Symposium

Advances in Enteric Neurobiology: The "Brain" in the Gut in Health and Disease

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The enteric nervous system (ENS) is a large, complex division of the peripheral nervous system that regulates many digestive, immune, hormonal, and metabolic functions. Recent advances have elucidated the dynamic nature of the mature ENS, as well as the complex, bidirectional interactions among enteric neurons, glia, and the many other cell types that are important for mediating gut behaviors. Here, we provide an overview of ENS development and maintenance, and focus on the latest insights gained from the use of novel model systems and live-imaging techniques. We discuss major advances in the understanding of enteric glia, and the functional interactions among enteric neurons, glia, and enteroendocrine cells, a large class of sensory epithelial cells. We conclude by highlighting recent work on muscularis macrophages, a group of immune cells that closely interact with the ENS in the gut wall, and the importance of neurological–immune system communication in digestive health and disease.

Introduction

The gut is the largest microbial, endocrine, and immune organ in both humans and mice. It contains its own intrinsic enteric nervous system (ENS) that regulates a variety of gastrointestinal functions and communicates bidirectionally with the CNS and extraenteric peripheral nervous system. The ENS is derived from neural crest progenitors that colonize the gut during fetal development to form two interconnected ganglionated plexuses that wrap around and integrate into the laminar structure of the digestive tract (Fig. 1). The myenteric plexus, which is the larger of the two plexuses, is located between two layers of smooth muscle and extends throughout the digestive tract. The submucosal plexus is located in the submucosa, closer to the intestinal lumen, and extends from the stomach through the rectum. There are estimated to be 100 million neurons in the human small intestine alone, making the ENS the largest collection of neurons and glia outside the brain, and by far the largest division of the peripheral nervous system (Furness, 2006). The ENS contains a diversity of neurons and glia (Zeisel et al., 2018), and virtually every CNS neurotransmitter is also found in the ENS (Furness, 2006). Nevertheless, the full extent of enteric neuronal heterogeneity and circuitry remain incompletely understood. The ENS exhibits a columnar topology along the radial axis of the gut (Lasrado et al., 2017), similar to the CNS; however, the logic underlying why certain enteric neurons are grouped into ganglia is largely unclear.

Recent advances using new model systems and genetic tools are dramatically changing the understanding of ENS development. A thorough description of ENS development is beyond the scope of this review but has been covered previously (Lake and Heuckeroth, 2013; Rao and Gershon, 2018). Here we focus on reviewing the latest work, particularly how the zebrafish model system has been leveraged to understand links between the CNS and ENS in human neurodevelopment. We also address how genetic lineage tracing and *in vivo* imaging approaches in mice have demonstrated unprecedented levels of adult enteric neurogenesis that are required to maintain ENS homeostasis in the face of continual neuronal loss. These models and tools have allowed us to understand novel cellular and molecular mechanisms by which the ENS is formed and maintained, thereby giving us a new understanding of the regulation of gastrointestinal functions. New genetic tools and approaches have also uncovered surprising roles for various gastrointestinal cell types that are important for modulating gut functions through their interactions with enteric neurons, such as enteric glia, enteroendocrine cells (EECs), and macrophages.

Glia closely associate with neurons in the gut and outnumber them by at least fourfold to sixfold (Gabella, 1981). We review recent studies that have challenged the dogma on glial functions in the gut and revealed surprising sex differences in the glial regulation of intestinal motility. Next, we review advances in the biology of EECs, which are specialized sensory epithelial cells that mediate chemosensation and mechanosensation across the gut

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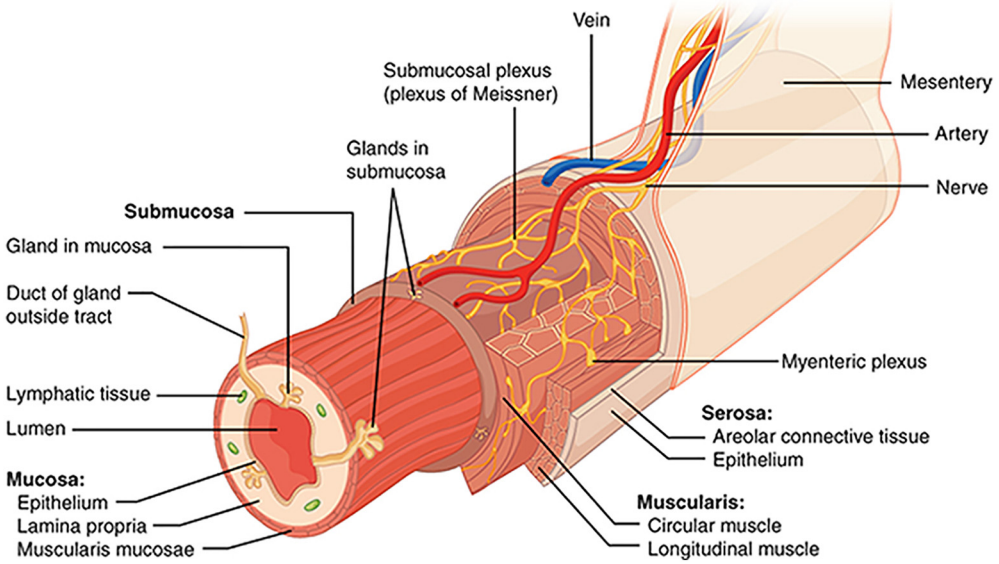

Figure 1. Organization of the enteric nervous system within the gut wall of the small intestine. The schematic illustrates the laminar organization of the bowel in three dimensions from the mesentery to the lumen. The two major plexuses of the ENS are the myenteric plexus, located between the circular and longitudinal muscle layers in the muscularis externa, and the submucosal plexus, located in the submucosa. Image was obtained from Wikimedia and reproduced under a Creative Commons Attribution-Share Alike 3.0 Unported license.

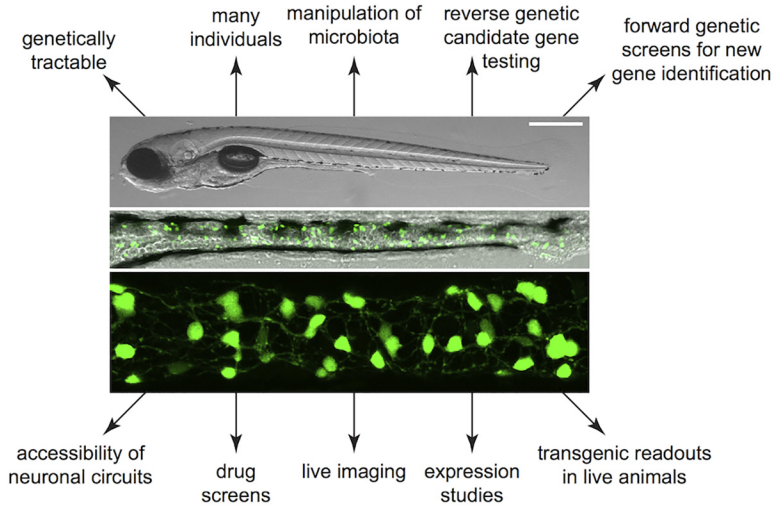

Figure 2. Zebrafish, a model system for investigating ENS development and function. Overview of distinguishing attributes of the zebrafish model system. Zebrafish larvae have an accessible ENS, labeled here with the *phox2b:GFP* transgenic line that marks enteric neurons (Taylor et al., 2016). Whole-mount side view of a 6 day post-fertilization zebrafish larvae in bright field (top), close-up image of the gut showing enteric neurons labeled with *phox2b:GFP* (middle), and *phox2b:GFP* $^{+}$ neurons and processes in higher magnification of the mid-gut (bottom). Scale bar, 500 μm.

epithelium. Understanding EEC biology is essential as they establish neurological–epithelial "synapses" with enteric afferent neurons and form a crucial part of the reflex microcircuits mediating intestinal motility. Finally, the gastrointestinal tract is also a primary site of cross talk among microbiota, the nervous system, and immune cells, such as macrophages. While macrophages are not formally part of enteric circuits, they closely interact with ENS cells to maintain and modulate their activity and hence help to regulate gut function. Here, we discuss how a subset of enteric macrophages regulates neural control of gut motility by secreting bone morphogenetic proteins (BMPs); and conversely how enteric neurons secrete signals that regulate macrophage homeostasis. This active cross talk between ENS and enteric macrophages is modulated by the presence and activity of the intestinal microbiota that signal to both macrophages and the ENS, and these interactions shift with aging to cause ENS degeneration. Along with reviewing these advances, we highlight important gaps in knowledge and opportunities in enteric neurobiology.

Zebrafish as an emerging new model system for ENS research

Zebrafish (*Danio rerio*) have long served as a model for studying developmental processes because of the following several unique attributes: they are genetically tractable; easy to maintain and breed; have large numbers of offspring that develop rapidly in an external environment; and are transparent, providing unparalleled opportunity for *in vivo* imaging (Fig. 2). In recent years, zebrafish have been used to study ENS development and function, as well as to evaluate candidate genes for human ENS diseases (Ganz, 2018). The zebrafish ENS is comparable in structure and neuronal complexity to the mammalian ENS, but it is much more accessible for imaging (Wallace et al., 2005; Ganz, 2018). Most genes are conserved between zebrafish and humans, and a set of conserved signaling pathways that regulate different aspects of ENS development has been identified in zebrafish and other vertebrate species (Wallace et al., 2005; Howe et al., 2013; Heanue et al., 2016a; Ganz, 2018). Thus, what we learn from zebrafish is directly applicable to mammalian ENS development and function.

A number of recent studies has successfully used morpholino antisense oligonucleotides, TALENs (transcription activator-like effector nucleases), or more recently CRISPR (clustered regularly interspaced short palindromic repeats)-based approaches in zebrafish to test the role of candidate genes in diverse human disorders, such as Hirschsprung disease, chronic intestinal pseudo-obstruction (CIPO), and autism spectrum disorder

(Bernier et al., 2014; Bonora et al., 2015; Cheng et al., 2015; Heanue et al., 2016b; Gui et al., 2017). The suitability of zebrafish for performing high-throughput multiplexed CRISPR/Cas9 screens (Shah et al., 2015) has become even more important with the explosion of data from single-cell RNA sequencing and genome-wide association studies, which are rapidly expanding the list of candidate genes requiring functional testing. Zebrafish are also uniquely suited for studying host–environment interactions, and small-molecule screens in zebrafish have already uncovered a variety of nongenetic factors that can influence ENS development (Lake and Heuckeroth, 2013; Lake et al., 2013; Schill et al., 2016). Zebrafish are relatively easy to breed "germ free" and have been used to study the role of the ENS in bacterial community assembly, illustrating novel connections between host gut motility and microbial colonization (Wiles et al., 2016; Rolig et al., 2017). These studies benefited from the large number of offspring readily available and the ease of *in vivo* imaging in zebrafish, which made it feasible to assess bacterial colonization in combination with functional analyses of gut motility. This ease of imaging has been exploited to develop new programs to quantify different gut motility parameters that had not been captured previously with standard assays (Ganz et al., 2018).

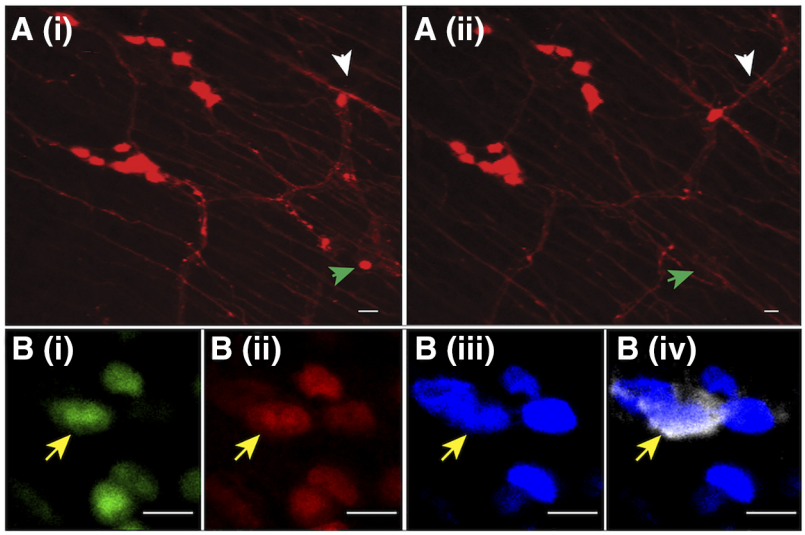

Figure 3. Continual neuronal turnover maintains the adult ENS. ***A(i)***, ***A(ii)***, 2D projection of 3D images captured using live animal two-photon microscopy (time 0 [***A(i)*** and time 24 h [***A(ii)***]) from the same region of the myenteric plexus of the NOS1-CreER^{T2}:tdTomato mouse that was induced with tamoxifen before the imaging shows the disappearance of a tdTomato^{+} nitrergic neuron (green arrow) within 24 h. The image also shows (white arrow) the appearance of new projections from a tdTomato^{+} neuron that rapidly extend to form new network connections within 24 h, showing the robust plasticity of neuronal networks in the myenteric ganglia. ***B(i)–B(iv)***, Using label-retaining halogenated thymidine analogs CldU and IdU in adult Nestin-creER^{T2}:tdTomato mouse, we observed the presence of CldU-labeled (green; [***B(i)***]) and IdU-labeled (red; [***B(ii)***]) HuC/D-expressing (blue; [***B(iii)***]) neurons, some of which also express tdTomato (cyan; [***B(iv)***]), providing evidence of the derivation of these neurons from Nestin-expressing cycling precursors (yellow arrow). Scale bars, 10 μm. (Kulkarni et al., 2017).

Several gaps remain in the understanding of cellular and molecular pathways implicated in ENS development and maintenance, with neuronal circuit formation being a major one. The accessibility of the ENS in zebrafish provides a unique opportunity to dissect neuronal circuit formation in concert with intestinal function. The remarkable ability of zebrafish to regenerate different parts of their nervous system has provided important insights into the molecular factors that promote neural regeneration (Kizil et al., 2012). Recent studies have found similar regenerative capacities in the murine ENS (Kulkarni et al., 2017; De Vadder et al., 2018; Saha et al., 2018; Yarandi et al., 2018; and detailed below). The regenerative capability of the zebrafish ENS remains to be determined, but, if conserved, zebrafish could offer an ideal model system for studying adult enteric neurogenesis.

Shifting paradigms of adult ENS maintenance

While ENS development has been heavily studied in both health and disease (Newgreen and Young, 2002a,b; Young, 2008; Burns et al., 2009; Hao and Young, 2009; Bergner et al., 2014; Uesaka et al., 2016), there has been limited examination of how the ENS is maintained in the healthy adult gut. This is important because the ENS is routinely subjected to significant and continual mechanical stress (Gregersen and Kassab, 1996) and chemical insults from luminal contents (Sherman et al., 2015; Hyland and Cryan, 2016). In intestines from healthy adult animals, previous studies reported conflicting data on neuronal loss (Gabella, 1971, 1989; Gianino et al., 2003; Anitha et al., 2006, 2016) and detected no enteric neurogenesis from proliferating label-retaining precursor cells (Liu et al., 2009; Joseph et al., 2011). The expression of the transcription factor Sox10 marks the enteric neural precursor cells (ENPCs) that generate neurons during development, but in adult mice, Sox10 expression labels enteric glia. These cells show limited neurogenic capability *in vivo*, and that is restricted to repair after significant chemical injury (Laranjeira et al., 2011). These observations established the dogma that there is no ongoing neurogenesis in the healthy, mature intestine.

Recently, a report (Kulkarni et al., 2017) provided a paradigm shift in this understanding of the mechanisms of adult ENS maintenance. Using novel tools, such as two-photon *in vivo* microscopy, adult myenteric neurons were observed to be lost at a significant rate (Fig. 3). Despite this loss, neuronal numbers in myenteric ganglia were maintained, suggesting the presence of ongoing adult enteric neurogenesis. Using an updated method for labeling and detecting label-retaining thymidine analogs, proliferating ENPCs were observed to replace ~90% of adult myenteric neurons within 2 weeks (Fig. 3), thereby maintaining the enteric neuronal numbers (Kulkarni et al., 2017). Further, these ENPCs were found to express the neural precursor marker Nestin, but not Sox10, suggesting that Nestin and Sox10 mark distinct neurogenic and gliogenic populations, respectively, in adult mice. The study also provided evidence of robust plasticity in the neural circuitry, the biology of which is yet unknown. These findings are directly relevant to deciphering the etiology of gastrointestinal diseases. While ablation of the cell cycle regulator protein PTEN selectively in adult Nestin^{+} cells caused enteric neuronal hyperplasia that mimicked the pathology of CIPO (Kulkarni et al., 2017), the ablation of PTEN selectively in adult enteric glia caused glial hyperplasia without altering neuronal number (Jonscher et al., 2018). These data suggest that the cells responsible for neuronal hyperplasia, which is prevalent in patients with long-term gut dysmotility (Kidane et al., 2015), might be Nestin^{+} ENPCs.

In this new paradigm of ongoing and rapid enteric neuronal turnover, chronic ENS pathologies could be due to ENPC defects, resulting in altered neuronal numbers and/or dysfunctional neurons. Accumulating evidence suggests that luminal factors such

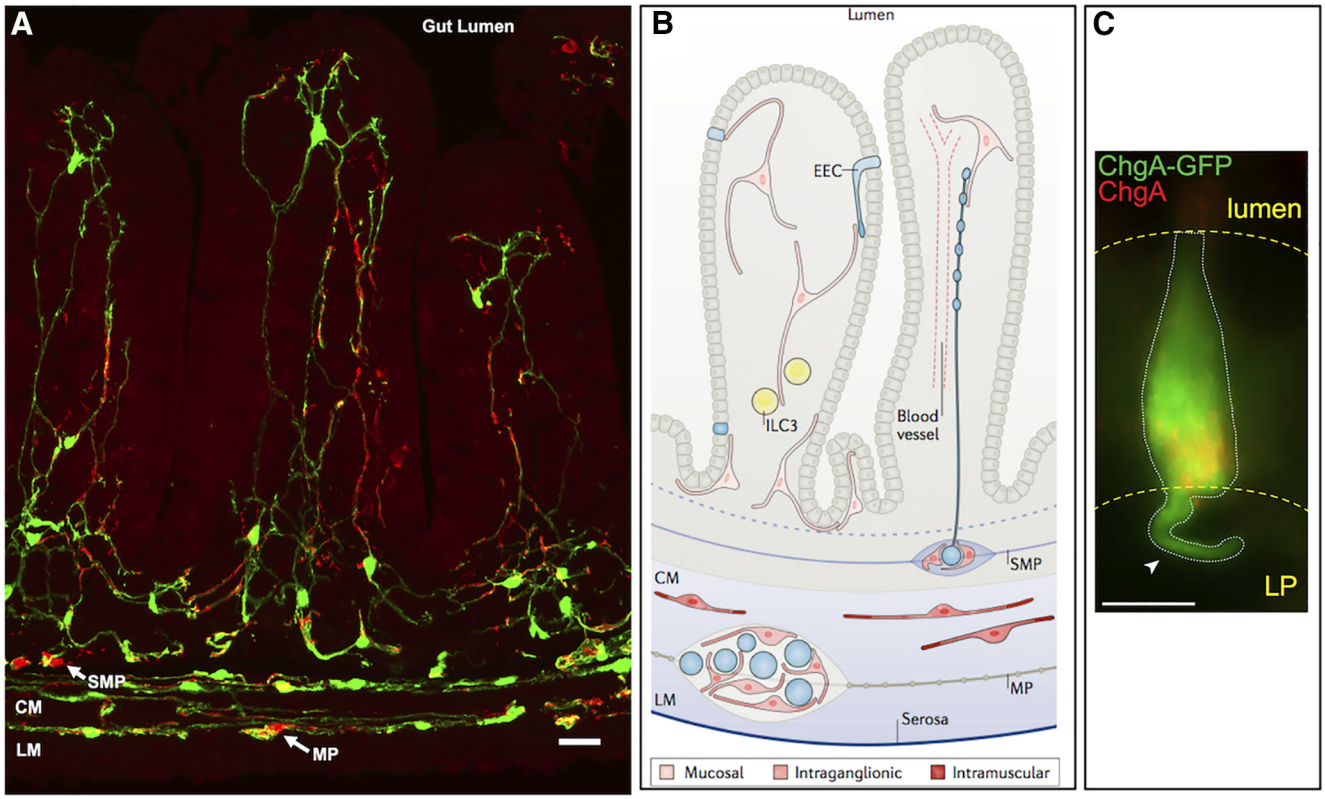

Figure 4. Glial diversity in the enteric nervous system. ***A***, Immunohistochemical staining of a cross section of small intestine from an adult PLP1-eGFP mouse in which green fluorescent protein (GFP) expression marks all enteric glia, illustrating the extensive glial network in the gut. Neurons are immunoreactive for PGP9.5 (red), and their soma are visible in the myenteric (MP) and submucosal (SMP) plexuses. The muscular externa consists of the longitudinal (LM) and circular (CM) muscle layers. Scale bar, 25 μm. ***B***, Schematic of enteric glial subpopulations and their interactions with other cell types. Mucosal glia are found in the lamina propria immediately underneath the epithelium and have been reported to interact with EECs, a subset of immune cells known as group 3 innate lymphoid cells (ILC3s; important for antimicrobial defense and maintaining tolerance to commensal microbiota), nerve fibers, and blood vessels. Intraganglionic glia are found within the enteric plexuses, closely apposing and partially ensheathing neurons (depicted as blue circles). Long, bipolar, intramuscular glia are found in the circular and longitudinal muscle layers, in close association with nerve fibers (data not shown) that innervate the smooth muscle. Image was reproduced with permission from SpringerNature (Rao and Gershon 2018). ***C***, Enteroendocrine cell marked by GFP expression (green) driven by the chromogranin A (ChgA) promoter colocalizes with ChgA immunoreactivity (red). Yellow dashes indicate luminal and lamina propria (LP) surfaces. Arrowhead indicates neuropod. Scale bar, 10 μm.

as diet and microbiota influence ENPC behavior to cause gut pathologies (De Vadder et al., 2018; Saha et al., 2018; Shannon and Vanden Berghe, 2018; Yarandi et al., 2018). An in-depth understanding of the cellular and molecular pathways that regulate neurogenesis and subtype specification in the adult ENS needs to be determined so that the etiology of disorders such as achalasia, caused by a loss of a specific subset of enteric neurons, can be better defined (Mearin et al., 1993; Lui et al., 1997).

Neuron–glia interactions in the ENS

Our comprehension of glial biology in the ENS beyond its role in maintaining neurons has also evolved considerably over the past several years. Enteric glia were initially referred to as the Schwann cells of the gut because of a common developmental origin in the neural crest. But ultrastructural features (Gabella, 1971), along with the expression of molecular markers typically associated with astrocytes, such as GFAP and S100β (Jessen and Mirsky, 2005), led to these cells subsequently being characterized as "astroglia of the gut" (Gershon and Rothman, 1991). More recently, transcriptional profiling has challenged this long-standing characterization and suggested instead that enteric glia are most similar to myelinating glia, despite the lack of myelination in the ENS (Rao et al., 2015). It is possible that these conflicting characterizations of enteric glia reflect heterogeneity among enteric glia, with some types being more similar to astrocytes, while others resemble Schwann cells. Consistent with this possibility, enteric glia exhibit a variety of distinct morphologies and are located in markedly different microenvironments depending on whether they reside in enteric ganglia, along fiber tracts, or scattered in the smooth muscular or mucosal layers of the gut (Gulbransen and Sharkey, 2012; Fig. 4). Recent studies suggest that distinct subpopulations of enteric glia can be identified even within a single ganglion, based on molecular marker expression and activity profile (Boesmans et al., 2015; Zeisel et al., 2018). Despite this evident heterogeneity, clear functional classes of enteric glia have yet to be defined; determining these subtypes and understanding how they relate to other classes of glia in the nervous system remains an important task ahead.

While much has been learned about enteric glia over the past 20 years, their primary role in gut function remains incompletely understood. Initial studies based on genetic ablation of GFAP-expressing enteric glia suggested that they are essential for the maintenance of the intestinal epithelium, and that glial disruption compromises intestinal epithelial barrier integrity, causing fulminant intestinal inflammation (Bush et al., 1998; Aubé et al., 2006). Recent studies have questioned these findings, showing that functionally antagonizing enteric glia or ablating them in adult mice with a cell-autonomous toxin does not alter intestinal epithelial permeability *in vitro* or *in vivo*, or cause inflammation (Grubišić and Gulbransen, 2017; Rao et al., 2017). While enteric

glia might not directly modulate the integrity of intercellular junctions in the epithelium, it is possible that they influence these junctions indirectly through their interactions with immune cells (Ibiza et al., 2016).

The role of glia in ENS-mediated behaviors has been characterized best in intestinal peristalsis, the coordinated propulsion of ingested nutrients and intestinal secretions from mouth to anus. While this motor behavior is thought to be the output of a seemingly simple microcircuit embedded in the gut wall, the peristaltic reflex is complex and incompletely understood. Moreover, it has a major role in important features of energy homeostasis such as blood glucose regulation. Enteric glia participate in bidirectional communication with enteric neurons to regulate motility (McClain et al., 2014; Rao et al., 2017), and this role is seemingly sex dependent, suggesting that much remains to be learned about neuron–glia interactions in even the most fundamental of ENS-mediated behaviors.

Enteric neuroepithelial interactions

The intestinal epithelium represents one of the largest surfaces of the human body that is exposed to the outside environment and is continuously challenged by a variety of both exogenous and endogenous stimuli. Positioned at this luminal interface are EECs, specialized sensory epithelial cells that are scattered throughout the intestinal epithelium and are responsible for sensing this complex luminal environment (Engelstoft et al., 2013; Fig. 4). EECs assume a characteristic flask or pyramidal shape consisting of a narrow apical neck reaching to the luminal surface, a wide base housing large eosinophilic granules, and an axonal-like process extending laterally from the basolateral surface (Reimann et al., 2008; Bohórquez et al., 2011). Recently termed "neuropods," these neurofilament-containing processes can extend up to 70 μm from the soma and possess secretory vesicles reminiscent of those found in neuronal synapses (Bohórquez et al., 2015).

At least a subset of EECs are electrically excitable and show spontaneous bursting activity in intestinal organoid cultures; these cultures have offered an exciting new approach to studying EEC activation by chemical and mechanical stimuli (Bellono et al., 2017; Alcaino et al., 2018). Sensory activity of EECs is mediated through their expression of cell surface receptors for nutrients, microbial byproducts, and host signaling molecules (Dyer et al., 2005; Samuel et al., 2008; Akiba et al., 2015; Bellono et al., 2017; Lund et al., 2018). A subclass of EECs called enterochromaffin cells (ECs) express voltage-gated Na^+, K^+, and Ca^{2+} channels (Bellono et al., 2017; Strege et al., 2017), which mediate chemical stimulus-dependent serotonin release, and Piezo2 channels, which mediate mechanical stimulus-dependent serotonin release (Wang et al., 2017; Alcaino et al., 2018). ECs are the major source of serotonin in the body, and EC-derived serotonin triggers the peristaltic reflex circuit upon mucosal deformation.

The mechanism of signal transduction from EECs to underlying neurons was thought to occur via paracrine or hormonal signaling. However, recent evidence using rabies virus tracing has demonstrated the presence of a potential synapse between a subpopulation of EECs and intestinal mucosal nerve fibers (Bohórquez et al., 2015). This is further supported by the presence of transcripts for synaptic machinery in EECs (Bohórquez et al., 2011; Bellono et al., 2017). These synaptic proteins have been found at the interface between $5HT_3R^+$ sensory afferent nerve fibers and juxtaposed ECs (Bellono et al., 2017). Complementing these anatomic studies, EC activation led to serotonin-dependent mucosal afferent nerve activation and increased mechanical sensitivity in an *ex vivo* gut–nerve preparation (Bellono et al., 2017). Recent optogenetic studies, furthermore, have found that stimulation of the colonic epithelium evokes the visceromotor response, a brainstem-mediated pain withdraw reflex triggered by noxious intestinal stimulation (Makadia et al., 2018). EEC–nerve communication may be bidirectional because EECs express postsynaptic proteins and can be activated by catecholamines, presumably from adjacent sympathetic nerve fibers (Bohórquez et al., 2015; Bellono et al., 2017).

Given the continual turnover of intestinal epithelial cells every few days, how EEC–mucosal nerve fiber synapses form remains an unanswered question. EECs migrate toward isolated trigeminal neurons *in vitro* and receive neuronal projections (Bohórquez et al., 2015), suggesting that dynamic synaptogenesis might occur in the mucosa. The average life span of most intestinal epithelial cells is only 5–7 d, but some EECs can persist for >60 d (Bohórquez et al., 2015). It is possible that the EEC–nerve connection supports this observed increase in cellular longevity to favor synaptic stability. The biology of neurological–epithelial synapse formation and enteric sensory transduction are exciting areas for future work.

Enteric neuroimmune interactions

One of the most exciting developments in ENS biology has been in the realm of neuroimmune cross talk between enteric neurons and intestinal macrophages. Macrophages regulate organ development, postnatal homeostasis, and remodeling, and they induce the inflammatory response (Lavin et al., 2015). In the gut, muscularis macrophages (MMs) are a phenotypically and transcriptionally distinct population of macrophages that reside in the outer smooth muscle layer of the intestines (muscularis externa; Mikkelsen et al., 1985), where they are positioned along nerve fibers of the myenteric and the deep muscular plexuses and within the myenteric ganglia, adjacent to the cell bodies of enteric neurons (Fig. 5; Muller et al., 2014; Gabanyi et al., 2016). MMs in the healthy gut modulate intestinal motility via (1) secretion of BMP2, a growth factor that acts on enteric neurons (Muller et al., 2014); and (2) by maintaining ENS homeostasis, by phagocytosing adult neurons (Kulkarni et al., 2017). This is known to maintain ENS structure because an absence of macrophages in colony-stimulating factor 1 (CSF1)-deficient $Csf1^{op/op}$ mice results in increased myenteric neuronal numbers and a less organized ENS architecture (Muller et al., 2014). Whether MMs also regulate differentiation of ENPCs is unknown (Fig. 5).

The ENS–MM interaction might be involved in the pathophysiology of several diseases. Mucosal inflammation leads to functional changes of the ENS, causing neuronal loss and/or hyperinnervation, altered neurochemical phenotype, and neuronal hyperexcitability, all of which may persist long after inflammation is resolved. Development of plexitis, an inflammatory infiltrate of the neural plexuses in patients with inflammatory bowel disease suggests that MMs and their derivative inflammatory factors provide local inflammatory signals within the ENS, leading to neuronal loss. However, the exact mechanisms of this remain unknown (Brierley and Linden, 2014). In contrast to the role of diseased MMs in neurodegeneration, healthy MMs play a role in ENS regeneration by producing anti-inflammatory and neurotrophic growth factors that promote ENS regeneration after mucosal inflammation is resolved (Margolis et al., 2016). This suggests the presence of a complex context-dependent neurological–immune cross talk in health and disease.

Complementing the role of MMs in maintaining ENS homeostasis, the ENS regulates MM homeostasis and function. The vast

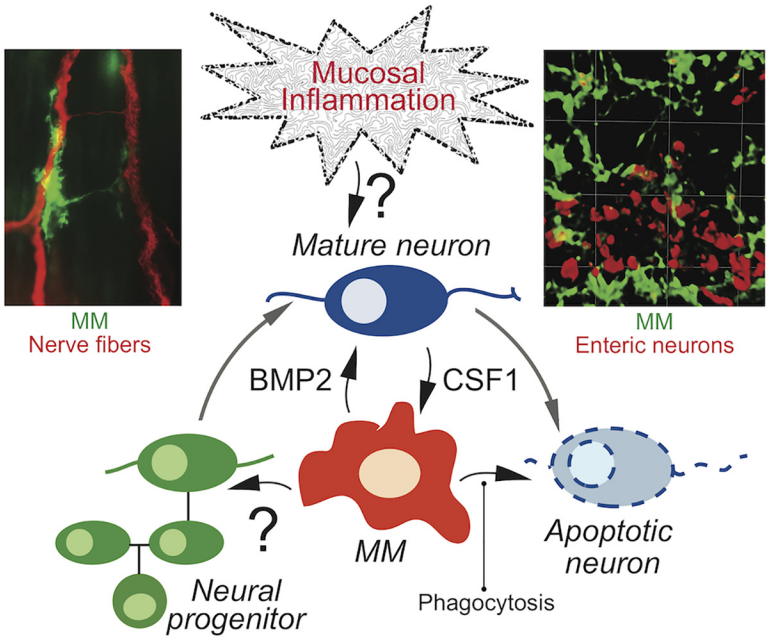

Figure 5. MMs are anatomically and functionally associated with the enteric nervous system. MMs are positioned along nerve fibers (top left) or within the enteric ganglia (top right) of the intestinal myenteric plexus. They control homeostasis and function of adult enteric neurons through the production of neurotropic growth factor BMP2 and phagocytosis of mature enteric neurons, and indirectly modulate neuromuscular function. Enteric neurons, in turn, control MM numbers through the production of macrophage-specific growth factor CSF1.

majority of murine MMs are derived from bone marrow progenitors, although a fraction of self-maintaining MMs has been described recently; all MMs are radiosensitive and are completely replaced by donor bone marrow cells after lethal irradiation followed by bone marrow transplantation (Muller et al., 2014; De Schepper et al., 2018). MM development is controlled by the presence of the cytokine CSF1, which is expressed by enteric neurons, suggesting the regulation of MM biology by the ENS. In addition, MM behavior is directly regulated by extrinsic neurons of the sympathetic nervous system (Gabanyi et al., 2016) and is indirectly regulated by the parasympathetic nervous system, likely through actions on enteric neurons, which then transduce signals to MMs (de Jonge et al., 2005; Cailotto et al., 2014; Matteoli et al., 2014). The physiological relevance of these signals in gastrointestinal health and disease, however, remains to be established. Furthermore, how the ENS controls MM function through neurotransmitter activity remains to be tested.

Aging and enteric neuroinflammation

Aging causes a decline in gastrointestinal neuromuscular function that contributes to a variety of common digestive disorders, including constipation, reflux, and fecal incontinence (Phillips et al., 2004; Camilleri et al., 2008; Saffrey, 2013). Age-related enteric neuronal loss and degeneration appear to play a role in these disorders (Camilleri et al., 2008; Saffrey, 2013). Studies in humans and animal models have described an age-associated decline in myenteric neurons in the small and large intestines (Saffrey, 2013). Although less studied, age-dependent loss of enteric glia has also been reported (Phillips et al., 2004). While there is evidence that oxidative stress, alterations in neurotrophic factors, and calcium dysregulation may contribute to these degenerative changes (Saffrey, 2013), recent discoveries have suggested that an aberration of enteric neurological–immune cross talk, which causes a chronic, low-grade inflammatory state known as "inflammaging" (Franceschi et al., 2007), plays a key role in the development of age-associated gut motility disorders (Becker et al., 2018a).

Increased inflammation is associated with an age-dependent rise in immune activation, apoptosis of enteric neurons, declining neuronal density within myenteric ganglia, and decreased intestinal transit (Becker et al., 2018a). Furthermore, ENPCs, resident stem cell populations that are key to maintaining ENS (reviewed in an earlier section), decline in number with age and inflammation (Molofsky et al., 2006; Becker et al., 2018a), suggesting that decreased neuronal density is partly due to an inflammaging-associated reduction in the neurogenic potential of the ENPC. Similarly, inflammatory processes, especially elevated interleukin-6 levels, increase enteric neuronal apoptosis, suggesting that inflammation creates a shortfall in neuronal numbers by increasing neuronal death while simultaneously reducing the rate of neuronal replacement (Becker et al., 2018a).

Multiple processes have been proposed as the cause for inflammaging, including increased production of proinflammatory cytokines due to a rise in senescent cells (Rodier and Campisi, 2011), alterations in the gut microbiota (Claesson et al., 2012; Thevaranjan et al., 2017), and changes to immune cells (Franceschi et al., 2007). Notably, several of these processes, such as the presence of senescent cells and altered MM behavior (whose "tone" tilts from an anti-inflammatory "M2" to proinflammatory "M1" state), have been detected in the ENS with aging (Jurk et al., 2012; Becker et al., 2018a). MMs from bone marrow chimeric mice, generated by transplanting bone marrow from old mice into lethally irradiated young recipients, demonstrate a loss of M2 phenotype similar to those from old mice (Becker et al., 2018a). Ongoing work suggests that the change in MM behavior with aging is associated with a significant change in intestinal microbiota composition, and both of these factors work in tandem to disrupt ENS structure and function and to cause intestinal dysmotility (Becker et al., 2018b). Recent work suggests the presence of a "triumvirate" of ENS, MM, and intestinal microbiota, whose cross talk is essential to regulate normal gut behavior. Given that there are limited therapeutic options for treating age-associated gut motility disorders, targeting functional interactions among this triumvirate to normalize age-associated aberrations in their cross talk could be a promising strategy for treating these disorders.

Conclusion

The recent advances in enteric neurobiology summarized above highlight the critical importance of continual cross talk among enteric neurons, glia, EEC, immune cells, and the many other component cells of the digestive tract in the regulation of gastrointestinal functions. Aberrations in the development and maintenance of the ENS, or communication between any of these cell types, could be associated with disease pathogenesis. This review provides a small glimpse of the complex cellular and molecular pathways involved in maintaining gut homeostasis and highlights some of the current gaps in knowledge. These gaps serve as exciting opportunities to improve our understanding of basic and translational enteric neurobiology. Any gains made are sure

to advance CNS biology as well, given the bidirectional communication between the ENS and CNS along the gut–brain axis.

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