206]. The synchronized firing of VFNs was identical to that which occurs across the myenteric plexus to generate CMCs — a rhythmic 2 Hz firing pattern[207]. Simultaneous recordings with gut sympathetic efferents revealed that the ~2 Hz firing pattern is faithfully transmitted via sympathetic neurons back into the gut, which suggests a previously unrecognized level of integration of the ENS, whereby VFN–sympathetic circuits enact rapid and direct ENS excitation-limiting self-regulation across otherwise distant gut segments.

VFNs and gut–brain satiety signalling. Two major studies applied extensive neurogenetic techniques to examine VFNs for the first time. The first by Muller et al. reported that a subset of small and large intestine VFNs express the neuropeptide CART and form circuits with liver-projecting and pancreas-projecting prevertebral sympathetic neurons. Through these circuits, chemogenetic activation of CART neurons increased blood glucose and decreased insulin levels, causing reductions in short-term food intake in mice.

CART neuron ablation through DTA expression or microbial depletion evoked opposing glucoregulatory effects. In a second major study, Zhang et al. supported earlier findings that suggested VFNs mediate the ileal brake[208], a well-known response to nutrients in the small intestine that acutely suppress appetite[209]. In this mechanism, nutrient-evoked GLP-1 release onto ileal VFNs activated sympathetic neurons to induce gastric relaxation in mice[170]. This suppressed feeding through spinal afferent detection of gastric volume, leading to modulation of hypothalamic circuits involved in appetite regulation. By contrast, subsequent studies demonstrated insensitivity among ileal enteric neurons to mucosally applied GLP-1, raising doubt as to the role of VFNs[210].

Human VFN and multiplexed immunolabelling. VFNs in human gut were identified in 2023 and characterized extensively by multiplexed immunolabelling, as noted earlier[179]. A remarkable observation was that VFNs lacked unique combinations of neurochemicals (a neurochemical code). Rather, their neurochemical codes resembled those of previously described human enteric neurons, which included codes characteristic of all major classes such as excitatory and inhibitory motor neurons, ascending and descending interneurons and sensory neurons. Furthermore, the neurochemical code of a VFN was associated with the cell body morphology and axonal projections congruent with the major class matching that code. For example, when human VFNs expressed a neurochemical code of intrinsic sensory neurons, they also had the multiaxonal nerve cell body morphology that is characteristic of intrinsic sensory neurons; those with codes matching excitatory motor neurons or ascending interneurons had uniaxonal morphologies and, indeed, their axons typically ascended along the gut before exiting via mesenteric nerve trunks. Thus, it must be asked whether viscerofugal neurons derive from major enteric neuronal classes, retaining many of their typical features but acquire a new axonal target. If so, recruitment might not be random, as 69% of human VFNs resembled excitatory motor neurons, which is disproportionately high compared with the 30% that excitatory motor neurons represent among all myenteric neurons in the same region[211].

Activation by noxious stimuli. Stebbing et al.[212] have shown in anaesthetized rats that acute gut stimulation with intraluminal 2,4,6-trinitrobenzenesulfonic acid increases firing of gut-projecting prevertebral sympathetic neurons, driven predominantly by VFNs. The response, if any, on the effector side of the VFN–sympathetic circuits during activation by 2,4,6-trinitrobenzenesulfonic acid remained elusive, as it was not associated with a detectable motility response. The authors speculated that sympathetic activity evoked by noxious stimuli could recruit sympathetic neurons involved in regulating immune function and inflammation.

Outstanding questions

Topography of VFN–sympathetic circuits. Physiological studies and the density gradient of VFN populations along the gut suggest that VFN–sympathetic circuits tend to operate in a distal-to-proximal direction (Fig. 5). However, intestinointestinal reflexes have been shown to operate in both directions along the gut[213]. Additionally, the study by Muller et al.[191] suggests cross-organ VFN–sympathetic pathways from gut to liver and from gut to pancreas. It is unknown whether this feature represents the full extent of VFN influence on other organs via sympathetic neurons, which highlights a need for comprehensive input–output mapping of the VFN–sympathetic circuits along the gut, across organs and into the CNS.

Physiological roles of VFN–sympathetic circuits. Although major studies into the physiological role of VFNs have revealed novel and unexpected findings, they address relatively obscure or minor populations, including those with projections to liver and pancreas-projecting sympathetic neurons[191], and ileal VFNs[170]. A demonstrated physiological role remains elusive for most VFNs, which are found in the greatest numbers in the large intestine[214,215] and which synapse with gut-projecting motility and secretion-inhibiting sympathetic neurons[216]. Traditional approaches, such as transection of viscerofugal axons in mesenteric nerve trunks, cannot be applied without ablating sympathetic axons as well as spinal and/or vagal afferent axons. Thus, although their location and semblance to major ENS neuronal classes present a major challenge to selective control or lesion of VFNs, what the papers by Zhang and Muller demonstrate is that combinations of localized, genetically targeted approaches now make possible a physiological interrogation of the major VFN–sympathetic circuits. Finally, functional investigations of VFNs beyond those that project to prevertebral ganglia are yet to be made.

Conclusions

The bidirectional communication between the gut and brain, once considered predominantly a conduit for regulating digestion, is now recognized as a critical axis underpinning overall health and well-being. Although vagal afferents have been relatively well characterized, advances in high-resolution anterograde tracing and neurogenetic tools have begun to illuminate the previously obscure landscape of spinal afferent innervation, revealing an unexpected level of complexity. Gut-projecting spinal afferents deploy an extraordinarily diverse array of sensory endings, particularly evident in the distal colon and rectum, which exhibit the highest density and morphological variety. Among the most striking discoveries are IGVEs embedded within myenteric ganglia and the observation that single spinal afferent neurons can possess multiple, morphologically distinct endings across different layers of the gut wall, suggesting a high degree of integrative sensory capacity. Piezo2 has emerged as a key mediator of spinal afferent responses to both physiological distension and noxious mechanical stimuli from the colon, marking a key transduction mechanism of visceral sensation. Alongside direct neuronal sensing, paracrine communication from EECs to both spinal and vagal afferent terminals represents an additional and potentially synergistic modality of sensory activation. Establishing a comprehensive map of these sensory architectures and their transduction mechanisms along the gut–brain axis in the healthy state is a prerequisite for deciphering the broader physiological and pathological contexts, including interactions with the gut microbiome. Future research must focus on correlating the newly identified morphological and genetic classes of afferents — including the multi-ending neurons and distinct IGVE subtypes — with specific sensory modalities and physiological roles. Elucidating how the nervous system differentiates between noxious and non-noxious visceral stimuli to trigger appropriate responses, whether pain behaviours or physiological reflexes, remains a critical challenge. Additionally, the functional significance of the third pathway involving enteric viscerofugal neurons, implicated in reflexes such as the ileal brake, requires further investigation to fully appreciate the multifaceted nature of gut–brain signalling.

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