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The gut, its microbiome, and the brain: connections and communications

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Review Series

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The gut, its microbiome, and the brain: connections and communications

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Modern research on gastrointestinal behavior has revealed it to be a highly complex bidirectional process in which the gut sends signals to the brain, via spinal and vagal visceral afferent pathways, and receives sympathetic and parasympathetic inputs. Concomitantly, the enteric nervous system within the bowel, which contains intrinsic primary afferent neurons, interneurons, and motor neurons, also senses the enteric environment and controls the detailed patterns of intestinal motility and secretion. The vast microbiome that is resident within the enteric lumen is yet another contributor, not only to gut behavior, but to the bidirectional signaling process, so that the existence of a microbiota-gut-brain "connectome" has become apparent. The interaction between the microbiota, the bowel, and the brain now appears to be neither a top-down nor a bottom-up process. Instead, it is an ongoing, tripartite conversation, the outline of which is beginning to emerge and is the subject of this Review. We emphasize aspects of the exponentially increasing knowledge of the microbiota-gut-brain "connectome" and focus attention on the roles that serotonin, Toll-like receptors, and macrophages play in signaling as exemplars of potentially generalizable mechanisms.

Early study of the gastrointestinal tract

The application of the scientific method to the study of the bowel revealed long ago that the gastrointestinal (GI) tract is more than a repulsive set of entrails. It is a highly sophisticated complex organ that is under exquisite neuronal control (1, 2) (Figure 1). The efferent side of this control operates on two levels. One is a large, intrinsic, enteric nervous system (ENS), comprising the myenteric (Auerbach's; ref. 3) and submucosal (Meissner's; ref. 4) plexuses, which is able to function as a "local nervous mechanism" controlling the behavior of the bowel independently of input from the brain or spinal cord (5–8). The other is an extrinsic innervation, which emanates from the central nervous system (CNS; brain and spinal cord) and communicates with the gut via sympathetic and parasympathetic inputs. The independent nature of the ENS led Langley, in his classical definition of the autonomic nervous system (9), to include the ENS as a separate autonomic division. Not only is the ENS independent, it can also communicate via intestinofugal nerves with the prevertebral sympathetic ganglia that innervate it (10–12) and directly with the CNS (13). Intestinofugal neurons may be mechanosensitive, but they appear to be mainly driven by other intrinsic neurons through cholinergic synapses and, in the colon, provide a rhythmic output to sympathetic ganglia during an intestinal behavior called the colonic motor complex (11). Intestinofugal neurons and sympathetic ganglia also provide a potential pathway for long, entirely peripheral intestino-intestinal reflexes (11).

The complexity of the dual control of the bowel implies that coordination of intrinsic and extrinsic neuronal signaling is necessary. It is equally necessary that both intrinsic and extrinsic nervous systems receive sensory input from the bowel so that their efferent signals are based on contemporaneous information from within the gut. Both the ENS and the CNS also require accurate and rapid feedback so that their output remains realistic and useful in GI function. We now provide a timely, yet comprehensive review of the means by which this feedback is accomplished. We also include the relatively recent realization that the enteric microbiome is an active participant in a bidirectional information loop. Because knowledge has exploded, we have not tried to be exhaustive, but have focused our attention on particular signaling molecules (serotonin; 5-HT), receptors (Toll-like receptors), and cells (enterochromaffin cells and macrophages) as examples that are relatively well understood.

Enteric sensation

Two different afferent neural pathways transmit enteric information to the CNS (14, 15) (Figure 1). One of these pathways is spinal and segmental, while the other is vagal. The cells that provide the relevant axons are all extrinsic visceral afferent neurons that can collectively be called ExPANs (extrinsic primary afferent neurons) because their cell bodies are located outside of the gut. Spinal ExPANs are situated in thoracolumbar and lumbosacral dorsal root ganglia (DRGs) (14–17), while vagal ExPANs reside in the nodose and superior (jugular) ganglia of the vagus nerves and project to the nucleus of the solitary tract (NTS) and, to a lesser extent, the area postrema (AP) in the brainstem (18). ExPANs are distinguished from their intrinsic counterparts, IPANs, which provide the ENS with sensory information and lie within the submucosal (19) and myenteric (20) plexuses of the gut wall. For the most part, nociceptive and other signals of discomfort arising in the bowel, such as bloating and urgency, are detected by processes of ExPANs in DRGs and are conveyed to the CNS in spinal nerves

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(16, 17). In contrast, GI sensors that monitor nutrient composition and gastric volume transmit non-painful information to the CNS through the vagal processes of ExPANs, resulting in sensations such as satiety and nausea. Vagal afferents may also carry information resulting from the sensing of microbe-associated molecular patterns (MAMPs) (18).

Gut-projecting spinal afferent neurons

Spinal ExPANs have been subdivided into classes based on where they project within the bowel wall and stimuli to which they respond (refs. 21–23 and Table 1). The enteric terminals of ExPANs were recently visualized as a result of exquisite experiments that applied anterograde tracers to murine DRGs (24). This technique, which used high–molecular weight biotinylated dextran as the tracer, surpassed earlier methods in which tracers were applied to the severed peripheral ends of nerves to the gut (25) or that used the calcitonin gene–related peptide α (CGRPα) promoter to drive expression of a GFP reporter (26, 27). Although many, if not all, nociceptive visceral afferent fibers express CGRP, and CGRPα-driven GFP expression demonstrates cell bodies in DRGs, GFP is not well visualized in the enteric terminals of these neurons (26, 27); moreover, a CGRP-driven GFP reporter is not selective for spinal visceral afferents in gut because intrinsic enteric neurons also produce CGRP (28–31). When axon terminals from injected DRGs are visualized within the bowel, however, their identity as visceral afferent axons cannot be questioned; moreover, the tracer can be detected simultaneously with immunocytochemically demonstrated neuropeptides, allowing exploration of the chemical coding of visceral afferent nerve endings (32).

Injections of anterograde tracer into the lumbosacral DRGs have revealed a complex set of nerve endings in the colon and rectum. The thoracolumbar DRGs also project to the colon and rectum; however, this innervation is less complex. As many as 13 different morphologies of terminal axons from lumbosacral DRGs have been described (33), and single neurons can give rise to multiple types of endings in multiple layers of gut (34). Most of these (~82%) are located in myenteric ganglia, the submucosa, and circular muscle. Very few terminals are located within submucosal ganglia, longitudinal muscle, or within walls of blood vessels; however, some terminal axons also enter the mucosa. The most common type of terminal axon is varicose, and these ramify in a meandering way through myenteric ganglia. Varicose terminals, or "intraganglionic varicose endings" (IGVEs), are CGRP immunoreactive. The IGVEs are distinct and different from much more sparse flattened "intraganglionic laminar endings" (IGLEs), each of which is located within a single myenteric ganglion (35), and which resemble vagal sensory IGLEs of the proximal bowel (ref. 18; see below). In contrast to IGVEs, IGLEs formed by spinal nerves in the colon and rectum are nonpeptidergic and thus lack CGRP. Most spinal nerve terminals, potentially including IGLEs, express VGLUT2, and these extensively remodel in inflammation (36). IGLEs in the esophagus (37) and stomach (38) have been reported to be low-threshold mechanoreceptors (see below), and it is likely that their rectal equivalents (rIGLEs) are similar. One might imagine that aspects of vagal innervation are mimicked by sets of lumbosacral spinal neurons innervating the distal bowel below the coverage of the vagus nerves.

Most gut-projecting spinal afferent fibers are slowly conducting unmyelinated C fibers. A transient receptor potential (TRP) channel, particularly vanilloid member 1 (TRPV1), a nonselective cation channel that is also the receptor for capsaicin, is expressed in most of those fibers (39). The terminals of these fibers usually contain peptides such as CGRP or substance P (39). Actually, a very high proportion of ExPANs express TRPV1 channels, including 40% to 70% of vagal afferents and, depending on the axial level examined, 65% to 95% of spinal afferents (16). Many stimuli unrelated to capsaicin also activate TRPV1 channels, including inflammatory signals, heat, acidification, lipid peroxides, and exogenous ligands (of which capsaicin is an example) (40, 41). Activated TRPV1 channels induce membrane depolarization, triggering action potentials and thus pain transduction. TRP ankyrin member 1 (TRPA1) is expressed by another population of visceral afferent neurons that innervate the gut; chemical irritants such as garlic, mustard oil, and menthol activate TRPA1 channels (42). The DRG neurons that innervate the mouse colon have recently been subjected to single-cell sequencing (43), which distinguished seven classes of gut-projecting visceral afferent neurons, and TRPV1 is expressed in six of them.

The TRPV1-expressing neurons that innervate the bowel are major drivers of the visceral hypersensitivity that often accompanies bouts of colitis (44), and they enhance the abilities of hosts to fend off bacterial infection of the gut (45). To do so, they express Toll-like receptors and other pattern recognition receptors that allow the neurons to detect not only changes in tissue due to infection, but MAMPs on the surface of bacteria (46, 47). In their defensive role, visceral afferent neurons, or at least their terminals, act to mimic efferent terminals (axon reflex) (45). Although they normally transmit information from the intestine to the spinal cord, TRPV1-expressing nociceptive neurons are also able to release CGRP from their distal terminals in the bowel. Secreted CGRP regulates the number of microfold ("M") cells in the mucosal domes over Peyer's patches. This axon reflex–like action limits the ability of pathogens, such as *Salmonella enterica* serovar Typhimurium, to invade the gut wall and spread beyond the bowel. TRPV1-expressing nociceptive neurons are even able to maintain luminal filamentous bacteria that

Table 1. Classes of spinal afferents (ExPANs)

Type of afferent ExPAN	Stimulus
Mucosal	Deformation of the mucosa
Muscular	Intestinal distension
Mucosal/muscular	Mucosal deformation + intestinal distension
Vascular (close proximity to blood vessels)	Chemical mediators of inflammation and tissue damage + intense mechanical
Serosal	High pain threshold
Silent	Normally quiescent but become mechanosensitive when exposed to inflammation

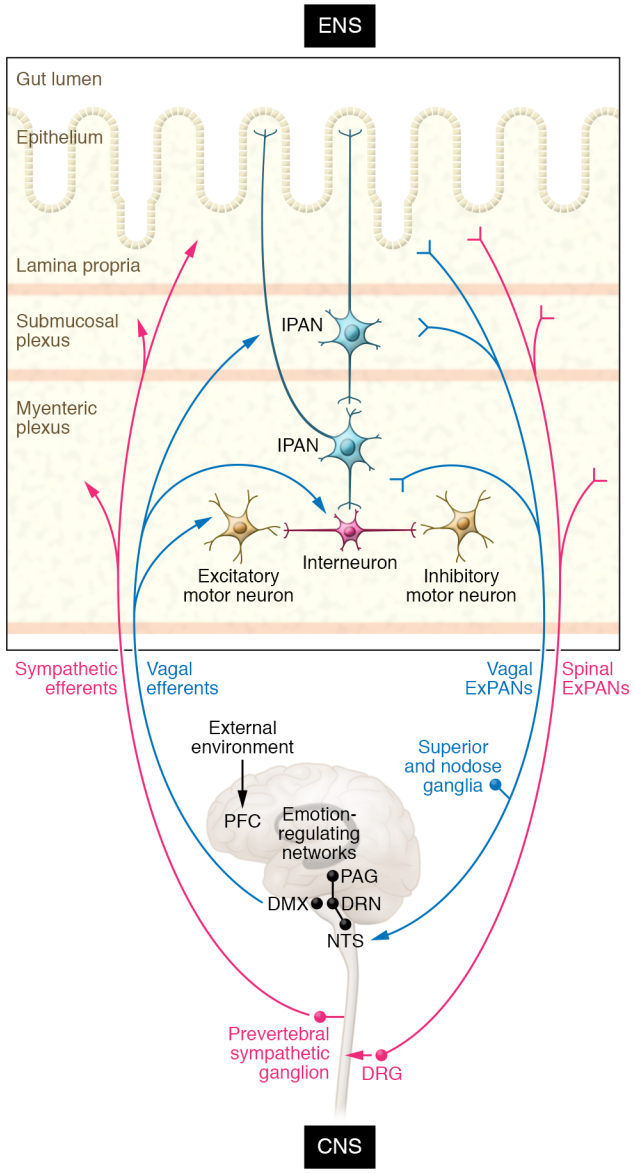

Figure 1. Neural pathways that carry the bidirectional signaling traffic between the gut and the brain. The brain-to-bowel efferent signals (pink and blue arrows, left) are mostly autonomic. Parasympathetic axons (blue) depart predominantly from the dorsal motor nucleus of the vagus (DMX) in the brainstem and are conducted through the vagus nerves to the bowel, where they terminate on selected neurons within the two plexuses of the ENS. Additional parasympathetic fibers exit the sacral spinal cord to terminate on enteric neurons of the mid- to distal colon (not shown). Sympathetic preganglionic axons (pink) leave the spinal cord at thoracic and lumbar levels, synapse with postganglionic neurons, primarily in prevertebral sympathetic ganglia, and terminate within the bowel. The bowel-to-brain afferent signals are carried by two types of ExPANs. Spinal ExPANs (pink) have their cell bodies in dorsal root ganglia and project into the CNS at spinal levels. Vagal ExPANs (blue) have their cell bodies in the nodose and superior ganglia of the vagi and project mainly to the nucleus of the solitary tract (NTS). From the NTS, signals emanating from the gut can be referred to the dorsal raphe nucleus (DRN) and periaqueductal gray matter (PAG) and to emotion-regulating networks that include the limbic system. The details of gut behavior are largely controlled by the intrinsic neurons of the ENS. This system contains IPANs, which project to the mucosa and receive information from epithelial sensors or respond directly to stimuli impinging on the bowel. The ENS also comprises intrinsic excitatory and inhibitory motor neurons and ascending and descending interneurons. Both enteric plexuses contain IPANs; secretomotor neurons are largely in the submucosal plexus, while the motor neurons that control the smooth muscle of the muscularis externa are in the myenteric plexus. The enteric plexuses reciprocally project to one another.

reside in close proximity to ileal villi and the mucosal domes over Peyer's patches that oppose the growth of *S.* Typhimurium. In contrast, genetic deletion of TRPV1 and administration of TRPV1 antagonists attenuate inflammation but diminish the ability of the gut to oppose infection (48–51). TRPV1-expressing neurons thus modulate intestinal inflammation, but intestinal inflammation also changes the neurons. Proinflammatory mediators alter the sensitivity of TRPV1-expressing neurons and recruit otherwise silent visceral afferents to promote visceral hypersensitivity, which may be useful as a defense against microbial invasion, but also potentiates adverse symptoms in irritable bowel syndrome and inflammatory bowel disease (52–54).

IPANs within the ENS resemble their ExPAN equivalents in DRGs (55–57). Protein kinase G1α (PKG1α) is selectively expressed in DRG nociceptive neurons and has been linked to long-term hyperexcitability (58). PKG1α is also expressed in subsets of intrinsic neurons in each enteric plexus (57). PKG1α immunoreactivity colocalizes with the IPAN markers calbindin (in myenteric plexus) and cytoplasmic NeuN (in submucosal plexus). Gut-projecting visceral afferents in DRGs, identified by retrograde transport, are also PKG1α immunoreactive. N46, a selective antagonist of PKG1α, impairs the ability of cholera toxin–stimulated IPANs to activate Fos in enteric neurons. N46 also inhibits luminally evoked peristaltic reflexes in isolated preparations of distal colon. These observations suggest that PKG1α is present and functionally important, both in IPANs and in visceral afferent nociceptive ExPANs. IPANs thus appear to play a dual role, initiating intrinsic secretory and peristaltic reflexes and also serving as intrinsic nociceptors (57, 59).

Gut-projecting vagal afferent neurons

The vagal sensory pathway to the bowel has been extensively investigated (18, 60–63) (Figure 1). Vagal afferents are a highly eclectic class of sensory neurons that keep the microenvironments of various regions of the GI tract under the strict surveillance of the brain. The nodose ganglion, which houses most of the vagal ExPANs that innervate the stomach and intestine, provides a convenient portal of entry for their study. Anterograde tracers can be introduced bilaterally into the nodose ganglia, enabling visualization of afferent terminals. Among these, mucosal endings provide the brain with chemical and nutrient information from the GI lumen, although this information must be conveyed to the nerve endings across the mucosal epithelium, because no nerve fibers enter the enteric lumen (62) (Figure 2). Vagal sensory axons also terminate in IGLEs (61), which are morphologically similar to those of spinal sensory nerves discussed above, and similarly have been found to be mechanosensors, most likely responding to dilation of the stomach or intestine (64, 65). Additional vagal terminals take the form of intramuscular arrays, which are also likely to be mechanotransducers, although they have not yet been thor-

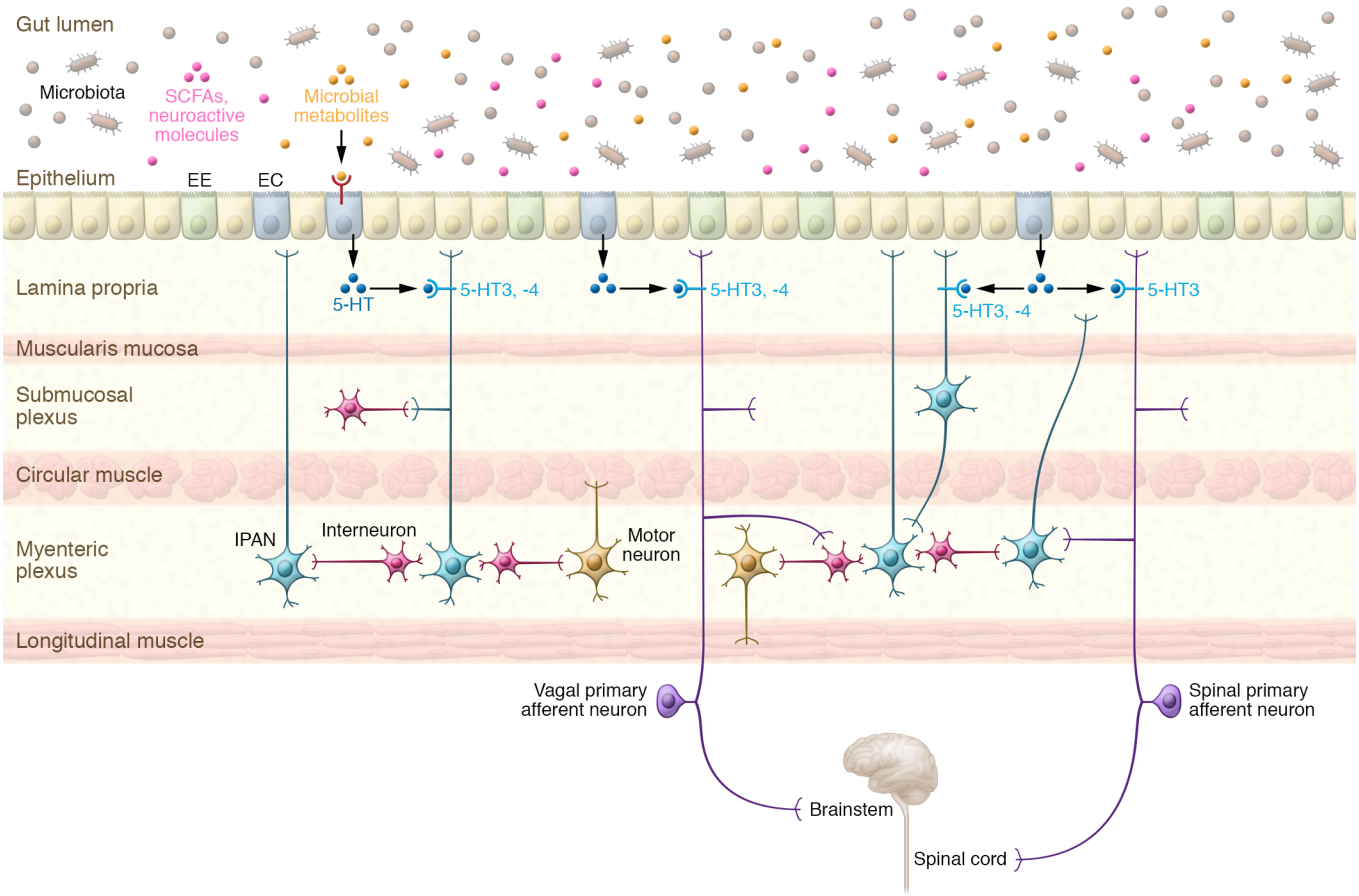

Figure 2. Paracrine transmitters, such as 5-HT, are enablers of microbiota-gut-brain "connectome" signaling. Microbiota within the lumen of the bowel produce metabolites, which include short-chain fatty acids (SCFAs) and other neuroactive molecules that can lead to the stimulation of IPANs and ExPANs. This stimulation can be direct, following the absorption of the microbial metabolites, or it can be indirect, involving stimulation of receptors on mucosal epithelial cells. Epithelial cells also have receptors for MAMPs that allow them to react to contact with the microbial surface. Activation of EC cells, the most common of the enteroendocrine (EE) cells of the gut, causes these cells to secrete 5-HT into the underlying lamina propria. EC cells, which express Piezo2, are mechanosensitive and can also be stimulated to secrete by increases in intraluminal pressure or sympathetic nerve stimulation. Terminals of IPANs and ExPANs both express 5-HT_3 and 5-HT_4 receptors, allowing 5-HT from EC cells to stimulate IPANs and ExPANs. The activated IPANs thus result in the manifestation of peristaltic and secretory reflexes, while activated vagal ExPANs transmit sensations of nausea or satiety and spinal ExPANs transmit the sensation of pain or discomfort to the CNS. Interneurons are present in both submucosal and myenteric plexuses and presumably are critical for the ability of the ENS to manifest integrated neuronal activity and reflexes in the absence of CNS input.

oughly characterized (65). GI stimuli, including mucosal stroking, gastric or intestinal distension, hormones, GI luminal nutrients, osmolytes, and pH alterations, initiate electophysiologically recordable responses in nodose ganglion neurons.

Single-cell RNA sequencing (scRNA-Seq) has exponentially expanded knowledge of the complexity and diversity of nodose neurons (66). Remarkably, revelation of that diversity has not merely produced a catalogue of neurons. Instead, the scRNA-Seq technology has succeeded in linking knowledge of the molecular heterogeneity of vagal ExPANs to the anatomy of their terminals both within the bowel and in the brain; moreover, this anatomy has also been coupled to function (67). This work, while exciting, needs to be replicated, and caution should be applied to conclusions. The major advance has been to go from simple scRNA-Seq (66) to target-specific scRNA-Seq, which uses retrograde tracing from specific targets, in combination with whole nodose scRNA-Seq to obtain a comprehensive view of the genetic makeup of individual nodose neurons projecting to particular GI regions (67). This analysis has demonstrated, for example, that vagal sensory neurons that give rise to mucosal endings in the stomach contain transcripts encoding either somatostatin or CALCA (alternative spicing encodes calcitonin and CGRP), while neurons that innervate the intestinal mucosa express either vasoactive intestinal peptide (VIP) or GPR65 (68), and all four express combinations of receptors for nutritionally regulated hormones. These observations are consistent with the view that these types of vagal sensory neuron all project to the mucosa and are equipped to respond to paracrine signals from enteroendocrine, tuft, and other mucosal epithelial cells that act as sensors for luminal contents (62) (Figure 2). They are thus also likely to be the neurons that inform the brain about the luminal environment of the bowel.

In contrast to mucosa-projecting neurons, nodose neurons that give rise to IGLEs in the stomach and intestine do not express the four genes that define the mucosal afferents, but instead express transcripts encoding the receptor for glucagon-like peptide-1 (GLP-1R) in the stomach or the oxytocin receptor (OXTR)

in the intestine (67). Surprisingly, the vagal afferent neurons that most potently induce satiety (62) are OXTR-expressing neurons (which give rise to IGLEs in the intestine) and not the expected mucosa-directed cells specialized to respond to nutrient intake (67). Stimulation of GLP-1R–expressing neurons that form gastric IGLEs also produces satiety, but much less so than stimulation of their OXTR-expressing counterparts. Stimulation of intestinal IGLE mechanoreceptors activates brainstem satiety-promoting pathways that inhibit hunger-promoting hypothalamic neurons marked by agouti-related peptide (AgRP) and neuropeptide Y (NPY). Interestingly, intestinal IGLEs and the OXTR-expressing neurons that give rise to them also express CCKAR, a receptor for the potent satiety-inducing hormone cholecystokinin (CCK); moreover, CCK potentiates the ability of intestinal distension to cause satiety. Thus, a single genetically identifiable class of sensory neuron may be able to integrate hormonal and mechanical control of food intake.

Many brain regions involved in the regulation of feeding have been identified. To investigate how these regions incorporate vagus-derived signals from the stomach and intestine, the four subtypes of vagal sensory neuron (GPR65, VIP, GLP-1R, and OXTR) were activated chemogenetically in vivo and responses of the hypothalamic hunger-producing AgRP-expressing neurons were recorded (67). OXTR-expressing mechanoreceptive cells (intestinal IGLEs) strongly inhibited AgRP neurons in hungry mice. Chemogenetic activation of GLP-1R–expressing cells (gastric IGLEs) had lesser effects, while activation of GPR65- or VIP-expressing cells was without effect. Consistent with these data, non-nutritive volumetric distension of the intestine, but not the stomach, also inhibits hypothalamic AgRP neurons. Hypothalamic hunger circuits, which were previously considered the domain of long-term nutritional hormones such as leptin, are thus also subject to regulation by mechanical stimuli emanating from the intestine and relayed to the brain via the vagus nerves.

How information is relayed from the terminals of vagal afferents in the NTS and AP to the hypothalamus is not totally clear; however, NTS neurons project directly to the hypothalamus (69, 70) and to the parabrachial nucleus (71, 72), which also projects to the hypothalamus. In fact, stimulation of OXTR-expressing vagal neurons activates cells in the NTS, AP, and parabrachial nucleus (67). One type of activated NTS neuron, which expresses tyrosine hydroxylase (TH), and one in the parabrachial nucleus, which expresses CALCA, inhibit food intake. Food ingestion, furthermore, activates these cells; moreover, the TH-expressing NTS neurons project to and stimulate CALCA-expressing parabrachial neurons (71, 72). These observations are consistent with the idea that the OXTR-expressing intestinal mechanoreceptors antagonize feeding by stimulating a satiation pathway involving the TH-expressing NTS neurons and CALCA-expressing parabrachial neurons (67).

The importance of intestinal mechanoreceptors in the process of satiation means that the rate of gastric emptying is critical to cessation of normal feeding. Intestinal load, which triggers IGLE mechanoreceptors, is a function of the rate at which the stomach empties. That rate, in turn, depends on the properties of consumed food. Liquids, solids, high caloric density, lipid content, and osmolarity all affect the gastric emptying rate (73, 74). Bariatric surgeries, such as Roux-en-Y and vertical sleeve gastrectomy, however, greatly accelerate gastric emptying and intestinal distension (75), suggesting that they may utilize intestinal IGLEs to activate the satiety mechanism, decrease food intake, and combat obesity. The location of a major generator of satiation in the intestine also may account for the delay in satiety during meals. Slowing the intake of food during eating to give the stomach a chance to empty and allow intestinal IGLEs to become engaged may provide a physiological basis for the dieter's dictum to leave the table while still a little hungry; wait a bit and intestinal IGLEs will take care of it.

The microbiota-gut-brain axis

The intestinal microbiome was recently found to be a surprising contributor to the regulation of GI motility (76) and mood (77). Because of its residence within the lumen of the bowel, the gut microbiome, together with its enteric container and associated pathways to the brain, is now referred to as the "gut connectome" (78). The mechanisms underlying the bidirectional interactions encompassed in the "gut connectome" are beginning to be understood (77, 79–82) (Figure 2). Enteric microbes communicate with the CNS through neuronal, endocrine, and immune signaling pathways. The CNS, moreover, does not just passively receive information from enteric microbiota. It can also initiate interactions that impact the gut microbiota, via stress mediator–induced virulence gene expression and through sympathetic and parasympathetic control of GI motility, secretion, and immunity (83). The ENS is an important participant in this conversation, because by regulating intestinal secretion, motility, permeability, and immunity the ENS controls the environment and thus the composition of enteric microbiota. Pathways of microbiota-gut-brain signaling involve bacterial metabolites, immunoeffectors (84), paracrine messengers, neurotransmitters, and vagus nerve transmission (83, 85, 86). Although "gut connectome" signaling is complex (87), its elucidation is clinically important because enteric microbiota and their metabolites may contribute to the pathogenesis of neurological and psychiatric disorders, such as depression, autism spectrum disorder, and Parkinson and Alzheimer diseases (88).

Immune mechanisms for enteric microbiota–gut–brain signaling

To prevent resident bacteria from invading the bowel wall, an equilibrium must be established between microbiota tolerance and host protection. Immune mechanisms are vital to this equilibrium and also participate in mediating communication between the enteric microbiota, ENS, and brain. Although multiple mechanisms have been linked to interactions between immune cells, the enteric microbiota, and the ENS, we will focus on components of the innate immune response (e.g., Toll-like receptors) and macrophages as examples that have been well studied and that also interact with serotonergic signaling (89) (Figure 2).

Toll-like receptors. Toll-like receptors (TLRs) act as sensors for microbe-associated molecular patterns (MAMPs) and thus can initiate immune responses that serve as conduits for communication with the ENS (90, 91). For example, LPS, a cell wall component of Gram-negative bacteria, activates an intestinal immune cascade that is initiated by binding to TLR4 on enterocytes (92). The data implicating TLRs in microbial-ENS communication suggest that enteric neuronal responses to stimuli from distinct types

of microbes affect its physiology. Enteric neurons and glia express TLR2 and TLR4, which have been shown to mediate microbiota-ENS communication (93, 94). Numbers of nitrergic neurons are decreased and GI motility is slowed in TLR4-deficient mice, a phenotype similar to that observed in germ-free (GF) and antibiotic-treated mice, implicating LPS in sculpting and function of the ENS (94, 95). Similarly, neurochemical coding of enteric neurons, epithelial chloride secretion, and smooth muscle GDNF are abnormal in GF mice and animals deficient in TLR2 signaling (93). These defects, and the accompanying intestinal dysmotility, are completely reversed by administration of GDNF or a TLR2 agonist. Bacterially driven TLR2 signaling can thus regulate intestinal neuromuscular function.

The mechanisms by which microbe-TLR communication affects ENS morphology and function, as well as how changes induced in TLR signaling by exposure to antibiotics affect gut-brain signaling, are unknown (96). There is evidence, however, that 5-HT and TLR play reciprocal roles in their regulation. TLR activation appears to be linked to decreased activity of the serotonin transporter (SERT) (92). SERT-mediated 5-HT uptake is the major means of terminating 5-HT's action; therefore, anything that decreases SERT activity enhances 5-HT signaling (97). Intestinal SERT expression, for example, is increased in *Tlr2*$^{-/-}$ mice, and, in vitro, LPS treatment decreases SERT activity in a dose- and time-dependent manner (92). Apical TLR2 activation, moreover, inhibits SERT activity in Caco-2/TC7 cells (used as a model of colonic epithelium) (98). 5-HT's role in ENS changes that are seemingly modulated by the gut microbiota and TLR signaling is unknown; however, 5-HT and its regulation by SERT have been shown to drive neurogenesis and development of the ENS (97, 99).

Macrophages. Innate immune cells, particularly macrophages, are influenced by enteric microbiota and, in turn, send signals to the ENS (Figure 3). Macrophages are present throughout the gut, where they play essential roles in innate immunity and maintenance of homeostasis through pathogen phagocytosis, uptake of bacterial products, facilitation of repair, and interaction with smooth muscle, telocytes, and glia (100–103). Of the macrophage populations, intestinal monocyte-derived and tissue-resident macrophages are decreased in quantity in mice that are GF or depleted of microbiota with antibiotics, implying that microbiota contribute to intestinal recruitment and differentiation of macrophages (104). A distinct population of muscularis macrophages (MMs; in the intestinal muscularis externa) also regulate motility; moreover, enteric microbiota facilitate this regulation. MMs alter peristaltic activity through the secretion of BMP2, which activates a receptor on enteric neurons (Figure 3A). Enteric neurons, reciprocally, secrete CSF1, a macrophage growth factor. Importantly, stimuli from enteric microbiota regulate expression of BMP2 as well as enteric neuronal expression of CSF1. There is thus a readily changeable, microbiota-driven crosstalk between MMs and enteric neurons that regulates GI motility. Interestingly, GI motility, as well as CSF1 and BMP2 production, is decreased after antibiotic treatment, implying that the crosstalk between MMs and enteric neurons is at least partly dependent on enteric microbiota (104). Extrinsic vagal cholinergic (α7 nicotinic) antiinflammatory effects on the gut also involve MMs (refs. 105, 106, and Figure 3B).

MMs play an important role in modulating the effects of infection-induced inflammation on intrinsic enteric neurons. In murine enteric infections (*Salmonella*, *Toxoplasma*, *Yersinia* species), long-term GI symptoms occur, including reduced GI motility and loss of excitatory enteric neurons (107). This effect depends on enteric neuronal NLRP6 inflammasome– and caspase-11–mediated cell death (Figure 3A). NLRP6 is a member of a family of proteins that patrols the cytosolic compartment of cells to detect pathogen- and damage-associated molecular patterns (108). In contrast, a β_2-adrenoceptor–mediated signaling mechanism enables MMs to protect neurons from death in response to luminal infection by a mechanism involving the arginase-1/polyamine axis (ref. 107 and Figure 3C). The responsible catecholamine is norepinephrine from stress-activatable sympathetic axons in the gut, rather than a circulating adrenal hormone. The intrinsic enteric neuronal death that would otherwise follow infection by a pathogen can thus be limited by resident MMs.

Enteric microbiota and macrophages communicate with and regulate extra-enteric autonomic neurons, including those of the sympathetic and parasympathetic nervous systems. Extrinsic sympathetic activity is enhanced and GI motility is slowed in GF mice; moreover, transfer of feces from specific pathogen–free donors to GF mice normalizes sympathetic activity (109). Blockade of catecholamine release, furthermore, rescues mice from GF-associated slowing of their GI motility. These data imply that enteric microbiota participate in regulation of sympathetic nerve activity. The pathogen *S*. Typhimurium also causes sympathetic neurons to secrete norepinephrine, which stimulates β_2-adrenoceptors on MMs, which limits enteric neuronal damage, supporting the importance of MMs in neuroprotection during enteric infection (ref. 110 and Figure 3C). The ENS may also protect itself from invasive *S*. Typhimurium by producing IL-18, which both drives goblet cell antimicrobial peptide production and reinforces the mucosal barrier (111–113).

Microbial metabolites. Tryptophan metabolites have been studied better than other enteric microbiota–generated metabolites in the regulation of CNS and ENS physiology and function. These metabolites communicate with the brain and the ENS by way of the intestinal mucosa and the vagus nerves (97, 114–118) (Figure 2). The afferent vagus nerves serve as major communication highways connecting the gut to the emotion-regulating centers of the brain. Enterochromaffin (EC) cells can communicate with the mucosal projections of IPANs and ExPANs through synapse-like connections of extensions that extend below the basal lamina of the mucosal epithelium and have been called "neuropods" (119). Vagal afferent fibers express 5-HT receptors (5-HT$_3$, 5-HT$_4$) that enable them to respond to the 5-HT that EC cells secrete (120, 121). Absorbed bacterial metabolites, including short-chain fatty acids (SCFAs), can also activate free fatty acid receptors present on vagal afferents (122). In fact, SCFAs and secondary bile acids (such as deoxycholic acid, produced by the action of luminal bacteria on secreted primary bile acids) have been shown to influence intestinal 5-HT production (123). Specifically, in both humans and mice, increased dietary tryptophan availability causes spore-forming species of Clostridiales to induce SCFAs and secondary bile acid synthesis that upregulates 5-HT production in, and release from, EC cells, a process that can enhance GI motility (76, 124–126).

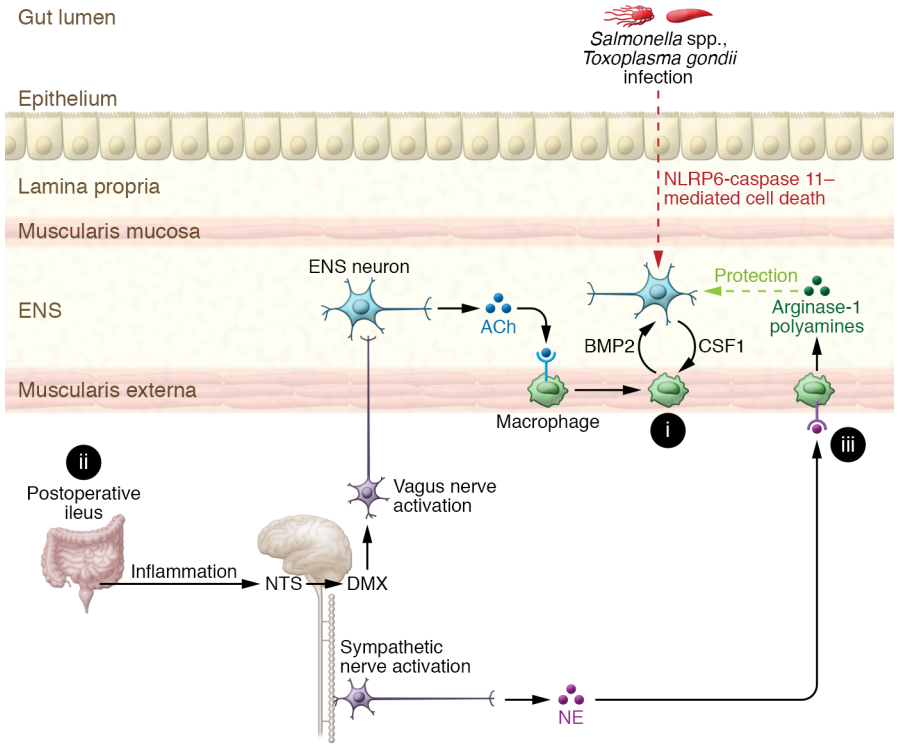

Figure 3. Interactions between macrophages, enteric neurons, and parasympathetic and sympathetic nerves contribute to the maintenance of intestinal homeostasis in inflammation and bacterial infection. **(i)** Muscularis macrophages (MMs) secrete BMP2, which activates BMP receptors on enteric neurons and thus affects intestinal motility. Enteric neurons reciprocally secrete CSF1, a growth factor required for macrophage development. Enteric microbiota stimulate secretion, of both BMP2 and CSF1, and thus enhance the crosstalk between enteric neurons and MMs. **(ii)** Provocation of intestinal inflammation, for example by postoperative ileus, signals to the NTS in the brain via vagal afferent nerves. This leads to activation of vagal efferent nerves, originating in the dorsal motor nucleus (DMX), which stimulate cholinergic enteric neurons to secrete acetylcholine (ACh). This ACh activates α7 nicotinic receptors on MMs to downregulate their inflammatory effects. **(iii)** Infection of the bowel with bacteria, including pathogens such as species of *Salmonella* or *Toxoplasma*, can cause NLRP6 inflammasome– and caspase-11–mediated cell death of enteric neurons. Stress activation of sympathetic nerves leads to the release of norepinephrine (NE) from sympathetic nerve terminals in the gut. NE stimulates β_2-adrenoceptors on MMs, which in turn activates the arginase-1/polyamine axis, leading to the release of polyamines, such as spermine, which are neuroprotective. Macrophages can thus protect enteric neurons from infection-induced cell death.

The autonomic innervation of the bowel has also been shown to activate EC cells to release 5-HT into the gut lumen, where it can influence gut microbial function or be taken up by SERT-expressing enterocytes (127). The 5-HT released from EC cells interacts with enteric microbiota, specifically with *Turicibacter sanguinis*, a bacterium that expresses a transporter with structural and functional similarities to SERT (128). This bacterium takes up 5-HT, which contributes to its ability to colonize the bowel. The organism reciprocally alters steroid and lipid metabolism in the host, reducing triglyceride levels and decreasing the size of inguinal adipocytes. The selective serotonin reuptake inhibitor (SSRI) fluoxetine antagonizes all of these bacterial actions. These findings suggest that select bacteria within the enteric microbiome interact bidirectionally with host 5-HT to improve their own fitness within the bowel and, in doing so, make contributions that either are, or may be, beneficial to their hosts. The ability of enteric bacteria to interact with 5-HT may also explain the bidirectional interactions between 5-HT–based psychotropic drugs (such as SSRIs) and the intestinal microbiota (129).

In addition to its direct effects on sensors in the epithelium of the intestine and nerves in the gut wall, the enteric microbiota may also influence serotonergic neurotransmission in the brain by regulating the availability of the 5-HT precursor tryptophan. TPH2, the rate-limiting enzyme in brain 5-HT biosynthesis, is not normally saturated (130); therefore, the rate of 5-HT biosynthesis in the brain is highly dependent on the availability of tryptophan. Tryptophan availability depends on diet and transport from the blood into the brain. Alterations in tryptophan metabolism have been reported recently in several neurological, psychiatric, and intestinal diseases, indicating its potential involvement in gut-brain diseases (131). Enteric microbiota influence three different pathways of tryptophan metabolism in the GI tract. One such pathway leads to 5-HT production in EC cells. Another pathway leads to opening of the indole ring to produce kynurenine, which occurs in both immune and epithelial cells (132). The third pathway involves direct transformation of tryptophan by gut microbiota into molecules that include ligands of the aryl hydrocarbon receptor (AhR) (133), a ligand-dependent transcription factor capable of influencing DNA transcription.

The AhR is recognized as a biosensor that is critical for intestinal epithelial cell and immunoeffector cell homeostasis; moreover, AhR signaling is a vital component of the immune response at sites, like the intestinal lining, that act as barriers between the body and the external environment. Enteric neurons also express the AhR, which may serve as an integration center between the luminal microbiota and intestinal motility (134). Neuron-specific deletion of the AhR, or constitutive overexpression of its negative-feedback regulator CYP1A1, reduces colonic peristaltic activity; moreover, expression of the AhR in enteric neurons of antibiotic-treated mice partially restores their intestinal motility (135). These studies suggest that the ENS can monitor the metabolites released from enteric luminal microbes and adjust neuronal activity and motility accordingly. 5-HT increases CYP1A1 expression via a SERT-dependent process in epithelial cells, but it is not clear whether this same regulation occurs in neurons or how serotonergic regulation of CYP1A1 in epithelial cells affects epithelial-neuronal communication (136). Further research is required to understand the mechanisms that underlie ENS monitoring of the luminal environment and whether 5-HT signaling can be manipulated to modulate the AhR intestinal response to microbial metabolites.

Circulating tryptophan concentrations are significantly higher in male GF mice than in conventional control animals (137); these altered tryptophan levels result in an increase in hippocampal 5-HT and the 5-HT metabolite 5-hydroxyindole acetic acid (137). Much more dietary tryptophan is metabolized to kynurenine than to 5-HT; moreover, unlike 5-HT, kynurenine traverses the blood-brain barrier and exerts a deleterious effect on brain health by inducing neuroinflammation, neurodegeneration, and, in models of chronic stress, depression-like behavioral alterations (138, 139). As a result, the balance between the proportions of tryptophan that are left intact, diverted to produce 5-HT, or directed to kynurenine production may be important in brain function.

Intestinal microorganisms metabolize unabsorbed tryptophan. Among the metabolites they produce are indole derivatives, including indole-3-aldehyde, indole-3-acetic acid, indole-3-propionic acid, indole-3-acetaldehyde, indole-3-lactic acid, and indole acrylic acid. Indoles exert beneficial actions on intestinal and systemic homeostasis by their actions on intestinal permeability, regulation of inflammation, and host immunity; nevertheless, some indole derivatives have been associated in animal studies with depressive-like phenotypes (125, 140, 141). One means by which microbiota-derived indoles affect CNS and ENS function is to influence the production and release of 5-HT from EC cells (141, 142). Specifically, the fish intestinal bacterium *Edwardsiella tarda* produces indoles from tryptophan that activate Trpa1 channels in enteroendocrine cells, leading to the production/secretion of 5-HT, which stimulates enteric neurons, enhances intestinal motility, and stimulates vagus nerve activity (142). The same indoles also stimulate TRPA1 channels in humans and mice. Although *E. tarda* itself is a human pathogen (143), this phenomenon highlights the ability of specific bacteria to affect the physiology of the gut and brain simultaneously. These actions suggest that targeting specific microbiota or tryptophan-driven pathways may be valuable in therapies of disorders, such as irritable bowel syndrome, that may affect both the gut and the brain.

Microbiota and ENS development

Enteric microbiota–driven effects on ENS development and function have been demonstrated in GF mice, which have deficits in GI motility as well as smaller numbers and different subtype distributions of enteric neurons as compared with conventionalized mice (94, 144, 145). Excitability of IPANs, nodes for gut-to-brain communication, is also abnormal in GF animals (146, 147). Further, conventionalization of adult GF mice with specific pathogen–free microbiota, probiotics, or specific bacterial strains reduces ENS-associated deficits, including those in intestinal transit time (89, 145), neuronal excitability (146), chemical coding of enteric neurons, and enteric glial cell density (135, 145, 148–150). Enteric microbiota are actually essential for the movement of glia to, and maintenance of glia in, the mucosa. The mechanisms that have been studied and found to affect microbiota-mediated enteric neuronal activity and plasticity include GPCR-mediated signaling pathways (151), 5-HT, tryptamine (152), 5-HT_4 receptor activation (89), SCFAs (153), microbial-epithelial interactions (154), and the AhR (153–155).

The substantial limitations in translating observations made in GF animals to humans necessitate the implementation of functional studies to increase understanding of specific microbiota-driven activity and host metagenomics (156–166). The microbiota's important roles in ENS and CNS plasticity, however, make it a potentially valuable research direction. Finally, the ENS contributes to the composition of the microbiome, as alterations in the colonic and/or fecal microbiota have been observed in mice or zebrafish with congenital aganglionosis (167, 168). Whether these abnormalities represent direct effects of ENS circuits on microbiota or whether they are consequences of abnormal peristalsis due to aganglionosis remains to be established.

Conclusions

The conventional view of the gut-brain relationship has undergone considerable change in the time since the Second World War. At that time, the ganglia in the wall of the bowel were considered to be parasympathetic relays enabling the CNS to control the gut (169). The realizations that the ENS is truly massive (1, 2), that it also is complex with neurons of many different phenotypes and intricate microcircuits (13), and that it contains IPANs that allow it to monitor luminal contents (19, 20, 57) returned the early insights about the nature of the ENS to scientific prominence. In fact, the ENS has popularly been called the "second brain" to emphasize its independence from the CNS (1) or even the "first brain" to emphasize its presumed early evolution (13). More recently, the bidirectional nature of the gut-brain axis and, even more, the prominence of enteric microbiota in these interactions have brought us to our present excitement over the tripartite microbiota-gut-brain "connectome" and the reciprocal traffic in information among its components (77–82). Understanding human biology now requires that we look not only within our heads, but also deeply into our abdomens and to our microbial partners as well.

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