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Unexpected Roles for the Second Brain: Enteric Nervous System as Master Regulator of Bowel Function

Sabine Schneider,[1,*] Christina M. Wright,[1,*] and Robert O. Heuckeroth[1,2]

[1]Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA; email: heuckerothr@email.chop.edu

[2]The Children's Hospital of Philadelphia Research Institute, Abramson Research Center, Philadelphia, Pennsylvania 19104, USA

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*These authors contributed equally to this article.

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Abstract

At the most fundamental level, the bowel facilitates absorption of small molecules, regulates fluid and electrolyte flux, and eliminates waste. To successfully coordinate this complex array of functions, the bowel relies on the enteric nervous system (ENS), an intricate network of more than 500 million neurons and supporting glia that are organized into distinct layers or plexi within the bowel wall. Neuron and glial diversity, as well as neurotransmitter and receptor expression in the ENS, resembles that of the central nervous system. The most carefully studied ENS functions include control of bowel motility, epithelial secretion, and blood flow, but the ENS also interacts with enteroendocrine cells, influences epithelial proliferation and repair, modulates the intestinal immune system, and mediates extrinsic nerve input. Here, we review the many different cell types that communicate with the ENS, integrating data about ENS function into a broader view of human health and disease. In particular, we focus on exciting new literature highlighting relationships between the ENS and its lesser-known interacting partners.

INTRODUCTION

Digesting nutrients that fuel our survival requires complex integration of many bowel functions, and all must run smoothly to maintain a normal quality of life. Food must be broken into small particles and chemically digested for nutrient absorption. Indigestible components must efficiently pass through the gastrointestinal tract for elimination, while fluid and electrolyte balance is maintained. All of these processes occur in the setting of a complex microbiome that aids nutrient absorption but can trigger inflammation and even infection should the system go awry. The intestine could not coordinate these functions without the enteric nervous system (ENS), a complex network of neurons and glia that reside in the bowel wall and send nerve fibers throughout the bowel. Like an orchestra conductor, the ENS is a critical regulator of the many processes described above and interacts with an astounding array of cell types to facilitate bowel function.

The ENS is distributed all the way along the bowel in two layers called the myenteric and submucosal plexus (1). Each plexus is comprised of diverse enteric neuron and glial cell types that interact closely with each other and with other intestinal cells. Myenteric plexus cells cluster into ganglia between the outer longitudinal and inner circular smooth muscle of the bowel. Myenteric neurons provide the majority of direct innervation to the bowel's motor apparatus and the final output controlling bowel relaxation and contraction. Myenteric neurons interact closely with the tissue-resident macrophages (muscularis macrophages) that influence motility. Submucosal plexus ganglia reside between muscle and epithelium, where they regulate epithelial secretion and local blood flow. Neurons in both plexi respond to input from mucosal enteroendocrine cells and the autonomic nervous system. The ENS also interacts with immune and epithelial cells to promote barrier function that protects the bowel from pathogens in the gut lumen. Enteric neurons are currently classified by function, axon number, direction of axonal projections, synaptic connectivity, neurotransmitters, receptors, and electrophysiologic signatures. Approximately 20 enteric neuron subtypes and four glial subtypes have been characterized thus far, but a wealth of single-cell sequencing data is expected to redefine enteric neuron and glial subtypes in the next few years. Many excellent recent reviews detail known ENS circuitry, cell types, transmitters, and functions (2–5). Instead of duplicating those efforts, our goal is to show how the ENS interacts with non-ENS cell types and the implications of these interactions for human disease.

To facilitate communication, enteric neurons extend an elaborate network of neurites with associated glia. These ENS components contact almost all bowel cells, including muscle, epithelial cells, pacemaker cells [called the interstitial cells of Cajal (ICC)], blood vessels, and immune cells. Enteric neurons synapse on each other, but they also release neurotransmitters from varicosities along neurites to regulate smooth muscle cell (SMC) and ICC activity. The ICC act as pacemakers because they have intrinsic slow waves of depolarization and hyperpolarization (6).

The contractile force for bowel motility is provided by SMCs, which must coordinate activity to mix luminal contents or move undigested food toward the distal bowel for eventual elimination (7). To contract smooth muscle, myenteric plexus excitatory motor neurons project their axons predominantly orally. In contrast, inhibitory motor neurons project axons distally and cause smooth muscle relaxation. Simultaneous activation of excitatory and inhibitory motor neurons in a bowel region causes proximal bowel contraction and distal relaxation, a pattern called peristalsis that is frequently observed in the small intestine.

Additional enteric neuron types control epithelial secretion (secretomotor neurons), epithelial secretion and blood vessel dilation (secretomotor/vasodilator neurons), epithelial proliferation, or innervate enteroendocrine cells and lymphoid follicles. Enteric neurons also send intestinofugal fibers to the pancreas, gallbladder, prevertebral sympathetic ganglia, and the central nervous system (CNS) (4). Finally, although the ENS can control many aspects of bowel function autonomously, in vivo ENS activity is modulated by luminal contents (nutrients and microbes);

muscularis macrophages; parasympathetic neurons; sympathetic innervation from the celiac, superior, and inferior mesenteric ganglia; and hormonal signals (e.g., adrenaline, thyroid hormone, corticotrophin-releasing hormone, oxytocin). Many of these extrinsic signals are influenced by CNS activity, which explains how emotional responses like anxiety and fear can alter bowel function. The remainder of this article explores cell types that interact with the ENS, integrating basic science with human disease to examine how ENS function and dysfunction impact human health.

THE ENTERIC NERVOUS SYSTEM INTERFACES WITH THE SIP SYNCYTIUM TO REGULATE MOTILITY

ENS interactions with SMCs, ICC, and platelet-derived growth factor receptor (PDGFR)α+ cells are critical for bowel motility. Many excellent publications describe these interactions in detail (6–8), so here we highlight a few important interactions and recent developments.

Gap junctions connect SMCs, ICC, and fibroblast-like PDGFRα+ cells into a multicellular syncytium commonly called the "SIP syncytium," whose name derives from the first letter of each cell type (**Figure 1*a***) (6). The SIP syncytium receives input from excitatory and inhibitory motor neurons whose cell bodies reside within the myenteric plexus. Although SMCs were once considered the main targets of excitatory and inhibitory ENS motor neurons, neural input onto ICC and PDGFRα+ cells is likely also critical for mediating smooth muscle contractility and for generating complex motility patterns necessary for life.

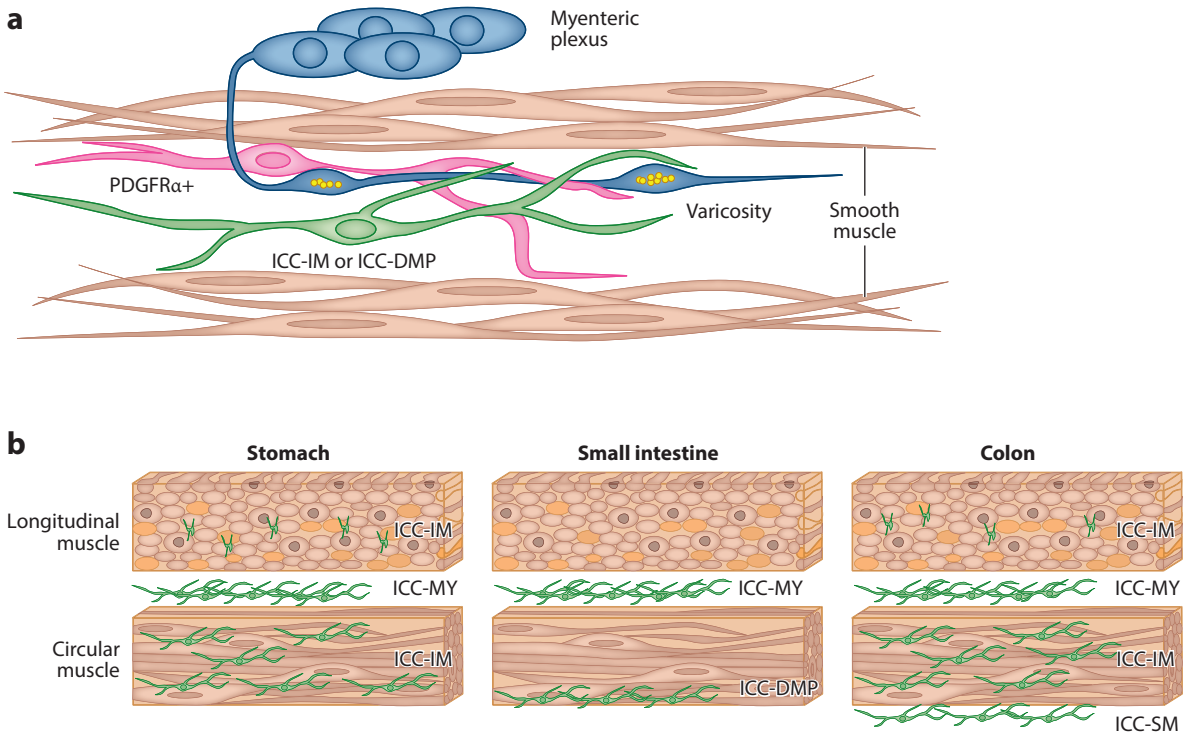

Figure 1

(*a*) The SIP syncytium is composed of SMCs, ICC, and PDGFRα+ cells that receive input from ENS varicosities. (*b*) ICC localization and composition varies with bowel region. Abbreviations: ENS, enteric nervous system; ICC, interstitial cells of Cajal; ICC-DMP, deep muscular plexus ICC; ICC-IM, intramuscular ICC; ICC-MY, myenteric ICC; ICC-SM, submucosal ICC; SMC, smooth muscle cell.

There are at least five types of ICC in the bowel: ICC-MY, located between the circular and longitudinal muscle layers; ICC-IM, located within the circular muscle layer in colon and stomach; ICC-DMP, between the inner and outer parts of the circular muscle layer of the small intestine; ICC-SM, on the submucosal surface of the circular muscle layer of colon; and ICC-SS, in the subserosal layer (**Figure 1*b***). ICC-MY and ICC-SM, the "pacemaker" cells of the bowel, generate rhythmic electrical slow waves of depolarization and hyperpolarization that propagate passively to SMCs via gap junctions and synchronize SMC contraction. This baseline electrical rhythm is present even when the ENS is absent. ICC-IM and ICC-DMP are closely associated with nerve varicosities (8), and electron microscopy suggests more nerve-ICC-IM contacts than nerve-SMC contacts (9). ICC-IM and ICC-DMP are believed to be the primary ICC that receive input from the ENS (8).

To determine how ICC contribute to neuromuscular transmission, many early studies compared SMC recordings in wild-type and ICC-IM–deficient animals during electric field stimulation. Although some investigators observed attenuated excitatory and/or inhibitory junction potentials in ICC-depleted smooth muscle, results were inconsistent (9–11). Possible explanations for the disparate findings include incomplete absence of ICC in certain model organisms [e.g., commonly used W/W^V mouse has inconsistent and incomplete ICC loss (6)], strain and species differences, differences in level of tissue precontraction or prerelaxation (12), use of nifedipine (12), differences between bowel regions, and developmental changes in innervation or smooth muscle reactivity in the absence of ICC. More recent studies test contributions of transduction pathways for specific neurotransmitters, often in genetically modified conditional knockout mice (10, 12, 13). These studies support the presence of parallel complementary pathways for signal transduction involving SMCs, ICC, and PDGFRα+ cells (**Figure 2*a*,*b***).

Inhibitory Signaling

Inhibitory junctional potentials (IJPs) recorded from colon smooth muscle are composed of a fast purinergic phase that is followed by a slower nitrergic phase (13). The purinergic contribution to the IJP is probably mediated by PDGFRα+ cells, which express high levels of the purine receptor $P2Y_1$. The ligand for $P2Y_1$ receptors ($P2RY_1$) in the bowel was once thought to be adenosine 5′ triphosphate (ATP), but in recent years, β-nicotinamide adenine dinucleotide (β-NAD) and adenosine 5′-diphosphate-ribose (ADPR) have emerged as more likely candidates (14). Applying β-NAD and ADPR to PDGFRα+ cells activates apamin-sensitive, small-conductance Ca^{2+}-activated K^+ (SK) channels, leading to potassium efflux and membrane hyperpolarization (15). The change in membrane potential spreads via gap junctions to nearby SMCs. Purinergic receptors are also expressed on SMCs and ICC, but they are unlikely to mediate the strong fast hyperpolarization associated with the IJP (14, 16).

Nitric oxide (NO) is a major signaling molecule in inhibitory motor neurons that acts on both ICC and SMCs. NO binds and activates nitric oxide–sensitive guanylyl cyclase (NO-GC). Guanylate cyclase converts GTP to cGMP, which activates GMP-dependent protein kinase 1 (PRKG1). PRKG1 in turn phosphorylates serines and threonines on many intracellular proteins, causing hyperpolarizing via mechanisms that remain incompletely understood. Molecules downstream of NO (e.g., NO-GC, *Prkg1*) have been deleted from subsets of ICC and SMCs using conditional knockout mice. Although inconsistent findings have been reported, the results suggest that both ICC and SMCs likely mediate NO signaling (10, 13). Interestingly, one study found that conditionally deleting NO-GC from ICC in mouse colon reduced IJP amplitude in response to electric field stimulation, but conditionally deleting NO-GC from ICC in mouse fundus completely abolished IJPs (13). This underscores an important and underemphasized issue in the literature: Regulation

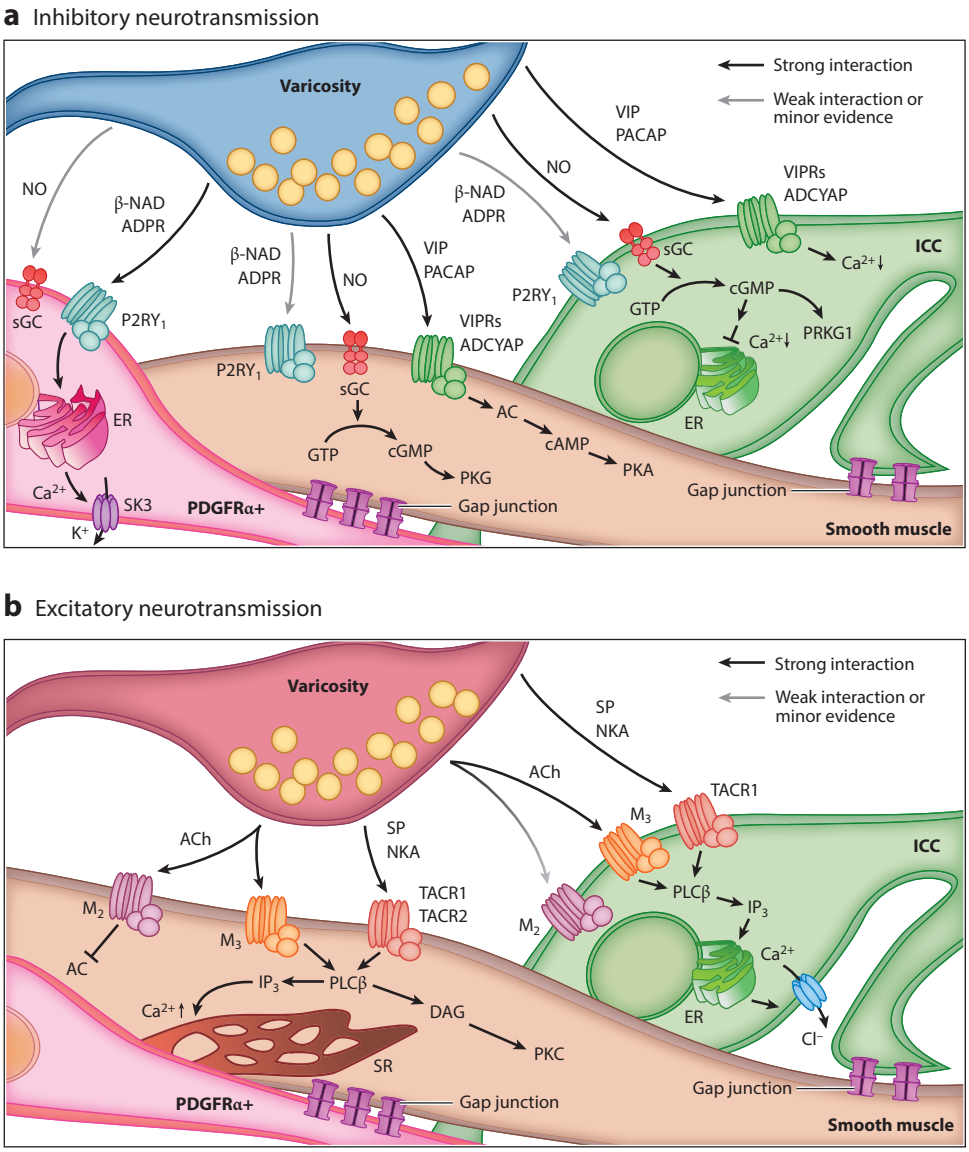

Figure 2

(*a*) Inhibitory neurotransmission involves a combination of purine, NO, and VIP/PACAP signaling. (*b*) The primary neurotransmitters involved in excitatory neuromuscular transmission are acetylcholine and tachykinins. Abbreviations: AC, adenylate cyclase; ACh, acetylcholine; ADCYAP, pituitary adenylate cyclase-activating polypeptide; ADPR, ADP-ribose; β-NAD, β-nicotinamide adenine dinucleotide; cAMP, cyclic AMP; cGMP, cyclic GMP; DAG, diacylglycerol; ER, endoplasmic reticulum; GTP, guanosine triphosphate; ICC, interstitial cells of Cajal; IP_3, inositol triphosphate; M_2, muscarinic receptor 2; M_3, muscarinic receptor 3; NKA, neurokinin A; NO, nitric oxide; PACAP, pituitary adenylate cyclase–activating peptide; PKA, protein kinase A; PKC, protein kinase C; PKG, protein kinase G; PLCβ, phospholipase C beta; PRKG1, GMP-dependent protein kinase 1; sGC, soluble guanylate cyclase; SK3, small conductance calcium–activated potassium channel 3; SP, substance P; SR, sarcoplasmic reticulum; TACR1/2, tachykinin receptor 1 or 2; VIP, vasoactive intestinal peptide; VIPR, VIP receptor.

of ICC, SMCs, and PDGFRα+ cells by neural signaling may vary considerably depending on bowel region. Consistent with this observation, ICC localization and receptor expression differ in various bowel regions (**Figure 1*b***). For instance, ICC-MY in stomach do not express the NO-GC subunit sGCβ1, whereas most ICC-MY in colon express sGCβ1, and some ICC-MY in colon express sGCβ1 at high levels (17).

A few recent articles suggest that PRKG1-independent (but NO-GC– and cGMP-dependent) NO signal transduction may occur in ICC (18, 19). Pacemaker rhythms in cultured colon ICC were slowed by NO donors and cGMP but not by a PRKG1 inhibitor (18). Small-molecule activation of NO-GC decreased spontaneous calcium transients in ICC-DMP in mouse small intestine, but PRKG1 inhibition had no effect (19). These findings are difficult to reconcile with data showing absent NO-mediated IJPs in *Prkg1* conditional knockout mice (10). Possible explanations for the discrepancy include failure of the PRKG1 inhibitors to penetrate tissue or differences between the ICC subtypes studied.

Although the literature on how NO affects smooth muscle and ICC is sometimes contradictory, NO clearly has strong effects on both ICC and SMCs in a guanylate cyclase–dependent manner. Further research is needed to determine (*a*) how members of the SIP syncytium in various bowel regions differ in receptor expression and response to signaling, (*b*) how innervation of the SIP syncytium differs in each bowel region, and (*c*) the significance of PRKG1-independent pathways in NO-mediated responses. Defining these characteristics may elucidate molecular mechanisms that generate diverse motility in each bowel region.

In addition to NO, inhibitory enteric neurons release vasoactive intestinal peptide (VIP) and pituitary adenylate cyclase–activating peptide (PACAP). These neuropeptides likely signal through VIP receptors VIPR1 and VIPR2 and the PACAP receptor ADCYAP1R1, which are expressed on ICC and SMCs (7). Typically, neuropeptides are released at high-stimulus frequencies, so VIP and PACAP may play less prominent roles in direct motor neurotransmission than NO (7). In SMCs, VIP/PACAP signaling is mediated via G_s, which activates adenylyl cyclase, cyclic AMP(cAMP), and protein kinase A (PKA) (20). This increases Ca^{2+} transients that should increase the opening probability of Ca^{2+}-activated K^+ channels, causing membrane hyperpolarization (20). In ICC, VIP may act via a different pathway. VIP decreases calcium transients dramatically in some ICC, and calcium transients increase when a VIP inhibitor is added. These calcium transients likely control the activity of calcium-dependent chloride channels (CaCCs) that regulate slow waves (19). The mechanism behind this response is currently unknown.

Excitatory Signaling

The major excitatory neurotransmitters involved in direct motor neurotransmission are acetylcholine (ACh) and tachykinins [neurokinin A (NKA) and substance P]. ACh binds muscarinic M_2 and M_3 receptors on SMCs and ICC, while tachykinins bind tachykinin receptor 1 (TACR1) and 2 (TACR2) on SMCs and TACR1 on ICC (**Figure 2*b***). In ICC-DMP, signal transduction via M_3 receptors likely occurs through G_q, leading to Ca^{2+} release from the endoplasmic reticulum and activation of CaCCs such as anoctamin 1 (Ano1). Opening of CaCCs causes a depolarizing current predicted to enhance the likelihood of action potential generation and increase contraction amplitude (21, 22). M_2 receptors are also expressed on ICC but may play a less important role than M_3 receptors (21, 23). In contrast, evidence from knockout mice suggests that both M_3 and M_2 receptors influence SMC contractility (24).

Like muscarinic signaling in ICC, tachykinin signaling via TACR1 is hypothesized to act via G_q signaling coupled to Ca^{2+} release and CaCC opening. Tachykinin signaling may be more important than muscarinic signaling in ICC-DMP. Applying TACR1 antagonists attenuated basal

Ca^{2+} transients in small intestine ICC-DMP, suggesting that ICC-DMP are tonically excited by tachykinins (21). The same may not be true in colonic ICC, which do not express TACR1 as highly (21). In SMCs, tachykinin signaling is transduced by both TACR2 and TACR1 (25). Second messenger signaling likely occurs through protein kinase C (PKC) and IP_3 (7), although other pathways may be involved (25).

The Enteric Nervous System, SIP Syncytium, and Bowel Motility: Putting It All Together

When considering direct neural input to the SIP syncytium, it is important to remember that diverse motor patterns are needed for food to be digested and absorbed and for waste to be eliminated. These motility patterns in human small intestine include peristalsis (waves of contraction and relaxation that propagate down the bowel), segmentation (alternating contraction and relaxation to mix food with digestive enzymes and bile), and the migrating motor complex (MMC) (where strong waves of contraction and relaxation propagate down the bowel during phase III to move luminal contents toward distal bowel for elimination). In the colon there are high-amplitude propagating contractions (HAPCs) that move stool over long distances toward the rectum and occur only occasionally. Generation and maintenance of these motor patterns require a complex interplay between neural signaling, ICC, PDGFRα+ cells, and SMCs. In broad strokes, ICC continually produce oscillating electrical slow waves in the bowel that set the rhythm for many motor patterns. Slow wave electrical activity propagates from ICC to SMCs, generating rhythmic SMC depolarization and contraction. Neural signaling onto the SIP syncytium generates and modulates ICC and SMC activity to produce the motor patterns described above. Stimuli from the environment alter ENS activity to determine which motor patterns should occur at specific times (7). For example, segmentation in the small bowel occurs after meals, but phase III MMC predominates once most nutrients are absorbed. Although the ENS is not essential for generating some motor patterns, the ENS is probably the primary inducer of complex motility patterns needed for survival (26).

To "decide" what to do, sensory neurons of the ENS [called intrinsic primary afferent neurons (IPANs)] respond to diverse stimuli including stretch and mucosal distortion (1, 4). Stretch sensing is mediated by mechanoreceptors located within the myenteric plexus, which fire in response to distention (27). Historically, it has been assumed that stimulation of enterochromaffin (EC) cells in the bowel mucosa and the resulting serotonin (5-HT) release onto IPANs play a major role in evoking enteric neural reflexes such as the peristaltic reflex. However, recent papers question the importance of serotonin release from EC cells for initiation of motility, as mice lacking a critical enzyme involved in 5-HT biosynthesis in EC cells [tryptophan hydroxylase 1 (TPH1)] survive to adulthood and have a normal gastrointestinal transit time (28). Further research has shown that these mice have larger fecal pellets and aberrant colonic migrating motor complexes (29), suggesting that in the absence of 5-HT from EC cells, a higher degree of stretch is required to trigger contractions (30).

We have focused thus far on neurotransmission to the SIP syncytium, but enteric glia also influence bowel motility. Female mice lacking enteric glia have reduced gastrointestinal transit time and increased colonic MMC frequency compared to controls. Intriguingly, the same effect was not seen in males, suggesting potential glia-intrinsic, sex-dependent differences or sexual dimorphism in glial interacting partners (31). Other studies also suggest that enteric glia influence bowel motility; for instance, mice lacking the glial-specific hemichannel connexin-43 had prolonged colonic transit, increased stool water content, and diminished contraction and relaxation amplitude (32). Mechanisms behind glial control of motility are not well understood. One

hypothesis is that trophic factors produced by glia may prevent ENS dysfunction. Another possibility is that enteric glia directly modulate ICC slow wave activity (33).

Clinical Relevance

Human bowel motility disorders include life threatening problems like Hirschsprung and chronic intestinal pseudo-obstruction (CIPO) syndrome. In these disorders, ENS or SIP syncytium function is so disrupted that surgical intervention or intravenous nutrition is often needed for survival.

Hirschsprung disease. The ENS forms from neural crest–derived precursor cells (ENCDCs) that colonize the bowel during first trimester fetal development. In approximately 1:5,000 children, ENCDCs never reach the distal bowel, resulting in a region where the ENS is completely absent. This problem is called Hirschsprung disease. In children with Hirschsprung disease, the region of bowel that lacks enteric ganglia (i.e., aganglionic bowel) is tonically contracted and does not have propagated motility, leading to functional obstruction. Because aganglionic bowel does not efficiently pass stool or air, Hirschsprung disease symptoms include distension, constipation, vomiting, abdominal pain, growth failure, and a predisposition to bowel inflammation (called enterocolitis) that may lead to death from sepsis. Hirschsprung disease provides absolute proof that the ENS is essential for life, as even a small region of aganglionosis can cause serious illness and premature death. Our recent review provides more detailed information about Hirschsprung disease symptoms and molecular mechanisms (34).

Chronic intestinal pseudo-obstruction syndrome. When the ENS is present throughout the bowel, but bowel motility does not consistently support survival or growth without at least intermittent intravenous nutrition, CIPO is the likely diagnosis. Symptoms of CIPO include repetitive episodes of abdominal distension and pain, vomiting, growth failure, and weight loss (35). CIPO may occur as a primary disorder, or it may be secondary to complications of another disease. CIPO should not be confused with transient bowel motility defects such as ileus (the absence of bowel contractions) that commonly accompany abdominal surgery, pancreatitis, appendicitis, or sepsis.

The etiology of CIPO remains poorly understood, although clues are emerging from human and mouse genetics. Because so many cell types, transmitters, and signaling molecules impact ENS development and intestinal function, there are likely many underlying causes of CIPO, potentially including dysfunction of the ENS, SMCs, ICC, and/or PDGFRα+ cells. Unfortunately, even with advanced genetic tools like whole-exome sequencing, causative genetic variants remain poorly defined. Only a few genetic causes of CIPO have been identified. These include mutations in the genes encoding filamin A (*FLNA*), DNA polymerase gamma (*POLG*), gamma smooth muscle actin (*ACTG2*), leiomodin 1 (*LMOD1*), myosin heavy chain 11 (*MYH11*), and myosin light chain kinase (*MYLK*) (35, 36). Identifying more genetic causes of CIPO is critical, as it will undoubtedly aid diagnosis. New medicines to treat CIPO are also desperately needed. Available prokinetic medications prevent ACh degradation (e.g., pyridostigmine), activate 5-HT4 receptors (enhancing ACh release from excitatory motor neurons and increasing bowel motility; e.g., cisapride, tegaserod, prucalopride), inhibit dopamine receptors (metoclopramide, domperidone), activate somatostatin receptors (octreotide), or activate receptors for motilin, a peptide that increases gastrointestinal motility (e.g., erythromycin). Unfortunately, many of these medicines have serious side effects or are only minimally effective at enhancing small bowel motility and resolving symptoms for people with serious motility disorders like CIPO (35, 37).

THE ENTERIC NERVOUS SYSTEM AND VASCULAR ENDOTHELIUM

During digestion, blood flow to intestinal mucosa increases as much as twofold owing in part to dilation of submucosal arterioles. Vasodilation and resulting hyperemia are needed to meet the high metabolic demands of the mucosa and to exchange nutrients, water, and solutes across bowel epithelium. Neurogenic vasodilation of submucosal arterioles is mediated by extrinsic and intrinsic (ENS) innervation. In contrast, vasoconstriction of bowel arterioles is wholly under control of extrinsic sympathetic innervation. Here, we briefly review ENS control of vasodilation, which has been extensively characterized.

Most studies of submucosal arteriole vasodilation evaluated guinea pig small intestine, using varied preparations, including isolated submucosa and full thickness intact bowel. These studies suggest many triggers for ENS-mediated vasodilation, including gently stroking bowel mucosa, distorting the mucosa with puffs of gas, and distending smooth muscle with a balloon. Small distortions of the mucosa (stroking, puffing gas) cause EC cells to release 5-HT onto enteric nerve terminals expressing 5-HT_3 [myenteric IPANS (38)] and 5-HT_4, and/or 5-HT_{1P} [submucosal IPANs (39)]. Stimulation of these IPANs by 5-HT activates short (1–2 mm) reflex pathways within the submucosa, as well as longer reflex pathways that span submucosal and myenteric plexus (39). Stretching the bowel activates 5-HT_3- and 5-HT_4-insensitive mechanotransducers in the myenteric plexus (40) and possibly submucosal plexus (41), leading to vasodilation. Mechanical deformation of the gut activates neurites and soma of a large number of mechanosensitive enteric neurons within the myenteric plexus (27). These mechanosensitive neurons are multifunctional (i.e., they may be afferents, interneurons, or efferents), and their responses to mechanical stimulation may be rapid adapting, slow adapting, or ultraslow adapting (42).

A final common pathway for ENS-mediated vasodilation in guinea pig small intestine is release of ACh onto endothelial cells, which activates muscarinic M_3 receptors, leading to NO release and vasodilation (43). In guinea pig distal colon, substance P and/or VIP release from submucosal plexus neurons may contribute to vasodilation (44). The ENS may also stimulate mast cells to release the vasodilator histamine directly onto submucosal blood vessels (43) via substance P and/or calcitonin gene–related peptide (CGRP) (45).

Clinical Relevance

Impaired vascular control has been implicated in a number of inflammatory conditions, including necrotizing enterocolitis (NEC) and inflammatory bowel disease (IBD). NEC is a dangerous bowel disease in premature neonates, characterized by severe inflammation, ischemic necrosis, and sometimes bowel perforation. It is tempting to hypothesize that neuron dysfunction may contribute to development of necrotizing enterocolitis, for instance through dysregulation of vasodilation that occurs in response to feeding. Indeed, altered microcirculation involving constricted arterioles has been demonstrated in NEC (46), and damage to the ENS also occurs with NEC (47). However, there is not yet convincing evidence that ENS dysfunction is the primary cause of altered blood circulation in babies with NEC. Arguing against this hypothesis, vessel endothelial cells from bowel with NEC failed to generate NO in response to ACh, but vessels dilated in response to exogenous NO administration, suggesting that dysfunction of endothelial cells may be paramount (48). This is similar to IBD, where vessel endothelium does not appropriately produce NO even when stimulated by ACh (49). Unfortunately, because defects seem intrinsic to endothelial cells, neuromodulators (e.g., AChE inhibitors) are unlikely to have therapeutic value in these disorders, although gut-derived neural stem cells do appear to prevent NEC-like injury in a rodent model (47).

EPITHELIAL SECRETION AND THE ENTERIC NERVOUS SYSTEM

5-HT released from enteroendocrine cells in response to mucosal stroking not only elicits peristalsis and supports vasodilation, but it also activates fluid and electrolyte secretion into the gut lumen to facilitate digestion. 5-HT activates IPANS that in turn synapse on secretomotor neurons that release ACh or VIP. ACh acts via muscarinic receptors and VIP via VIPR1 receptors on crypt epithelial cells to increase intracellular Ca^{2+} (via phospholipase C and IP_3) and cAMP (via G_s), respectively. Cyclic AMP activates the cystic fibrosis transmembrane regulator (CFTR) chloride channel. Calcium activates the HCLCA1 chloride channel, inducing more transient chloride flux. Movement of chloride into the gut lumen is accompanied by sodium and water. The details of these circuits are much better understood than this brief description suggests, and they are beautifully described in reviews (50, 51).

Interestingly, a recent publication has also defined a role for enteric glia in the regulation of epithelial ion transport. When glial cells lack the glial-specific hemichannel, connexin-43 neuron-regulated electrogenic ion transport was reduced, while transmural conductance and epithelial permeability were not significantly changed. Activation of glial fibrillary acidic protein (GFAP)-expressing glial cells stimulated electrogenic ion transport similar to that observed with direct neuronal stimulation. Inhibiting neuronal activation with tetrodotoxin only partially reduced glial-induced electrogenic ion transport, indicating that the enteric glia interactions with the epithelium do not require neuronal activity (52).

Clinical Relevance

Dysfunctional regulation of epithelial secretion may lead to increased stool water content. VIP-producing tumors and serotonin reuptake inhibitor (SSRI)-induced serotonin syndrome cause diarrhea by directly increasing neurotransmitters in the ENS circuit that controls epithelial secretion (53). Cholera, rotavirus, *Clostridium difficile*, *Cryptosporidium*, and enterotoxin producing *Escherichia coli* all cause profuse watery diarrhea at least in part by activating ENS circuits (54, 55).

THE ENTERIC NERVOUS SYSTEM, EPITHELIAL PROLIFERATION, AND REPAIR

Bowel epithelium is replaced every few days via proliferation of stem cells and transit-amplifying cells in the crypt. Newly generated cells differentiate into absorptive epithelial cells, goblet cells, and EC cells (among other cells types) before being lost via apoptosis. Regulation of epithelial proliferation and differentiation is carefully controlled because too little epithelial replacement reduces absorptive capacity, and too much epithelial proliferation causes cancer. Among other regulatory mechanisms, accumulating data suggest that the ENS influences epithelial proliferation and repair, as well as epithelial barrier function, but that effects of the ENS on bowel epithelium are complex.

For example, chemical ablation of the myenteric plexus with benzalkonium chloride increases epithelial proliferation, crypt depth, and villus height (56), suggesting inhibitory effects of the ENS on epithelial renewal. Consistent with the hypothesis that ENS components reduce epithelial proliferation, mice with a hypomorphic ENS because of mutations in the tyrosine kinase receptor *Ret* have enhanced small bowel epithelial proliferation after small bowel resection during the adaptive response (57). In contrast, loss of the hepatocyte growth factor receptor *Met* within the ENS leads to reduced epithelial proliferation after dextran sodium sulfate (DSS)-induced bowel injury (58). Because *Met* and *Ret* are expressed in different subsets of myenteric neurons, these

observations suggest that some enteric neurons enhance and others suppress bowel epithelial proliferation depending on context.

Consistent with this hypothesis, ACh activates muscarinic receptors on intestinal stem or progenitor cells to enhance epithelial proliferation (59–62). Neuron-derived serotonin activates 5-HT(2A) receptors on cholinergic neurons, enhancing both ACh release (63) and epithelial proliferation. Interestingly, glucagon-like peptide 2 (GLP-2), a potent stimulant for epithelial proliferation, might also work via the ENS because GLP-2 receptors are expressed by enteric neurons but not by intestinal epithelium (64). Alternatively, GLP-2 might support epithelial proliferation via subepithelial myofibroblasts that express GLP-2 receptor and release insulin-like growth factor-1 (IGF-1) in response to GLP-2. In support of this hypothesis, IGF-1 is required for GLP-2 intestinotrophic effects, and this mechanism could bypass the ENS (65).

In addition to regulating epithelial cell proliferation, enteric neurons appear to enhance epithelial barrier function. Co-culture of primary enteric neurons with primary intestinal epithelial stem cells increases expression of tight junction–associated protein zona occludens-1 (ZO-1), increases transepithelial resistance across the monolayer, and reduces apical to basolateral dextran permeability (66). Parallel studies suggest that enteric glia may also influence intestinal epithelial barrier function (67–70). For these studies, enteric glia were eliminated using a transgenic GFAP-Cre driver to induce expression of HSV-TK or using the GFAP promoter to drive expression of the neoantigen haemagglutinin. In the former case, treatment with ganciclovir induced cell death in any cell that expressed the transgene by conversion of the antiviral agent to a toxic nucleotide analog. In the latter case, neoantigen expression led to $CD8^+$-mediated cell death of any neoantigen-expressing cell. Eliminating glia in these ways led to massive bowel inflammation. Co-culture of epithelial cells and glia also changed gene expression (71) in epithelial cells, increasing tight junction proteins (like ZO-1 and occludin) and decreasing transepithelial permeability in cultured epithelial monolayers (69–71). *S*-nitrosoglutathione was identified as a soluble glial-derived factor that increased epithelial barrier function in vitro. This compound drastically reduced the development of enterocolitis in vivo after HSV-TK–mediated damage to the ENS and epithelium (69). Another study found that mucosal glia strongly upregulate their expression of glial cell line–derived neurotrophic factor (GDNF) in states of inflammation (72, 73). GDNF reduced epithelial apoptosis in vitro (74, 75), and in a mouse model of DSS-colitis, GDNF overexpression increased tight junction protein expression and decreased epithelial permeability (76). Interestingly, enteric GDNF also activates RET on type 3 innate lymphoid cells to induce release of interleukin (IL)-22, which enhances epithelial expression of genes that reduce bacterial translocation (77). Collectively these studies provided substantial support for the hypothesis that enteric glia enhance epithelial barrier function.

Surprisingly, an elegant set of experiments showed that enteric glial cells are not required for maintenance of epithelial integrity in mice. The authors eliminated all enteric glia by inducing cholera toxin subunit A expression under the control of the PLP1 promoter. In this system, no enterocolitis developed, and epithelial cell ultrastructure, proliferation, and permeability were unaffected despite a dramatic loss of enteric glia. The absence of glia in the setting of DSS-induced colitis did not worsen symptom severity or specifically affect transepithelial permeability. Lastly, the authors showed that the discrepancy between their study and the previous in vivo studies could be explained by the aberrant GFAP-Cre transgene expression in a small number of epithelial cells that caused direct injury to the epithelial cell layer after ganciclovir treatment or induction of neoantigen expression (31).

Overall, it is clear that the ENS influences intestinal barrier function in a normal physiologic state and during inflammation. The exact contributions of the different enteric neuron subtypes and of enteric glia have not yet been conclusively elucidated.

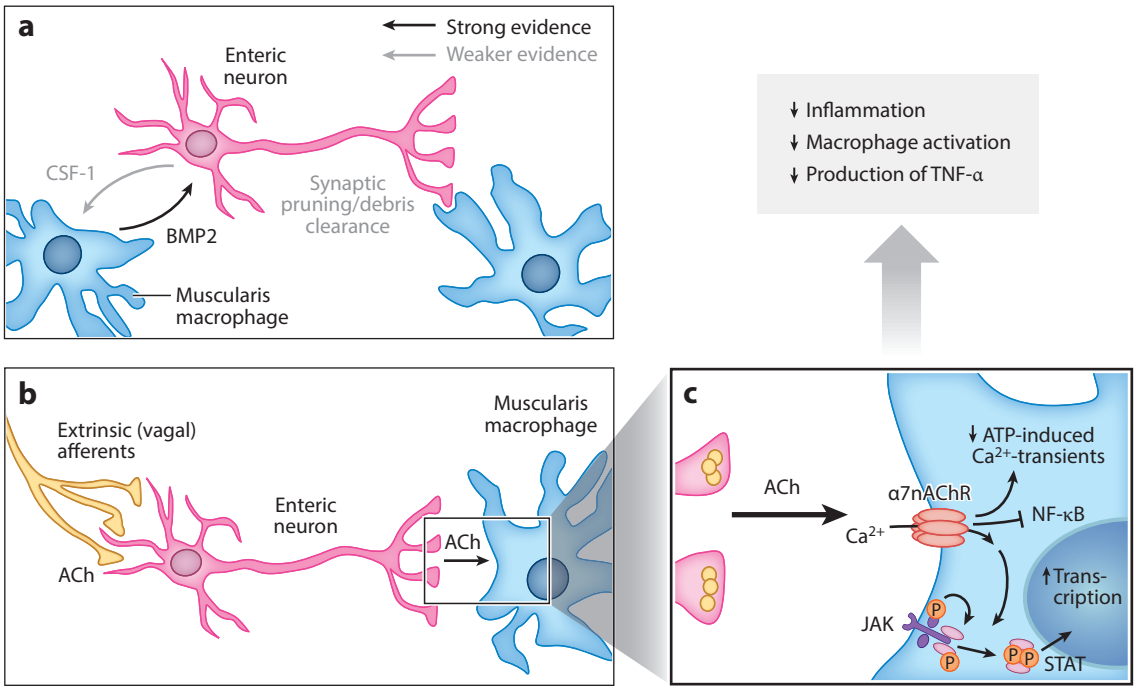

Figure 3

(*a*) Muscularis macrophages support enteric neurons through BMP2 signaling and possibly synaptic pruning and clearing of debris. (*b*) Enteric neurons are a key part of the CAIP, reducing the activation of macrophages through vagal stimulation. (*c*) Activation of muscularis macrophages by ACh decreases macrophage activation and decreases production of TNF-α. Abbreviations: α7nAChR, α7 nicotinic acetylcholine receptor; ACh, acetylcholine; ATP, adenosine triphosphate; BMP2, bone morphogenetic protein 2; CAIP, cholinergic anti-inflammatory pathway; CSF-1, colony stimulating factor 1; JAK, Janus kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; STAT, signal transducer and activator of transcription; TNF-α, tumor necrosis factor alpha.

THE ENTERIC NERVOUS SYSTEM AND MACROPHAGES

Two broad classes of tissue-resident macrophages contribute to intestinal immune function. The most abundant macrophage (Mφ) class reside in the lamina propria (LpMφ) directly beneath bowel epithelium (78) and are surrounded by dense submucosal neuron projections that innervate the epithelial layer. Direct functional interactions between ENS and LpMφ have not yet been documented but seem plausible. The second, relatively understudied group called muscularis macrophages (MMφ) are very closely associated with the myenteric plexus, and emerging literature suggests significant cross talk and even developmental interdependence between these cell types (**Figure 3**).

The Enteric Nervous System Depends on MMφ for Normal Structure and Function

MMφ produce bone morphogenetic protein 2 (BMP2), a morphogen that binds its receptor BMPRII on neurons and influences ENS precursor differentiation, neurite fasciculation, and ganglion formation (79, 80). Mice lacking MMφ during development have increased numbers of enteric neurons and a disorganized ENS. Interestingly, this phenotype is similar to what occurs when the BMP antagonist noggin is overexpressed in vivo (79, 81). Acute depletion of MMφ from

the bowel in adulthood enhanced colon contractility in response to stretch ex vivo, but it delayed expulsion of a bead by the colon in vivo, suggesting problems with coordinating muscle activity. The motility changes were not limited to the colon, as gastric emptying was also accelerated two days after acute MMφ depletion. The effects of MMφ depletion on ex vivo colon contractility could be mimicked by dorsomorphin (a BMP-signaling inhibitor) or rescued by adding BMP2. Consistent with these observations, nuclear localization of pSMAD1/5/8 (a downstream effector complex for BMP signaling) was reduced in enteric neurons from MMφ-depleted bowel. Interestingly, antibiotic treatment to reduce luminal microbes reduced bowel MMφ, BMP2 levels, and nuclear pSMAD1/5/8 in enteric neurons. Antibiotics increased whole-bowel transit time and enhanced colon contractility ex vivo, suggesting dysfunctional bowel motility (81). Taken together, these results offer strong evidence that enteric neurons rely on BMP2 produced by MMφ for normal morphology and function. These observations also suggest that postnatal acquisition of intestinal microbes critically shapes neuroimmune interactions in the bowel. Some authors have suggested that MMφ also contribute to normal ENS morphology by phagocytosing neuronal debris (82). This function of MMφ would be similar to that of microglia, the tissue-resident macrophages of the brain, which clear apoptotic neurons and eliminate unnecessary axons and synaptic connections (i.e., synaptic pruning). Further research is needed to determine the extent to which phagocytosis by MMφ shapes the ENS.

Activated MMφ Regulate Enteric Neuron Excitability During Inflammation

Activated murine MMφ release chemokines, cytokines [IL-1, monocyte chemoattractant protein 1, IL-6, tumor necrosis factor-alpha (TNF-α)], and other bioactive compounds (NO, prostaglandins) (83, 84). NO regulates neuron excitability and directly relaxes SMCs, so it is not surprising that increased NO reduces bowel contractility. Some enteric neurons also produce TNF-α receptors, and TNF-α activates neuropeptide Y (NPY) expression. NPY increases bowel epithelial permeability during inflammation (85), suggesting a neuroimmune interaction that might be modulated to reduce intestinal inflammation.

The Enteric Nervous System Modulates MMφ Activation and May Affect MMφ Survival

Macrophages express many neurotransmitter receptors, including α7 nicotinic acetylcholine (α7nAChR), tachykinin, glycine, and P2 purine receptors, all of which may alter MMφ function (84). The most well-characterized example of ENS modulation of MMφ is the cholinergic anti-inflammatory pathway (CAIP), a circuit involving the vagus, ENS, and MMφ. Stimulating the vagus nerve activates cholinergic enteric neurons near MMφ, leading to the release of ACh and stimulation of α7nAChR on MMφ. This reduces inflammation by decreasing the ATP-induced calcium transients in MMφ, decreasing macrophage activation (86) and reducing TNF-α production by macrophages (87). α7nAChR also activates the Jak2-STAT3 signaling pathway, which likely plays a role in attenuating macrophage activation (88). ENS-MMφ signaling is likely not limited to ACh-α7nAChR interactions given the large number of neurotransmitter receptors expressed by macrophages. Extrinsic nerves also modulate MMφ activation directly through norepinephrine signaling onto adrenoreceptor β2, but this pathway is probably independent of the ENS (78).

Intriguingly, adult enteric neurons express colony-stimulating factor-1 (CSF-1), the primary survival factor for MMφ (81). Because other CSF-1–expressing cell types had not been identified in the bowel, we hypothesized that MMφ rely on CSF-1 produced by enteric neurons for normal

development. Surprisingly, MMφ appear normal in neonatal bowel even in the absence of an ENS. Other intestinal cell types (endothelial cells, ICC) produce CSF-1 perinatally and may be the main cells supplying MMφ with CSF-1 in early life (89). As a considerable ENS development occurs after birth, ENS-MMφ interactions likely mature postnatally, and it remains possible that CSF-1 expressed by neurons is critical for MMφ survival in adulthood. Global knockout models of CSF-1 (i.e., *Csf1*$^{op/op}$ mice) lack all MMφ, but thus far, conditional depletion of CSF-1 from enteric neurons has not been reported. This experiment would be needed to confirm that ENS-derived CSF-1 influences adult MMφ number or function.

Clinical Relevance

Postoperative paralytic ileus is a common condition characterized by transiently impaired bowel motility after abdominal surgery. Postoperative ileus may be partially mediated by low-grade inflammation leading to impairment of muscle contractility. Supporting a role for MMφ in postoperative ileus, depletion of MMφ by clodronate or genetic MMφ loss in *Csf1*$^{op/op}$ mice protects against postoperative ileus (90). Intriguingly, vagal nerve stimulation reduced inflammation and improved postoperative ileus (91). This is likely mediated by the vagal-ENS-MMφ anti-inflammatory pathway described previously (86). MMφ activation has also been implicated in other gastrointestinal motility disorders such as gastroparesis, which also may be helped by vagal stimulation. This topic is summarized in a number of excellent recent reviews (83, 84).

ENTERIC NERVOUS SYSTEM INTERACTIONS WITH THE MICROBIOME

After birth, the bowel must grapple with drastic changes, as it is exposed to a diverse array of microbes seeking to establish themselves within the gut lumen. Microbial colonization occurs while the ENS is still maturing, and we now believe these microbes substantially affect ENS development. At least two studies have shown reductions in neuron density in the small intestine and/or colon of young germ-free mice compared to age-matched gnotobiotic controls (92, 93). Oddly, one study reported an increased proportion of nitrergic myenteric neurons in their 3-day-old germ-free mice (93), while the other study, which used 4-week-old germ-free mice, reported decreased nitrergic neuron numbers (92). The cause of this difference is unclear.

A few mechanisms for microbial effects on ENS development have been proposed. Interestingly, enteric neurons and glia express Toll-like receptors (TLRs), pattern recognition receptors that are activated by pathogen-associated molecular patterns (PAMPs) as part of the innate immune system. TLRs 3, 4, and 7 are expressed in enteric neurons and glia (94). Additionally, TLR2 may be expressed in the ENS and in surrounding cells including SMCs (95). Global deletion of TLR2 reduced the number of nitrergic neurons in colon and accelerated gastrointestinal transit (95). Mice with a global deletion of TLR4, which detects lipopolysaccharide (LPS, a major component of gram-negative bacteria), had reduced numbers of nitrergic neurons, slow bowel motility, and reduced bowel relaxation in response to electric field stimulation. Conditional knockout mice missing *Myd88* selectively in neurons and glia also had delayed colon transit and reduced nitrergic neurons (92) MYD88 is a mediator of TLR4 activity. Finally, a high-fat diet, which causes dysbiosis, also reduced NO-producing myenteric neuron number and slowed bowel motility in a TLR4-dependent fashion (96).

One possible explanation for these findings is that microbes could activate TLRs on enteric glia or mesenchymal cells, leading to release of the RET ligand GDNF. GDNF provides trophic support for enteric neurons expressing RET and its coreceptor GDNF family receptor alpha 1

(GFRα1) during ENS development (97). In addition, GDNF contributes to motility by stimulating the ascending limb of the peristaltic response (98). In support of this theory, mice lacking TLR2 had significantly reduced GDNF in muscle. Administering recombinant GDNF to these mice subcutaneously for seven days restored neuron numbers and reversed dysmotility (95). Some evidence suggests that enteric glia produce GDNF in response to inflammation (72), making them a plausible intermediary in this pathway, although SMCs or other mesenchymal cells could also be involved.

In addition to developmental effects, the microbiome can affect the ENS in adult life. Microbiome composition directly affects mature ENS function by altering electrophysiologic properties of neuron subtypes (99) and leading to changes in intestinal motility and neurally mediated secretion (100, 101). Furthermore, giving mice antibiotics reduces neuron numbers and slows gastrointestinal transit (92). The ENS can also influence the composition of the microbiome. In a zebrafish model of Hirschsprung disease, the pathogenic overabundance of proinflammatory bacterial strains could be corrected simply by restoring ENS function (102). These observations highlight just a few of the complex interactions between gut microbes and the ENS; a more detailed description of ENS-microbiome interactions can be found in recent reviews (103, 104).

ENTERIC NERVOUS SYSTEM INTERACTIONS WITH THE ADAPTIVE IMMUNE SYSTEM

Enteric neuron projections can be found within mucosal lymphoid follicles (Peyer's patches) (105, 106). When lymphocytes from Peyer's patches were exposed to neuropeptides, they significantly increased their proliferation rate and immunoglobulin synthesis (107). This suggests the exciting possibility that enteric neurons can directly influence the adaptive immune system. For example, the Y1 receptor that binds NPY is expressed on monocytes, macrophages, lymphocytes, and granulocytes (108). Y1-deficient mice have reduced antigen-presenting cell function, reduced effector T cells, and reduced production of TNF-α and IL-12 by macrophages. Similarly, $Npy^{-/-}$ mice have lower TNF-α levels, and $Y1^{-/-}$ mice resist colitis that occurs after epithelial injury with DSS (109). VIP is produced by inhibitory motor neurons, descending interneurons, and secretomotor neurons of the ENS to control motility and epithelial function. The VIP receptor is also expressed by T cells, where VIP promotes a Treg phenotype (110), by dendritic cells where VIP induces a tolerogenic phenotype (111, 112), and by macrophages where VIP inhibits production of TNF-α, IL-6, and IL-12p40 (110). These anti-inflammatory effects of VIP in vitro contrast with the observation that $VIP^{-/-}$ mice are resistant to TNBS-induced colitis (113) and LPS-induced endotoxemia (114). The reason for this discrepancy is not known.

ENTERIC NERVOUS SYSTEM INTERACTION WITH ENTEROENDOCRINE CELLS

Enteroendocrine cells are a diverse group of neuroendocrine cells in the bowel epithelium that produce hormones and neuropeptides in response to stimulation from the gut lumen. The molecules produced by enteroendocrine cells act locally on cells within the mucosa, as well as systemically via the bloodstream (115). Significant cross talk occurs between enteroendocrine cells and the ENS to modulate vasodilation, motility, and epithelial secretion. We already alluded to one mechanism by which EC cells, a subtype of enteroendocrine cells, signal enteric neurons through release of 5-HT onto nerve terminals. The triggers for 5-HT release are incredibly diverse and include mechanical deformation of the mucosa, macromolecules (e.g., glucose, fatty acids, amino acids), chemical irritants (e.g., allyl isothiocyanate), injury or stress (e.g., norepinephrine), and changes

in the bacterial milieu (e.g., butyrate and other short chain fatty acids) (115, 116). In response to stimulation, EC cells activate neurons through the release of 5-HT onto nerve terminals. In addition to 5-HT, enteroendocrine cells produce neuropeptides such as somatostatin, motilin, VIP, glucagon-like peptide-1 (GLP-1), and cholecystokinin, which modulate ENS activity (117–119). It has traditionally been assumed that these neuroactive peptides signal in a paracrine manner. However, presynaptic and postsynaptic markers have been identified on EC cells, suggesting that they communicate with nerves via synapse-like structures (116, 120). Even more compelling, monosynaptic transmission of modified rabies virus confirms the presence of functional efferent synapses (120). Unfortunately, the in vivo data do not address the question of whether the EC cells form synapses with enteric or extrinsic neurons (120). There is a paucity of research regarding enteroendocrine cell–ENS connections and particularly efferent ENS signaling onto hormone-producing enteroendocrine cells. Given the similarity in signaling molecules and receptors between enteroendocrine cells and the ENS, it seems very likely that these cells communicate through additional pathways that remain to be discovered.

THE ENTERIC NERVOUS SYSTEM, CENTRAL NERVOUS SYSTEM, AND THE AUTONOMIC NERVOUS SYSTEM

A variety of extrinsic nerves innervate the bowel, including sympathetic nerves, the parasympathetic vagus and sacral plexus nerves, and the dorsal root ganglia. Some of these nerves interact directly with their targets in the bowel, but they may also mediate their effects indirectly by synapsing on enteric neurons. We already mentioned the CAIP, where vagal nerve fibers signal ENS intermediaries that then signal macrophages to reduce inflammation. Here, we provide a brief overview of other important ENS interactions with the autonomic nervous system. Please see the following reviews for a more in-depth discussion of this topic (4, 121).

Sympathetic Neuron–Enteric Nervous System Interactions

Sympathetic nerve endings contact the vast majority of enteric neurons (122) and may also activate enteric glia via ATP release (123). Sympathetic nerves indirectly regulate epithelial secretion by inhibiting secretomotor neurons in the submucosal plexus. This inhibition is likely tonic, as transepithelial secretion is greatly enhanced after sympathectomy (122), and acute sympathetic inhibition effects on secretion are most apparent when the secretomotor reflex has been activated (124, 125).

The sympathetic nervous system coordinates motility across large segments of bowel. The changes in motility are mediated by reflex arcs that involve afferent sensory neurons of the ENS and efferent inhibition of enteric neurotransmission by the sympathetic nervous system. These reflex arcs may include or bypass the CNS, depending on context. Sympathetic reflexes have been worked out in detail and are well described elsewhere (122).

Parasympathetic–Enteric Nervous System Interactions

Parasympathetic innervation of the upper gastrointestinal tract is exclusively provided by the vagus nerve, and vagal projections are known to reach as far as the colon. Whether the entire bowel is innervated by the vagus nerve is still contested. Evidence suggests that the innervation includes the distal colon at least in some animal models (126). The pelvic nerves may provide additional parasympathetic innervation to the lower gastrointestinal tract. However, a recent study argues that these nerves are actually part of the sympathetic nervous system (127).

Most enteric ganglia in the small intestine and stomach are innervated by vagal nerve fibers (128). The vast majority of vagal nerve fibers are sensory and transmit afferent input to the CNS. It is unclear if afferent vagal signaling to the CNS is directly influenced by the ENS. Vagal-enteric (efferent) neurotransmission is predominantly cholinergic, although additional neurotransmitters have been identified in preganglionic vagal neurons (catecholamines and NO) and could have an auxiliary role (129, 130). Efferent vagal input affects all functions of the ENS, including the regulation of bowel motility, epithelial secretion, and vasoconstriction (4). Additionally, the vagus nerve mediates anti-inflammatory effects on the bowel through enteric neurons as part of the CAIP, as described earlier (86).

Clinical Relevance

The pathways described above confirm bidirectional communication between the ENS and CNS that has important effects on bowel function, but the gut also influences the brain, an interaction referred to as the gut-brain axis (131). An additional intriguing hypothesis is that these pathways may lead to CNS disease because protein misfolding events that occur in the ENS could be initiators for certain CNS diseases such as Parkinson's disease (PD). PD involves the prion-like misfolding of α-synuclein, which aggregates into deposits called Lewy bodies, leading to neurodegeneration and dementia. Genetic changes or ingested toxins like the pesticide rotenone could first cause α-synuclein aggregation in the ENS (132), which then travels to the CNS via the vagus, where they induce misfolding of additional α-synuclein to cause the characteristic Lewy body pathology. Support for this hypothesis includes the observation that Lewy body–type pathology is found within enteric neurons of people with PD (133), bowel symptoms often precede CNS symptoms and correlate with disease severity (134), and that α-synuclein aggregates can travel from the bowel to the brain via the vagus nerve (135, 136). In humans, truncal vagotomy may reduce PD risk (137, 138). Similar transit of misfolded protein from the bowel to the brain could also explain the spread of prion diseases like kuru, variant Creutzfeldt-Jakob, scrapie, chronic wasting disease, and spongiform encephalopathy, where ingestion of misfolded prion proteins initiates disease pathogenesis (139). More details are provided in excellent reviews (140–142).

The Enteric Nervous System and Central Nervous System

Because the ENS shares many neurotransmitters, receptors, and transcription factors with the CNS, it is not surprising that many people with CNS disease also have problems with bowel function. People with autism spectrum disorder (ASD) are 3–4 times more likely to have gastrointestinal symptoms than unaffected individuals; intriguingly, mutations in chromodomain-helicase-DNA binding protein 8 (CDH8), haploinsufficiency for the transcription factor TCF4, an activating mutation in the sodium-dependent 5-HT transporter (SERT/SLC6A4), and MET mutations are all directly linked to ASD and gastrointestinal motility disorders (58, 143–146). Mice expressing mutant forms of amyloid precursor protein associated with familial Alzheimer's disease accumulate amyloid beta in enteric neurons and have a reduced enteric neuron number, dysmotility, and increased vulnerability to bowel inflammation (147, 148). Mutations in TAR DNA–binding protein-43 (TDP-43) that cause familial amyotrophic lateral sclerosis (ALS) may also cause ENS defects, including intestinal obstruction in the Prp-TDP43^{Ala315Thr} mouse model (149, 150). There is much more to learn about the links between ENS and CNS disease.

SUMMARY

The enteric neurons and glia richly integrate sensory stimuli to control bowel motility, epithelial function, blood flow, and immune system activity. To do this, almost every cell of the bowel wall

closely interacts with the ENS, including SMCs, ICC, PDGFRα+ cells, enteroendocrine cells, epithelial cells, blood vessels, and many hematopoietic lineages. The ENS also interacts with extrinsic sympathetic, parasympathetic, and sensory nerves and is influenced by hormonal signals to modulate bowel function to meet systemic needs. Mechanisms and cell types that impact ENS activity remain underinvestigated. Defining molecular and cellular mechanisms of ENS activity promises new approaches to dangerous bowel motility disorders (Hirschsprung disease, CIPO, gastroparesis), common and less dangerous motility problems (irritable bowel syndrome, chronic constipation, functional dyspepsia), IBD, necrotizing enterocolitis, and ischemic bowel disease. Given the links between gut microbes, bowel motility, epithelial function, and CNS activity, ENS biology may also provide new approaches to addressing complex problems like anxiety, depression, autism, PD, ALS, and Alzheimer's disease as we begin to define the interplay along the gut-brain axis.

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