HHS Public Access

Author manuscript

Nat Rev Gastroenterol Hepatol. Author manuscript; available in PMC 2021 August 01.

Published in final edited form as:

Nat Rev Gastroenterol Hepatol. 2021 August ; 18(8): 571–587. doi:10.1038/s41575-021-00423-7.

Enteric glial biology, intercellular signalling and roles in gastrointestinal disease

Luisa Seguella[1,2], **Brian D. Gulbransen**[2,✉]

[1]Department of Physiology and Pharmacology, "V. Erspamer", Sapienza University of Rome, Rome, Italy

[2]Department of Physiology, Neuroscience Program, Michigan State University, East Lansing, MI, USA

Abstract

One of the most transformative developments in neurogastroenterology is the realization that many functions normally attributed to enteric neurons involve interactions with enteric glial cells: a large population of peripheral neuroglia associated with enteric neurons throughout the gastrointestinal tract. The notion that glial cells function solely as passive support cells has been refuted by compelling evidence that demonstrates that enteric glia are important homeostatic cells of the intestine. Active signalling mechanisms between enteric glia and neurons modulate gastrointestinal reflexes and, in certain circumstances, function to drive neuroinflammatory processes that lead to long-term dysfunction. Bidirectional communication between enteric glia and immune cells contributes to gastrointestinal immune homeostasis, and crosstalk between enteric glia and cancer stem cells regulates tumorigenesis. These neuromodulatory and immunomodulatory roles place enteric glia in a unique position to regulate diverse gastrointestinal disease processes. In this Review, we discuss current concepts regarding enteric glial development, heterogeneity and functional roles in gastrointestinal pathophysiology and pathophysiology, with a focus on interactions with neurons and immune cells. We also present a working model to differentiate glial states based on normal function and disease-induced dysfunctions.

The enteric nervous system (ENS) is an integrative neural network that provides local control over essential gastrointestinal functions through 'brain-like' neural circuitry composed of neurons and glia. The relative importance of intrinsic and extrinsic neurons in gastrointestinal reflexes and the characteristics of individual enteric neuron subtypes have been well described[1,2]. How enteric neurons organize into functional circuits[3], how these

✉ gulbrans@msu.edu.

Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

The authors declare no competing interests.

Peer review information

Nature Reviews Gastroenterology & Hepatology thanks W. Boesmans, M. Hao, K. Sharkey and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Supplementary information

The online version contains supplementary material available at https://doi.org/10.1038/s41575-021-00423-7.

circuits develop[4] and how intercellular interactions with non-neuronal cells, such as immune cells[5–8] and glia[9], shape the output of enteric neurocircuits is only beginning to be understood. In particular, new data showing that bidirectional communication between enteric neurons and enteric glia influences the output of enteric neurocircuits is beginning to transform views of how normal enteric circuits function and of the processes that lead to dysfunction in common functional gastrointestinal disorders such as irritable bowel syndrome (IBS)[9–15].

Enteric glia are a large population of peripheral neuroglia that are associated with the cell bodies and processes of enteric neurons throughout the digestive tract. Neuroglia, in general, fulfil homeostatic functions in the nervous system and this view is consistent with the known functions of enteric glia in the ENS. The latest advances in enteric glial biology have begun to define what these specific functions are and the mechanisms involved. The data emerging from these studies show that enteric glia are one of the most dynamic signalling components of the ENS and have revamped the concept of enteric glia as passive cells. Fast, bidirectional communication between enteric glia and neurons regulates enteric reflexes[10,14,16,17] and the communication between extrinsic and intrinsic neurons[12,18,19] that innervate the digestive tract. Likewise, enteric glia contribute to diverse disease processes, including neuroinflammation[12,13], cancer[20,21] and infection[22], in which glia acquire pro-inflammatory or pro-tumorigenic phenotypes. In these cases, both losses and gains of glial functions contribute to altered ENS homeostasis and gastrointestinal pathophysiology.

Although much progress has been made, the field of enteric glial biology is still young and answering some of the many remaining unknowns offers an opportunity for scientific growth and meaningful advances. Little is known regarding glia in digestive organs other than the colon or ileum and glial heterogeneity within even a single region is complex. The intent of this Review is to summarize some of the currently known functions of enteric glia and highlight areas in which additional work is needed. New findings that refine the concepts of glial development, the roles of enteric glia in physiological reflexes, and that expand the known roles of glia in disease will be presented. Although current data show the vast potential of glia to be involved in diverse functions, many questions remain regarding the necessity of glia, their specific roles in neural circuits and differences in the roles of glia throughout the digestive tract. Major progress in these areas should be expected as understanding enteric glia has the potential to unlock central mechanisms that regulate gastrointestinal function and disease.

Enteric glia are heterogenous neuroglia

The term ‘enteric glia’ collectively refers to all neuroglia associated with the ENS and substantial heterogeneity exists within this broad population of cells. Glial cells are phenotypically plastic and adapt their functions in response to cues in the microenvironment to maintain local homeostasis. Presumably, the different functional roles and regional specializations of different intestinal organs promote subpopulations of glia with unique characteristics to develop, yet little is known about regional or local glial heterogeneity and how glial specializations contribute to the various digestive functions. The current framework characterizing the major subpopulations of enteric glia segregates cells based on

morphology and anatomical location within the intestinal wall[23,24] (TABLE 1). This classification system is useful as a means of developing a common language to discuss major populations of enteric glia with differing anatomical locations but does not capture functional heterogeneity between intestinal regions or within the major subgroups themselves. Current evidence suggests ongoing dynamic regulation of gene and protein expression within subtypes of enteric glia as well as potential differences in responsiveness to intercellular mediators within and between subtypes[24,25]. Single-cell transcriptional profiling data show that glial diversity differs between regions of the digestive tract and suggest that glial diversity is greater in humans than in mice[26,27]. These studies also show that glial gene expression is affected by circadian rhythm and age. In addition, the extent to which 'enteric' glia derive from differing progenitor sources, such as Schwann cell precursors[28,29], and whether the current definition of enteric glia encompasses intestinal Schwann cells associated with extrinsic nerves innervating the intestine are unanswered questions[30,31]. Glial heterogeneity adds considerable complexity to the generalized view of enteric glia and understanding this area in more depth will be an important aspect of future work. Here, the discussion is limited to the current knowledge base suggesting local, regional and functional heterogeneity between enteric glial subtypes.

Local heterogeneity refers to differences among glia within a given region of the gastrointestinal tract. This aspect is currently the most well defined of glial heterogeneity and major subpopulations of glia are defined based on their anatomical location throughout the intestinal wall and their localization either within or outside of enteric ganglia (TABLE 1)[23,24]. Based on anatomical criteria, at least six main types of enteric glia are present in the intestine. These types include glia associated with neuron cell bodies in the myenteric and submucosal plexuses (Type I_{MP} and Type I_{SMP}, respectively), glia within nerve fibre bundles connecting myenteric ganglia (Type II), extraganglionic glia associated with nerve fibres at the level of the myenteric and submucosal plexuses (Type-$III_{MP/SMP}$) or the intestinal mucosa (Type III_{mucosa}), and glia associated with nerve fibres in the smooth muscle layers (Type IV) (FIG. 1). Current in vivo data suggest that all enteric glia within a given region of the intestine are derived from a common pool of progenitor cells within the myenteric plexus and are developmentally linked[4]. Nonetheless, Schwann cells present in the intestine could complicate this interpretation. In zebrafish, Schwann cell precursors that migrate from the spinal cord into the intestine give rise to postnatal enteric neurons[32]. Similarly, Schwann cells associated with extrinsic nerves are also capable of forming new enteric neurons and glia in mouse models of Hirschsprung disease following stimulation with glial cell-derived neurotrophic factor (GDNF)[29]. Understanding the extent to which Schwann cells contribute to glial diversity under normal conditions will be an important question for future work. However, distinguishing between enteric glia and Schwann cells within a similar microenvironment could prove difficult given that enteric glia are similar to Schwann cells at the transcriptional level[33] and that the two cell types are driven towards a common phenotype when exposed to similar factors in cell culture[28]. Such local cues, rather than specified developmental programmes, seem to be the main driver of glial heterogeneity within the intestine and environmental cues are responsible for producing certain glial subtypes such as mucosal glia. Lineage tracing shows that this population derives from enteric glial cells with progenitor potential in the submucosal and myenteric plexuses and

subsequently migrates to the mucosa in a process that requires cues from the microbiome[34]. The nature of the cues that drive this differentiation is not known. Mucosal glia are also diverse and subpopulations have specialized interactions with nerves, immune cells, enterocytes and/or enterochromaffin cells[35–38]. A particularly interesting subpopulation of mucosal glia is associated with the neuropod synaptic specializations of enterochromaffin cells[35]. These cells are ideally situated to modulate gut–brain communication and could have a major influence over intestinal sensory functions[39]. However, the functional significance of these cells is not currently understood and whether glia associated with the neuropods of enterochromaffin cells are actually enteric glia or Schwann cells that migrate to the intestine along extrinsic nerves is unknown[30,31].

Molecular and functional data suggest added complexity within and between the major glial subtypes defined earlier. For example, immunohistochemical and single-cell transcriptomics data from mice and humans show variability in the extent to which enteric glia express major glial markers such as glial fibrillary acidic protein (GFAP), S100β, proteolipid protein 1 (PLP1) and Sox10 (REFs[24–26,33]). GFAP is dynamic and its observed expression varies depending on glial state[12], subtype[24], genetic targeting strategy[10,40] and antibody used and therefore results should be interpreted with caution[41]. Differing transgene insertion sites cause variability in genomic models that use GFAP as a glial-specific driver[10,40] and enteric glia express multiple GFAP isoforms[42–44]. Thus, the ability of antibodies to identify one or more GFAP isoform will affect the observed extent of GFAP in immunolabelling experiments[41,44]. For example, GFAP is reportedly absent in intramuscular glia and in subsets of mucosal and submucosal glia in mice based on immunolabeling data, but it is unclear whether this is a true absence or if these data reflect variable antibody efficacy[33]. Single-cell transcriptional sequencing data show that all subtypes of myenteric glia within the mouse ileum express GFAP, albeit at differing levels[25]. Bulk RNA sequencing data of myenteric glia from the mouse colon also indicate comparable levels of GFAP, PLP1 and S100β transcription under steady-state conditions[33]. However, these data should be interpreted with caution given that they reflect population levels of RNA and RNA sequencing is a proxy for protein expression. It is unclear how well these data reflect variability within individual cells or functional protein expression.

Regional heterogeneity refers to differences among populations of enteric glia present in various locations of the gastrointestinal tract; however, the vast majority of work has focused on enteric glia located in the colon or distal ileum. The main subsets already discussed are prominent in these regions but fewer are present in the oesophagus and stomach since these regions lack a well-defined submucosal plexus[2]. Glial cells in the oesophagus and stomach could derive from a different pool of progenitor cells than glial cells in the intestine, as distinct populations of enteric precursors differently colonize the oesophagus and stomach than the intestine in mice[30]. Thus, both developmental programmes and functional specializations driven by the local environment could contribute to glial heterogeneity between the intestine and organs of the upper digestive tract. Distinct glial populations with unique transcriptional profiles have been described in the adult ENS of mice and humans[25,26]. The number of glial subsets varies according to species and location along the gastrointestinal tract and could relate to the complexity of motor circuits (FIG. 2). This area is still underdeveloped and would benefit from future work.

Local and regional functional heterogeneity among enteric glia remains poorly defined. Glia within the myenteric and submucosal plexuses support and modulate neuronal signalling in mice[11,14,16,45], whereas glia within the mucosa appear to influence enterocyte development[36,46,47] and immune responses in cell culture[19,37,48]. To what extent these roles are conserved between regions is unclear. Differences among protein expression, responsiveness to various intercellular mediators and transcriptional profiles have also been observed between myenteric glia in the ileum and colon of mice[24,25]. Functional specializations and differential distributions of enteric glial subtypes might operate in tandem with specified neuronal networks to regulate specific functions in different regions and segments of the digestive tract[49]. However, whether these differences represent true functionally defined subsets of glia or a snapshot of ongoing glial plasticity within a more homogeneous population is unknown.

Glial development and maintenance

The ENS of vertebrates derives mostly from a rostral portion of the neural crest, referred to as vagal. The vagal crest itself is composed of two subpopulations of precursors for the ENS: cells adjacent to somites 1 and 2 that produce Schwann cell precursors associated with the vagus nerve and contribute more to the autonomic ganglia in the oesophagus and stomach than the intestine, and cells adjacent to somites 3–7, the cervical region of the trunk crest, which contribute to form sympathetic ganglia and colonize most of the digestive tract[30]. Furthermore, enteric precursors also derive from the sacral neuronal crest, which contributes 20% of the neurons in the descending colon and rectum[30]. Enteric glia and enteric neurons are derived from enteric precursor cells that express the transcription factor Sox10 and undergo extensive proliferation as they colonize the bowel in a rostral to caudal progression[50,51]. Associated changes in the expression of transcription factors promote differentiation into neurons and glia. For example, Sox10 is maintained in glial precursors and in the majority of mature enteric glia but is progressively downregulated in neural crest precursor cells committed to a neural fate[52,53]. Subpopulations of cells within the migrating neural crest precursor cells include bipotential progenitors that can form both neurons and glia, fate-restricted gliogenic precursors with variable proliferative potential, and fate-restricted neurogenic precursors with limited proliferative capacity[4]. Together, these cells have the potential to give rise to multiple neuron subtypes and to all types of enteric glia in the intestine. However, Schwann cell precursors that invade the intestines with extrinsic nerves make a substantial contribution to neurogenesis later in development and produce many of the intrinsic sensory neurons that express the calcium-binding protein calbindin in mice[31]. Schwann cell precursors also contribute to post-embryonic neurogenesis in zebrafish[32] and to post-embryonic neurogenesis and gliogenesis in themouse ENS under certain circumstances[29]. Furthermore, given the different strategies engaged by ENS progenitors to migrate in the small intestine and the colon[54], the development and organization of neuron–glia networks could be driven differently in distinct gastrointestinal tract regions.

The development of the ENS is a dynamic process that continues postnatally and is influenced by the gut microbiota and the immune system[34,55,56]. Likewise, myenteric glia develop during the initial colonization of the myenteric plexus around E12.5 in mice and

subsequently give rise to submucosal and mucosal glia[4]. Enteric glia are not observed in the lamina propria at birth in mice and the colonization of the mucosa begins during the suckling period and does not reach adult levels until after weaning[34]. This population is continually replenished by enteric glia with progenitor potential within the myenteric plexus in a process that requires the gut microbiota. By contrast, myenteric glia are relatively stable and the rate of gliogenesis in the myenteric plexus is low under steady state conditions. Data from BrdU labelling studies suggest that only 2–3% of myenteric glia are either proliferating or newly born during a 12-week period in adult mice[57]. Whether the myenteric cells that incorporated BrdU over this period reflect enteric glia with progenitor potential that support glial turnover in the mucosa or local replenishment of glia in the myenteric plexus is unclear. The rates of glial turnover in the myenteric plexus, submucosal plexus and mucosa are currently uncharacterized and could be complicated by the fact that standard markers (such as BrdU and EdU) do not always capture cells that transdifferentiate or that migrate to the ENS along extrinsic nerves[29]. However, gliogenesis increases in response to injury, inflammation, exercise and other challenges under normal conditions and the maintenance of glia is supported by progenitor cells that are presumably the same subset of enteric glia with progenitor potential that form mucosal glia and exhibit neurogenic potential[58].

Role in gastrointestinal physiology

The fundamental function of neuroglia, a classification that encompasses enteric glia, is homeostasis of the nervous system[59]. In this regard, enteric glia can be defined as homeostatic cells of the ENS. Functional data support this definition and highlight multiple mechanisms whereby enteric glia set the tone of neurotransmission in the ENS and regulate the intestinal reflexes and processes underlying neuroinflammation in the intestine[10,14,60–62]. Multiple independent lines of evidence support a major role for enteric glia in gastrointestinal reflexive activities[10,14,63,64], but the contributions of glia to other functions, such as mucosal barrier regulation, remain debatable[11]. Here, discussion is focused on the current understanding of the role of enteric glia in the regulation of molecular, cellular and organ-level homeostasis in the intestine.

Regulation of enteric neural reflexes.

The bidirectional communication between enteric neurons and glia is a mechanism that regulates intestinal reflexes. Enteric glia surround enteric neurons and monitor multiple forms of neural activity through neurotransmitter receptor signalling[65]. The potential for enteric neuron-to-glia communication was originally described in 1972 by Gabella, who observed presynaptic specializations in nerve processes contacting enteric glia in the myenteric plexus of the guinea pig ileum[66]. Gabella noted that these “emi-junctions” are “so frequent that it seems unlikely that any glial cell is totally devoid of them”. The functional importance of neuron–glia junctions remained in question for nearly 40 years until Ca^{2+} imaging studies demonstrated functional responses in enteric glia evoked by exogenous neurotransmitters and neurotransmitters released from endogenous neurons in vitro[9,64,67–70]. It is now clear that multiple neurotransmitter systems evoke glial activity[67,71,72] encoded by intracellular Ca^{2+} responses and that glial Ca^{2+} responses, in turn, evoke the release of gliotransmitters such as ATP and GABA through membrane channels composed of connexin

43 (Cx43, also known as GJA1)[13,60] and/or through the reversal of neurotransmitter transporters[73] (FIG. 3). Glial Ca^{2+} responses are evoked in the myenteric plexus by neuronal activity during the active, contractile phase of colonic migrating motor complexes (CMMCs) that is dominated by activity in excitatory cholinergic neurons located in the myenteric plexus[64]. Myenteric glia are 'innervated' by cholinergic neurons, and neurotransmitters released by this class of neurons, such as acetylcholine and ATP, evoke Ca^{2+} responses in enteric glia in Ca^{2+} imaging studies of whole-mount preparations of myenteric plexus in mice[9,14,66]. By contrast, glia are relatively silent during the intervening quiescent periods of tonic inhibition that are driven by activity within myenteric inhibitory motor neurons that produce nitric oxide[64]. These data support the concept that myenteric glia in the colon are preferentially activated by excitatory neurotransmission and are either not responsive to or are under tonic inhibition by inhibitory neurons[74]. Little is known regarding enteric neuron–glia signalling in the submucosal plexus, although purinergic neuron-to-glia signalling is also prominent[16].

Several studies have attempted to test the functional significance of enteric glia in gastrointestinal reflexes by using glial metabolic poisons or glial ablation animal models. However, these approaches have proven inconsistent and produce widely variable effects in terms of gastrointestinal motility[17,45,75], secretions, barrier function[45,76,77] and survival[17,45,76–78]. For example, animals exposed to the glial metabolic poison fluorocitrate exhibit impaired small intestinal transit and ileal neurogenic contractions but do not exhibit marked changes in colonic motor function[17,75]. By contrast, depleting enteric glia with genetic targeting strategies does not overtly affect small intestinal transit but does impair colonic motility[45] and, in some models, is lethal[76,77]. Although these studies do support a role for glia in enteric reflexes, methodological complications limit a clear understanding of how it relates to active glial signalling. Neither technique specifically addresses glial signalling and the presence of confounding factors that do not directly involve glial signalling, such as reactive gliosis produced by fluorocitrate[79], variable efficacy of glial depletion[45], and adverse effects on epithelial and immune cells[45,76,77], limit the insight into the role of glial signalling in a physiological system. Variable efficacy in glial depletion[45,76] is particularly important when considering the potential effects on gastrointestinal motility given the extensive redundancy in the ENS. For example, neurons are integral to gastrointestinal motility but the ability to generate rhythmic propagating neurogenic colonic motor complexes is maintained in mice that have lost at least half of their enteric neurons[80]. Thus, the partial depletion of myenteric glia might not produce a robust effect on motility. Notably, current studies do not address the possibility that glial ablation models preferentially affect certain glial subtypes, which could add considerable complexity to the interpretation of these data as subtype-specific effects might produce effects that are more or less pronounced on certain functions, whereas a non-selective depletion of all glia could mask important contributions of relevant subpopulations. The effects of glial ablation on enteric neuron function and neurocircuitry are also uncharacterized. Similar issues have been partly responsible for fuelling debate surrounding the role of astrocytes in neural signalling in the brain and have made it clear that more selective methods are required to disentangle the roles of glia in neural signalling[81,82,].

Manipulating specific glial signalling mechanisms produces a clear effect on intestinal reflexes without the complications of altered cellular survival or phenotype. For example, reducing glial intercellular signalling by ablating glial *Gja1* slows intestinal motility, impairs CMMCs, impairs secretomotor reflexes and dampens neuromuscular functions in the mouse colon[11,13,60]. These observations suggest that glial signalling mediated by Cx43 exerts a modulatory effect that is required to maintain the normal strength of intestinal reflexes. Complementary studies using designer receptors activated by designer drugs (DREADDs) in mice to selectively activate Ca^{2+} responses in enteric glia extend this observation and suggest that glial cells have the potential to modulate but also to activate intestinal reflexes[12,14]. In these in vitro and in vivo experiments with mice, the chemogenetic receptor hM3Dq was expressed under the control of the *GFAP* promoter to model the activation of enteric glia by neurotransmitters acting through Gq-coupled receptors such as acetylcholine and purines[10,14]. The data show that this mechanism of glial activation is powerful and drives neurogenic contractions in the ileum and colon as effectively as stimulating neurons or smooth muscle[10,11]. In addition, augmenting glial activation during CMMCs potentiates CMMC strength, propagation speed and frequency[10,14]. These effects are absent in the presence of tetrodotoxin, indicating that the pro-contractile effects of glial activation are mediated by intercellular signalling with neurons. The same is true for secretomotor reflexes, where activating enteric glia via DREADDs indirectly drives secretomotor responses through neural signalling and possibly through direct signalling with epithelial cells[11]. Although the DREADD system relies on the expression of a foreign receptor to activate glia, it does replicate endogenous glial M3 acetylcholine receptor mechanisms[14] and shows that triggering these glial mechanisms affects the enteric neural circuitry that underlies motility and secretomotor reflexes in these models.

How enteric glia exert their effects on enteric neural reflexes is mostly uncharacterized, but it is possible that subsets of enteric glia are specialized to detect and modulate specific neural pathways. Astrocytes, a related type of neuroglia in the central nervous system, are circuit-specific cells and function to tune neurotransmission on a synapse-by-synapse basis[83–85]. Enteric glia express the specific molecular machinery to detect excitatory neurotransmitters[71], are recruited by excitatory neurons during reflexive activity, and release excitatory gliotransmitters, such as ATP and GABA[13,73], that activate P2X2/3 and $GABA_A$ receptors expressed by excitatory neurons in ascending motility pathways[86–88]. No corresponding positive interactions with inhibitory neurons or effects on inhibitory neurotransmission have been observed. By contrast, glia secrete mediators, such as prostaglandin E_2 (PGE_2)[89] and GABA[73], that suppress inhibitory pathways through EP_2 and $GABA_B$ receptors, respectively, expressed by nitrergic neurons[25]. These data support the concept that enteric neuron–glia signalling is circuit specific, but this hypothesis remains to be directly tested. Enteric glia are also recruited by some forms of activity in extrinsic sympathetic[18], parasympathetic[19] and sensory neurons[12] innervating the intestine. Additional work is needed to understand how enteric glia integrate synaptic information and modify enteric neural networks in response to these various forms of activation.

Supportive roles.

Enteric glia are often compared to astrocytes in terms of their homeostatic roles that support neural networks[90]. Supportive roles fulfilled by astrocytes are diverse and well characterized[91], but the evidence supporting the notion that enteric glia share similar functions is largely circumstantial or absent (Supplementary Table 1). Enteric glia do support enteric neurotransmission through astrocyte-like mechanisms that include supplying neurotransmitter precursors[92,93], regulating neurotransmitter availability[94–98], regulating oxidative stress[99,100], supplying neurotrophins[46,101] and, potentially, supporting neurogenesis[102,103]. Glia also extend these supportive roles to non-neuronal cells, such as intestinal epithelial cells, where glial factors influence the maturation and differentiation of the intestinal epithelium[36,104–106]. Furthermore, it is unknown whether enteric glia have a major role in other well-characterized astrocytic homeostatic functions such as ion homeostasis, regulation of pH, chemosensing, regulation of energy balance, sleep, control of blood–brain barrier and lymphatic systems, regulation of local blood flow, or glycogen synthesis and storage. The necessity of glial support in enteric networks is also unclear as depleting enteric glia in *Plp1*^{CreER};*Rosa26*^{DTA} mice does not seem to cause overt changes in neuron survival; however, this aspect has not been rigorously tested[45,107].

What limited evidence is available does suggest that enteric glia maintain homeostasis in the microenvironment surrounding enteric neurons. Glia regulate neurotransmitter availability through their expression of transporters such as the GABA transporter 2 (GAT2)[73,95] and by degrading neuroactive compounds in the extracellular space via cell-surface enzymes, including nucleoside triphosphate diphosphohydrolase 2 (NTPDase2)[96–98]. Enteric glia are immunoreactive for neurotransmitter precursors and synthesis mechanisms (including L-arginine[92,108] and glutamine synthetase[93]), which could indicate that glia support nitrergic and glutamatergic neurotransmission similarly to astrocytes[90], but this hypothesis has not been directly tested. Glutamine synthetase is also responsible for the detoxification of ammonia; enteric glia sense ammonia and modify neuromuscular transmission through GABAergic signalling with neurons in mice[73]. Type-III enteric glia in the extraganglionic region might also be involved in supplying nutrients to enteric neurons and/or shedding metabolic waste as they extend their processes to wrap around small blood vessels; however, neither role has been tested[24]. Enteric glia do express potassium channels[109–111] and voltage-gated potassium currents have been recorded in cultured enteric glial cells[109,110], although it is unknown whether enteric glia play a major role in potassium homeostasis in tissue. Enteric glia might also contribute to neurogenesis and neuronal network maturation given their ability to produce trophic compounds such as pro-epidermal growth factor[46], nerve growth factor[101,112] and vascular endothelial growth factor[113] in vitro as well as IL-1β[113–115] (which exhibits neurotrophic effects at certain concentrations[113]) and S100β[116] in vivo. However, the bulk of this evidence is based on cell culture studies and the relevance of glial trophic support in vivo is untested.

Data from targeted enteric glial ablation models are conflicting regarding the necessity of glial support in enteric networks. Glial ablation models that rely on glial-targeted $CD8^+$ and $CD4^+$ T cells in mice[77,117], transgenic mice expressing herpes simplex virus thymidine kinase from the mouse *GFAP* promoter[76] (*GFAP*$^{HSV\text{-}TK}$), and the gliotoxin 6-

aminonicotinamide[78,118] result in severe intestinal issues such as fulminant jejuno-ileitis, enterocolitis and death. By contrast, depleting glia in mice that express diphtheria toxin under the control of Cre recombinase directed to glia using the *Plp1* promoter (*PLP*^{CreER};*Rosa26*^{DTA}) does not cause overt changes beyond an increase in colonic migrating motor complex frequency in female mice[45]. The reason for this discrepancy between model organisms is not fully understood but could involve differing penetrance in model systems, differential effects on glial subtypes, and bystander and/or compensatory effects in the surrounding cells[45]. However, it is worthwhile to note that the specific attributes of neuron signalling and health have not been assessed in any of these models. Data from the *PLP*^{CreER};*Rosa26*^{DTA} mouse model show that the depletion of glia does not substantially alter enteric neuron survival under steady-state conditions, which argues against a requirement for glia in the maintenance of enteric neuron survival and ongoing neurogenesis[45,107]. This conclusion is strengthened by prior data that failed to observe any major level of neurogenesis in the adult ENS in steady-state conditions in mice[58]. By contrast, gliogenesis is consistently detected under steady-state conditions in the adult mouse gut and its rate increases after certain types of ENS injury, including inflammation, irradiation, benzalkonium chloride treatment and partial gut stenosis[57,58].

It is possible that the neurogenic potential of glia is only enacted during challenges that drive the system away from homeostasis and data showing that enteric glia contribute to the formation of new neurons during colitis[102] in response to chemical injury[58] and in the presence of oestrogen receptor-β agonists in mouse models of enteric neuronal damage[119] are in line with this conclusion. Interestingly, the depletion of glial cells in the *PLP*^{CreER};*Rosa26*^{DTA} mouse model does not substantially alter the susceptibility to dextran sodium sulfate-induced colitis[45], whereas genetic mouse models targeting specific glial mechanisms involved in inflammatory responses (such as the glial enzyme NTPDase2) do exhibit increased susceptibility to dextran sodium sulfate-induced colitis[97]. These differences suggest that future work assessing the specific glial maintenance functions might require subtle perturbations that only affect the mechanism of interest to avoid confounding factors in depletion models.

Enteric neurons could also directly fulfil some of the supportive roles attributed to glia in other systems. For example, glia regulate oxidative stress by contributing to glutathione synthesis but both enteric neurons and glia express components of the glutathione biosynthesis machinery and it is likely that neurons are able to fulfil this function in the absence of glia[99]. Other non-neuronal cells also support enteric neuron survival, such as intestinal smooth muscle, an important source of GDNF, which promotes neurogenesis and survival in the ENS[120]. Similar questions have arisen regarding the role of glia in the support of intestinal epithelial barrier function. Glia are the only neuron-type cell body at the level of the intestinal mucosa and are considered potent modulators of intestinal epithelial barrier function[104]. Enteric glia extend processes to the mucosal crypts and to the tips of the villi, ensheathing the neuronal processes involved in the control of mucosal secretions[121]. In addition, glia release barrierenhancing factors in vitro, including 15-hydroxyeicosatetraenoic acid[122], S-nitrosoglutathione[47] and GDNF[123], and levels of these factors are decreased in inflammatory bowel disease (IBD). Data from the *GFAP*$^{HSV\text{-}TK}$ mouse model show that ablating enteric glia produces a profound loss of intestinal barrier function[76,77]. However,

complications with toxicity to non-glial cells and bystander effects in intestinal epithelial cells in this model confound a clear interpretation of the results[45]. Experiments using the *PLP*^{CreER};*Rosa26*^{DTA} mouse model did not demonstrate toxicity in neighbouring cells nor that intestinal barrier function was affected[45]. Similarly, intestinal barrier function is unaffected in transgenic mouse models that have perturbed glial signaling[11] and in germ-free mice that lack mucosal glia[34,124]. Mice can tolerate a large loss of enteric glia, which makes the understanding of the contribution of enteric glia to intestinal epithelial barrier integrity and functions difficult to extrapolate. Glia clearly have the capacity to influence intestinal epithelial cells in culture systems[36,104,125]; however, whether this aspect is essential under homeostatic conditions in vivo remains contested.

Role in gastrointestinal pathophysiology

Neuropathology can be broadly defined as a failure of the nervous system to maintain local homeostasis. In this sense, it is essential to consider glia in any study of neuropathology given their contributions to maintaining local homeostasis in concert with other resident cells[126,127]. This aspect is particularly relevant in disorders of gut–brain interactions (previously known as functional gastrointestinal and motility disorders), in which abnormal motility and pain are considered disturbances of neurogastroenterology[128], and in age-related digestive disorders in which altered ENS homeostasis contributes to a decline in gastrointestinal neuromuscular function[60,129–131]. Enteric glia influence disease processes in intestinal disorders that are seemingly diverse in nature through mechanisms that regulate neuroplasticity and immune responses[12,27,113]. These mechanisms are also relevant for inflammatory disorders and new observations suggest that they also contribute to colon carcinogenesis[20], enteric neuroinflammation[132] and age-related gut motility disorders[60,129].

Activated, reactive and dysfunctional glia.

The interchangeable usage of terminology such as ‘activated’, ‘reactive’ and ‘gliopathy’ to describe different states of enteric glia has led to considerable ambiguity within the existing literature. These terms are used extensively despite a lack of clear defining criteria. Here, we provide a set of guidelines that summarize the defining characteristics of activated enteric glia, reactive enteric glia and enteric gliopathy (TABLE 2).

Activation describes a normal physiological process whereby enteric glia detect extracellular signals. Enteric glia express diverse receptors and are constantly monitoring their extracellular environment. In this sense, enteric glia are nearly always activated and only rarely, if ever, at rest. Glial activation triggers physiological intracellular signal transduction cascades such as those mediated by Ca^{2+} and cAMP[10,14,133]. The downstream effects of glial activation are generally considered beneficial and function to modulate intestinal reflexes and/or maintain homeostasis[14,17,19,44].

‘Reactive’ describes the status of enteric glia that are responding to a pathophysiological perturbation of any severity. Reactive enteric gliosis is a highly complex continuum of dynamic states that is context dependent and disease specific. Reactive gliosis is often considered a deleterious response in which glia exhibit an increase in pro-inflammatory functions at the expense of homeostatic functions such as during acute colitis in mice[12].

However, this assumption is misleading as reactive glia also exert beneficial effects and it could be argued that reactive gliosis is an attempt to maintain homeostasis. Criteria that define reactive enteric gliosis are currently lacking and experimental assessment relies on assessing changes in morphology and/or the expression of markers such as GFAP and S100β[134–136]. Whether or not changes in morphology or GFAP expression occur is not a definitive indication of a reactive glial phenotype as not all insults will produce detectable changes in morphology or GFAP expression and glial heterogeneity will affect how glial subtypes respond. More definitive guidelines for defining reactive astrogliosis have been proposed and are relevant when defining reactive enteric gliosis[137,138]. In general, reactive enteric gliosis encompasses four main features and reactive enteric glia can display one or more of the these changes depending on the severity and type of insult: (1) enteric glia can be considered reactive if they alter their molecular composition, structure and/or function in response to a pathophysiological perturbation of any severity; (2) the degree to which these changes occur varies with the nature and severity of the insult, with progressive alterations in molecular expression, progressive cellular hypertrophy and, in severe cases, proliferation; (3) the changes undergone by reactive enteric glia depend upon the type and severity of the insult, time following insult, the type of enteric glia, location within the intestine and the specific molecular signals received; and (4) the changes undergone during reactive gliosis have the potential to alter enteric glial activities through both the gain and loss of functions that can be either beneficial or detrimental to the surrounding neural and non-neuronal cells.

Enteric gliopathy refers to a dysfunctional or maladaptive response and/or survival of enteric glia. Gliopathies can result from genetic or acquired abnormalities that cause dysfunction specifically in glia (primary) or from effects in the surrounding tissue that produce glial dysfunction (secondary). An important difference between reactive gliosis and gliopathies is that gliopathies are considered maladaptive and are therefore always detrimental. Reactive gliosis is also considered a transient process, whereas a gliopathy is permanent. However, whether chronic reactive gliosis can produce a gliopathy is not understood.

Enteric glia in neuroplasticity.

Neuronal plasticity involving alterations to the sensitivity and/or survival of intrinsic and extrinsic neurons innervating the intestine produces disturbed intestinal motility and visceral pain in both functional and organic gastrointestinal disorders[139–142]. Key features of enteric neuroplasticity include the facilitation of presynaptic transmission, increased excitability of sensory neurons and interneurons, defects in purinergic neuromuscular transmission, the degeneration of inhibitory neurons and the development of reactive gliosis[13,14,139,143–147]. As noted, reactive gliosis can include deleterious effects that contribute to neurodegeneration and changes in neuromuscular function driven by acute inflammation[12,13,147], infection[148] or metabolic challenges[78]. In these examples, enteric glia are reacting to a potentially harmful environment to defend the nervous system from damage. Alternatively, reactive enteric gliosis can be triggered by intense activity in sensory neurons and enteric neurons in the absence of other inflammatory stimuli[12,13]. In this context, neurogenic inflammation, or inflammation arising from the nervous system itself, drives reactive gliosis through axon reflexes that can initiate a self-perpetuating process that results in permanent changes in neural circuitry. In either case, the development of reactive gliosis contributes to

neuroinflammation and, subsequently, to neuronal plasticity and functional abnormalities (FIG. 4a).

Enteric glia alter the survival and function of neurons through active signalling mechanisms and through more passive processes that regulate the secretion and availability of neuroactive compounds. Active glial signalling during neurogenic inflammation has a strong purinergic component that is required for the development of reactive gliosis, neuron death and long-term changes in neuromuscular function[12,13,147]. This process is initiated by ATP released from neurons through pannexin 1 channels and is subsequently perpetuated and amplified by the activation of the surrounding glial cells and their release of ATP through Cx43 hemichannels[13]. The ATP released by glia in this context acts on neuronal P2X7 receptors to promote neuronal death and neuroinflammatory processes[13,147] (FIG. 4b). Interestingly, similar mechanisms of glial recruitment by neurotransmitters and gliotransmitter release are involved in normal enteric reflexes but do not cause neuroinflammation[14]. One reason for this finding is that pro-inflammatory mediators, such as nitric oxide generated by glia during acute inflammation, interact with normal signalling mechanisms to enhance ATP release[13]. Normal neuron–glia signalling does not have a prominent nitric oxide component as the glial increase in inducible nitric oxide synthase expression only occurs during inflammation, thereby differentiating between physiological and pathological signalling. Additionally, physiological enteric neuron-to-glia signalling involves cholinergic signalling and the activation of glial cholinergic receptors does not cause neuroinflammation[14]. This finding implies that receptor-specific information is encoded in glial Ca^{2+} responses because glial purinergic and cholinergic receptors both signal through intracellular pathways that utilize Ca^{2+}. Furthermore, enteric glia do not seem to propagate Ca^{2+} responses throughout the glial network under normal conditions and glial activation is spatially restricted within so-called neuron–glia units in which only glial cells that surround a given neuron are activated in vitro[9]. How glia encode or interpret this information to differentiate between pathological and physiological signals is not yet understood.

Transcriptional profiling of mouse and human reactive enteric glia suggests that glial cytokine secretion, neurotrophin secretion and the regulation of neuromodulator availability contribute to neuroplasticity during inflammation[12,113,133] (FIG. 4b). Reactive enteric glia increase their production of pro-inflammatory and anti-inflammatory cytokines and chemokines but the specific effects of glial-derived cytokines and/or chemokines are not well defined. Certain glial proinflammatory proteins, such as IL-1β and IL-6, enhance neuronal excitability by modifying glial and neuronal signaling[149,150], while others, such as CXCL2, CXCL10, IL-22 and IL-23, might indirectly affect neuronal sensitivity through actions on local immune responses[12,133,151]. For example, an increase in the production of IL-1β by enteric glia is considered an important mechanism in the development of postoperative ileus by modulating ENS activity[152] and glial macrophage colony-stimulating factor (M-CSF) production influences visceral sensitivity following intestinal inflammation through Cx43-dependent signalling with muscularis macrophages[48]. Glial neurotrophins might also contribute to neuronal sensitization and links between enteric glia, nerve growth factor[112] and substances produced in response to brain-derived neurotrophic factor[153] have been proposed. In addition, comparing transcriptomics datasets to assess the potential

'interactome' between enteric glia and colon-projecting dorsal root ganglion neurons supports the concept that neurotrophins could be potential molecular substrates of interactions (published as pre-print[154]). However, these interactions have not yet been directly tested. More conclusive data show that S100β protein release via glial RAGE–NF-κB signalling pathways is associated with the onset and maintenance of nitric oxide-dependent inflammation in the human gut (rectal biopsy samples from patients with ulcerative colitis[155]) and that the S100β–RAGE interaction promotes the release of pro-inflammatory cytokines, including IL-1β, TNF and nitric oxide, as well as the recruitment of immune cells such as macrophages and mast cells to the intestinal mucosa in mice and humans. Mouse and human transcriptomics datasets indicate that inflammation also induces major changes in glial molecular mechanisms that regulate neuroactive compounds such as ATP, histamine and serotonin, but the effect of these changes on neuronal signalling is mostly undefined[12,97,133].

Enteric glial–immune interactions.

The intestine is the largest immune organ and glial cells are ideally positioned to bridge interactions between the immune and nervous systems[156]. Yet, little is known about bi-directional interactions between enteric glia and immune cells, their physiological relevance, or the specific mechanisms involved. Glial transcriptional responses to experimental colitis in mice and lipopolysaccharide in vitro exhibit an increased expression of genes associated with immune responses[12,113,148]. Many of these genes reflect the modulatory effects on innate immune responses, which is supported by data in mice showing that glia secrete neurotrophins that fine-tune innate IL-22 production from group 3 innate lymphoid cells[37]. Likewise, data show that enteric glia promote muscularis macrophages to adopt a pro-inflammatory phenotype through mechanisms that involve Cx43 and M-CSF release during chronic colitis in mice[48]. Enteric glia also modulate adaptive immune responses and have immunosuppressive effects on T cells in vitro[151]. The mechanisms whereby enteric glia influence T cells are not known, but human and mouse enteric glia do express major histocompatibility complex class II in response to pathogenic microorganisms in vitro[148] and glial antigen presentation contributes to interactions with T cells in a mouse transgenic model of Crohn's disease[77]. Enteric glia also modulate local immune responses and gut barrier integrity after injury by transmitting vagal anti-inflammatory signals to resident immune cells[157]. In addition to the modulatory effects, enteric glia might also initiate immune responses. Support for this concept comes from in vitro work showing that the exposure to pathogenic enteroinvasive *Escherichia coli* bacteria elicits protective responses in enteric glia that include the upregulation of immunomodulatory mechanisms[148]. The relative importance of glial-driven immune responses or modulation in vivo is still unclear and will be an important area for future research.

Enteric glia in cancer.

Glia have a central role in tumorigenesis in the nervous system and current data suggest that the same is true in the intestine, where enteric glia contribute to multiple aspects of tumorigenesis. For example, human duodenal gastrinomas associated with mutations in the *MEN1* gene are composed of cells expressing markers of enteric glia[21]. Under these circumstances, glial gastrin expression is induced in a feed-forward signalling loop that

involves the activation of cholecystokinin B receptors and results in the loss of menin protein and the de-repression of gastrin messenger RNA in mice[21]. Other mechanisms of tumorigenesis identified by in vitro studies show that bi-directional signalling between enteric glia and colon cancer stem cells drives tumorigenesis through mechanisms that involve IL-1 release from tumour epithelial cells and PGE_2 release from enteric glia[20]. In cultured biopsy samples, the increased expression of glial S100β also parallels tumour progression in the human colon and could contribute to the carcinogenic microenvironment by sequestering the pro-apoptotic factor wild-type p53 and upregulating pro-inflammatory and proangiogenic mediators[158]. Interestingly, enteric glia only acquire a pro-tumorigenic phenotype when exposed to tumour epithelial cell-derived IL-1 in vitro[20]. This finding suggests that glia might not initiate tumorigenesis but, once activated by a tumour, can stimulate further tumorigenesis. Tumour cells also utilize the ENS as a substrate for tumour invasion and, according to coculture studies, neurons are the predominant adhesion partner of tumour cells; the role of glia in this process is unclear[159].

Enteric glia in extraintestinal diseases.

Enteric glia are gaining substantial interest due to their role as an early ‘sentinel’ of neuroplastic changes that develop during various extraintestinal pathologies. Enteric glia exhibit a reactive phenotype during the earliest stages of extraintestinal diseases, such as Parkinson disease[160] and obesity[161], and enteric glia are the first site of entry to the nervous system in certain intestinal infections that spread to the brain such as prion disease and JC virus infection[22,162]. Glial GFAP expression and phosphorylation is elevated in colonic biopsy samples from patients with Parkinson disease and is associated with increased permeability and mucosal inflammation[163,164]. This local glial reaction might be driven by extracellular α-synuclein or other neurotropic agents that breach the intestinal epithelial barrier and trigger the initial α-synuclein aggregation in axon terminals in the enteric plexuses according to the Braak hypothesis[165,166]. Glial transition to a reactive phenotype might, in turn, exacerbate local inflammatory responses and synaptic dysfunction and facilitate the ascension of Parkinson disease pathology to the CNS[167]. Active parts played by enteric glia in the neuroinvasion process of prion disease provide support for this hypothesis. In mice, enteric glia act as a reservoir and replication site for misfolded prion protein before its replication in the brain and enteric glia exhibit the same alterations observed later in central astrocytes, including altered morphology and co-localization of misfolded prion protein in $GFAP^+$ cells[168]. The observation that JC virus preferentially infects myenteric glia in patients with chronic idiopathic intestinal pseudo-obstruction reinforces the concept that glia act as an initial site of entry for intestinal pathogens that disrupt neuromuscular function[22]. Thus, a growing body of evidence suggests that glia contribute to widespread nervous system disorders by facilitating the spread of pathogens that cross an impaired intestinal barrier. Whether glial changes are causative or reactionary to neuroplastic or barrier abnormalities remains to be clarified. Traumatic brain injury does increase colonic glial Sox10 and GFAP expression along with mucosal permeability in mice[169], suggesting descending modulatory mechanisms. By contrast, intracolonic administration of HIV1 Tat protein in rats triggers ascending dysfunction reflected by increases in Cx43 (GJA1) and inducible nitric oxide synthase expression in $S100\beta^+$ cells in the submucosal plexus followed by similar increases in the spinal cord and frontal cortex and, ultimately, cognitive

performance impairment[170]. These observations suggest that glia contribute to bi-directional communication between the periphery and the brain during pathophysiological states, although the mechanisms regulating glial crosstalk remain unknown.

Translational implications

The neuromodulatory and immunomodulatory properties of enteric glia are increasingly attractive as therapeutic targets for diverse gastrointestinal diseases (TABLE 3), including diseases with active inflammatory components such as IBD (to modulate immune responses), diseases that involve neuroplasticity such as IBS or IBD in remission (to tune the sensitivity of neurons), and diseases with unclear aetiology such as postoperative ileus or chronic intestinal pseudo-obstruction (to improve motor function). Given the evolving role of enteric glia in tumorigenesis, the targeting of glial mechanisms could be explored as a potential cancer treatment. Drugs or therapies that are specifically designed to affect glial mechanisms do not exist. However, glial mechanisms are affected by many of the recently approved or new emerging therapies for functional bowel disorders[171]. For example, at least some of the beneficial effects of drugs for IBS, such as the neurokinin 2 receptor antagonist ibodutant, muscarinic M3 receptor antagonists and the glutamine synthetase product glutamine, might involve effects on glia based on in vitro and in vivo data that show glial expression and the function of these mechanisms[12,14,73,171]. Similarly, the beneficial actions of P2X7 receptor antagonists on visceral pain in individuals with IBD[170] suggest that purinergic neuron–glia signalling in the context of acute inflammation contributes to visceral pain and experiments in animal colitis models support this mechanism[12]. The same might be true for pannexin 1 antagonists such as spironolactone, which is currently used as a treatment for hypertension but has not yet been explored for its effects in gastrointestinal disorders. The reduction of reactive gliosis in animal models of intestinal inflammation improves the survival of enteric neurons and protects against long-term changes in intestinal motor functions[12,62,172,173]. Based on these experimental data, drugs that reduce reactive gliosis, such as the PPARα agonist palmitoylethanolamide[62,172], cannabidiol[135], pentamidine[173], the RAGE inhibitors[174,175], the IL-1 receptor antagonist anakinra[152,176] or ibodutant[177], could be beneficial in the treatment of gastrointestinal diseases with inflammatory or neuroinflammatory processes. Data from animal models show that glia have the capacity to control enteric motor circuits and that targeting the conserved mechanisms in humans could be a powerful therapeutic approach for motility disorders.

Conclusions

Enteric glia are one of the most dynamic cell types in the gastrointestinal tract and fulfil diverse roles in neuronal support, mucosal integrity, neuroprotection, neurogenesis, neuroimmune interactions and synaptic transmission. Although the diversity of glial functions is only beginning to be unveiled, those currently known demonstrate incredible plasticity, wide heterogeneity and place enteric glia in a crucial regulatory role in all major gut activities. Increasingly sophisticated genetic and imaging technologies continue to facilitate the understanding of specific functions of enteric glia and have led to major progress in terms of understanding the functional relevance of glial Ca^{2+} response in motor circuits. However, the complexity of the signalling between enteric glia, neurons and non-

neuronal cells is still poorly defined and the influence of these interactions on gut health and disease is unclear. The specificity of glial signalling in the digestive tract, molecular signatures and functional characteristics of glial subtypes, and how enteric glia contribute to gastrointestinal dysfunction and disorders of the gut–brain axis remain open questions (BOx 1). Answering these questions represents a stimulus for growth in neurogastroenterology and an opportunity to identify new therapeutic strategies for gastrointestinal and extraintestinal disorders.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

B.D.G. receives support from grants R01DK103723 and R01DK120862 from the National Institute of Diabetes and Digestive and Kidney Diseases of the National Institutes of Health. The content is solely the responsibility of the Authors and does not necessarily represent the official views of the National Institutes of Health.

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Box 1 |

Outlook and open questions

Outlook

- Bi-directional enteric neuron–glia communication is a fundamental mechanism that regulates enteric nervous system functions.
- Enteric glia display phenotypic plasticity and glial heterogeneity is present within regions, between regions and during pathophysiological states.
- Enteric glia communicate with immune cells and influence immune homeostasis.
- Enteric glia have complex interactions with nerves, immune cells, microbiota, enterocytes and neuroendocrine cells at the mucosal interface.
- Enteric glia encode and decode physiological and pathological information in intracellular Ca^{2+} transients.
- Enteric glia have a key role in gastrointestinal tumorigenesis.
- Enteric glia regulate and are influenced by inflammation; changes in gut functions during inflammation affect local gastrointestinal functions and gut–brain signalling.

Open questions

- Are glial signalling mechanisms specific to individual neuron types and pathways? If so, how do glia encode this information and use it to influence neurons and other cell types that coordinate gastrointestinal functions?
- How extensive is glial heterogeneity in the digestive system? What are the combinatorial codes that define different glial subtypes by distinct protein expression, responsiveness to various intercellular mediators and transcriptional profiles?
- What is the role of enteric glia in gut–brain communication? Are specific glial subtypes devoted to intrinsic and extrinsic neurons and what is their role in gut–brain signalling?
- To what extent do glia contribute to neurogenesis and gliogenesis under normal and pathophysiological conditions? Is a specific progenitor subtype responsible? What are the mechanisms that promote neurogenesis versus gliogenesis?
- How do glia regulate immune homeostasis and how is this altered by disease?
- How do specific reactive glial phenotypes contribute to disease?

Example experimental approaches for open questions

- Functional imaging of enteric neuron–glia networks.

- Glial and neuronal directed cellular actuators; for example, designer receptors exclusively activated by designer drugs and indicators (such as genetically encoded Ca^{2+} indicators).
- Slide-seq, single-cell RNA sequencing and RNAscope to study distinct glial subtypes and assess molecular changes under diverse conditions.
- In vivo Ca^{2+} imaging of gut-projecting neurons, combined with glial designer receptors exclusively activated by designer drugs to study how enteric glia influence those neurons.
- Single-cell lineage tracing studies combined with pseudotime transcriptional analyses of molecular changes associated with progenitor-to-neuron and progenitor-to-glia differentiation.
- Models that perturb glial-specific immune-modifying signalling pathways and test the outcomes on immune homeostasis in health and disease.
- Single-cell transcriptional analysis of reactive glia throughout disease progression and resolution.

Key points

- Enteric glia are a heterogeneous population of peripheral neuroglia that regulate homeostasis in the enteric nervous system.
- Bidirectional communication between enteric glia and neurons modulates intestinal reflexes.
- Enteric glia are central players in neuroinflammation and contribute to neuroplasticity through interactions with neurons and immune cells.
- Enteric glia regulate disease processes involved in tumorigenesis and extragastrointestinal diseases.
- Therapies targeting glial mechanisms, such as gliotransmitter release or signalling pathways that promote gliosis, could substantially advance the treatment of common gastrointestinal diseases.

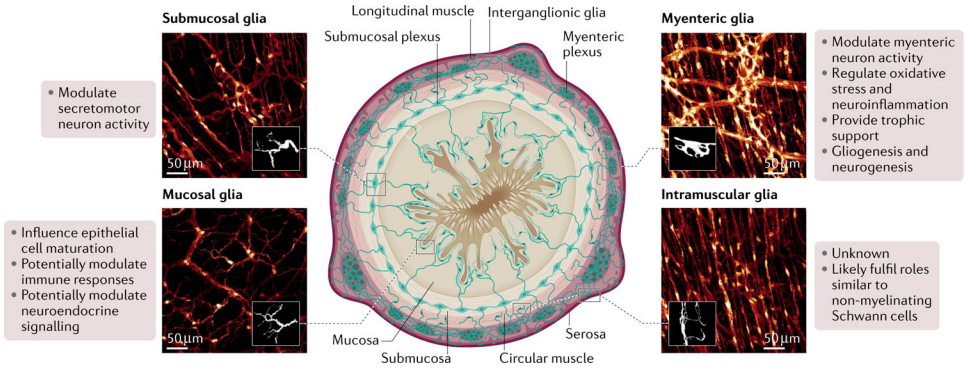

Fig. 1 |. Main populations of enteric glia and their known physiological functions.

Local subpopulations of glia are defined based on their morphology, anatomical location throughout the intestinal wall, and localization either within or outside of enteric ganglia. Based on these criteria, at least six main types of enteric glia are identified in any given region of the intestine: intraganglionic glia, including glia associated with neuronal cell bodies in the myenteric and submucosal plexuses (myenteric glia or Type-I_{MP} and submucosal glia or Type-I_{SMP}, on the top right and left, respectively); interganglionic glia, located within nerve fibre bundles connecting myenteric ganglia (Type-II); extraganglionic glia, including glia associated with nerve fibres at the myenteric and submucosal plexus but outside the ganglia (Type-$III_{MP/SMP}$) and in the intestinal mucosa (mucosal glia or Type-III_{mucosa}, bottom left); and glia associated with nerve fibres in the circular and longitudinal muscle layers (intramuscular glia or Type-IV, bottom right). Known functions of each subtype are listed beside each representative image.

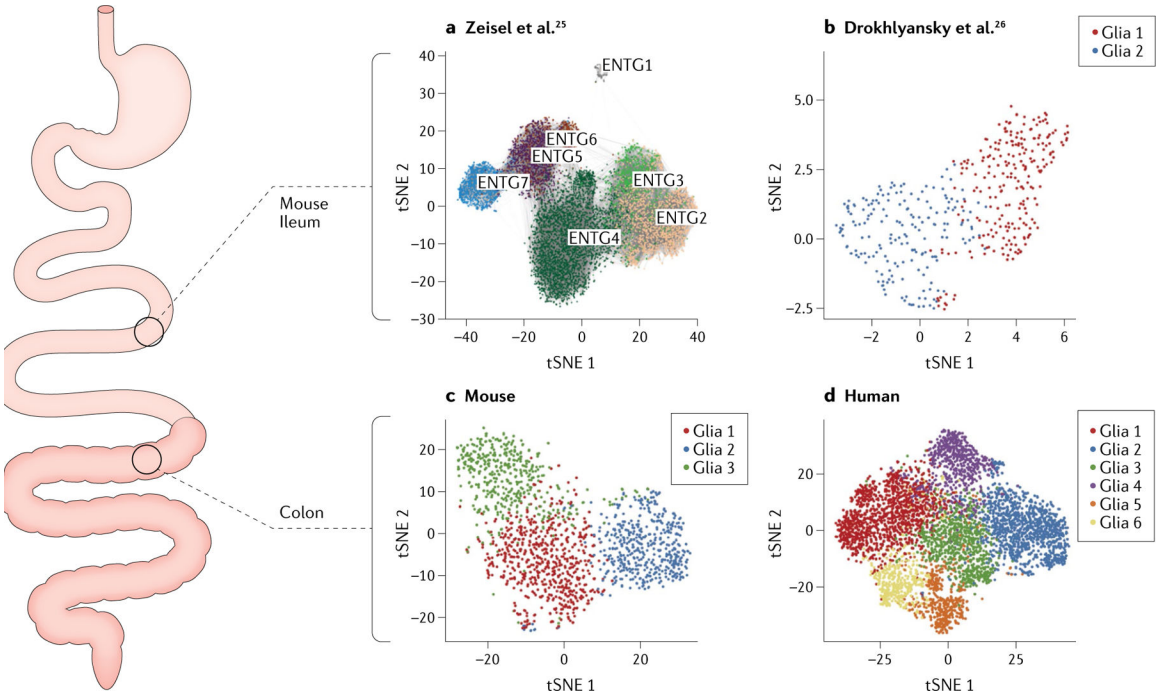

Fig. 2 |. Transcriptionally distinct glial subsets in the adult enteric nervous system.

Distinct subsets of enteric glia display unique transcriptional profiles in the enteric nervous system. Data from single-cell sequencing experiments indicate the presence of distinct glial populations that vary according to location along the gastrointestinal tract and species. The mouse ileum exhibits seven glial populations according to data from Zeisel et al.[25] (part **a**) and two glial subpopulations according to data from Drokhlyansky et al.[26] (part **b**). The mouse colon displays three glial subsets according to data from Drokhlyansky et al.[26] (part **c**) and the human colon displays six glial subsets (part **d**; also data from Drokhlyansky et al.). tSNE, t-distributed stochastic neighbour embedding. Part **a** was adapted from REF.[25], CC BY 4.0 (https://creativecommons.org/licenses/by/4.0/). Parts **b**–**d** were adapted with permission from REF.[26], Elsevier.

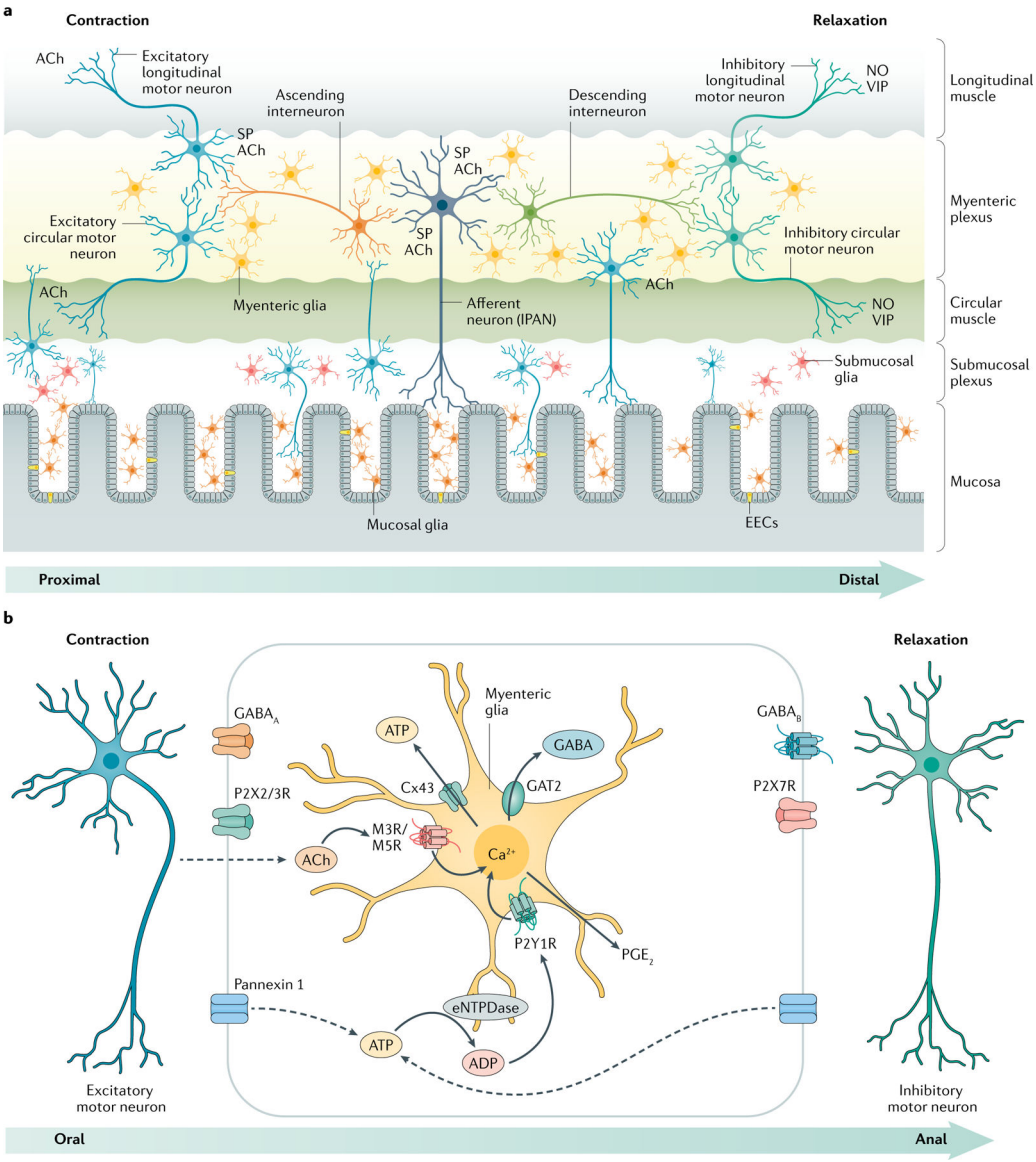

Fig. 3 |. Mechanisms of bidirectional communication between enteric neurons and glia in enteric circuits.

a | Enteric glia surround neurons in the myenteric and submucosal plexuses and are associated with nerve fibres in the mucosa. **b** | Glial Ca^{2+} responses are evoked in the myenteric plexus by neurotransmitters such as acetylcholine (ACh) and ATP, which, in turn, induce the release of gliotransmitters such as ATP and GABA through membrane channels composed of connexin 43 (Cx43) and/or through the reversal of neurotransmitter transporters (such as GABA transporter 2 or GAT2). These gliotransmitters act on receptors expressed by excitatory and inhibitory neurons to exert reciprocal effects on enteric neural circuits that control the intestinal motility. EEC, enteroendocrine cells; eNTPDase, ectonucleoside triphosphate diphosphohydrolase 2; NO, nitric oxide; SP, substance P; VIP, vasoactive intestinal peptide.

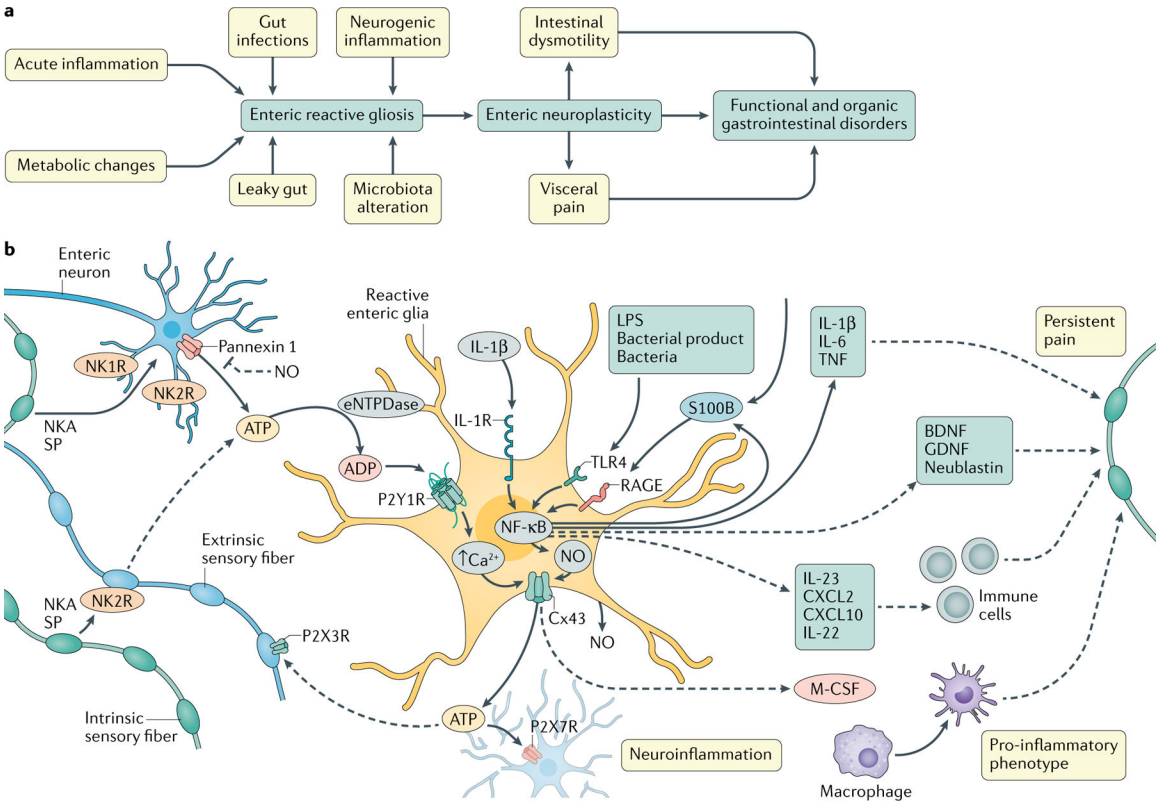

Fig. 4 |. Intercellular glial signalling mechanisms that contribute to neuroplasticity during gastrointestinal inflammation.

a | Reactive enteric gliosis is a protective response to potentially harmful stimuli. Reactive enteric glia contribute to functional abnormalities that underlie both functional and organic gastrointestinal disorders by promoting neuronal plasticity. **b** | Enteric glial signalling mechanisms active during acute inflammatory responses. Enteric reactive gliosis may be driven by adenosine triphosphate (ATP) release via pannexin 1 channels from enteric neurons intensively stimulated by neuronal mediators, such as neurokinin A (NKA), substance P (SP) or ATP. A self-perpetuating process derives from the activation of P2Y1Rs in the surrounding glial cells and their release of ATP through connexin 43 (Cx43) hemichannels. ATP released by glia drives P2X7R-mediated neuroinflammation and nociceptive neuron activation, likely via P2X3Rs. Glial activation also mediates the Cx43-dependent release of macrophage colony-stimulating factor (M-CSF) and other mediators that potentially regulate the activation of muscularis macrophages and visceral sensitivity in intestinal inflammation. Pro-inflammatory signals, such as S100β, IL-1β and bacterial products, increase the release of nitric oxide (NO) and other inflammatory signals via NF-κB that directly or indirectly affect the normal glia–neuron purinergic signalling and/or neuronal sensitivity through actions on local immune responses. eNTPDase, ectonucleoside triphosphate diphosphohydrolase 2; GDNF, glial cell-derived neurotrophic factor; LPS, lipopolysaccharide.

Table 1 |

Glial subtypes

Type	Enteric glial subtype		Defining features	Known or proposed functions
Intraganglionic	Myenteric	Type I_{MP}	Small somata and extend very short, irregularly branched processes that surround neurons; associated with neuron cell bodies in the myenteric ganglia (‘protoplasmic-like’)	Modulate myenteric neuron activity[10,11,14,17–19]; regulate oxidative stress[99,100]; provide trophic support[92–98,178]; regulate neuroinflammation[12,13,113,133,157]; gliogenesis[57], neurogenesis[58,102,103,119] and replenishment of mucosal glia[34]
	Submucosal	Type I_{SMP}	Associated with neuron cell bodies within submucosal ganglia	Modulate secretomotor neuron activity[16]
Extraganglionic	Interganglionic	Type II	Subtype that resides in interganglionic fibre tracts (‘fibrous’) with long processes that branch infrequently and run parallel to nerve fibres connecting enteric myenteric ganglia	Signal propagation in the glial network[70]; neuromodulation?
	Mucosal	Type III_{mucosa}	Extend a small number of long, fine unbranching processes; some follow nerve fibres and some terminate at the mucosal epithelium	Influence epithelial cell maturation[36,46]; potentially modulate immune responses[19,37]; probable modulation of neuroendocrine signaling[21,35]
	Enteric plexus	Type $III_{MP/SMP}$	Subtype located in the extraganglionic regions at the level of myenteric and submucosal plexuses that run along neuronal fibres and/or wrap around small blood vessels	Unknown
	Intramuscular	Type IV	Extend a small number of long, fine unbranching processes that follow nerve fibres in the muscularis	Unknown

MP, myenteric plexus; SMP, submucosal plexus.

Table 2 |

Criteria for and examples of activated glia, reactive glia or gliopathy

Glial phenotype	Criteria	Examples from experimental evidence
Activated enteric glia	Response to physiological stimuli; normal signalling mechanisms whereby enteric glia monitor the activity of cells in the surrounding tissue; primarily enacts glial mechanisms that exert beneficial, homeostatic effects	Enteric glia exhibit activity encoded by intracellular Ca^{2+} signalling in response to neurotransmitters released by intrinsic (enteric)[16,60,64,66,68,70,71,179] and extrinsic[12,18] neurons; enteric glial activation encoded by intracellular Ca^{2+} responses modulates enteric excitatory motor[10,14,17,60] and secretomotor[11] neurocircuits; enteric glia modulate local immune response in vitro and in vivo[37,48,151,157]; enteric glia influence the maturation and differentiation of the intestinal epithelium in vitro[46,104–106,180]
Reactive enteric glia	Response to pathophysiological perturbation of any severity; involves altered molecular composition, structure and/or function; changes undergone are progressive and depend on the type and severity of injury, glial subtype and specific molecular signals received; changes have the potential to alter glial activities through both gain and loss of functions that can be either beneficial or detrimental to the surrounding neural and non-neuronal cells	Enteric glia respond to intestinal inflammation[12,13,62,116,148,155] and infection in vitro and in vivo[22,148,170,172]; enteric glia undergo changes in GFAP expression, morphology, cytokine release and gene expression profile during intestinal inflammation in vitro and in vivo [12,113,133]; enteric glia contribute to neuron death during acute intestinal inflammation[13,147]; enteric glia contribute to vagal anti-inflammatory effects on resident intestinal immune cells following intestinal injury[19,157]; enteric glia influence visceral sensitivity through interactions with muscularis macrophages[48]
Enteric gliopathy	Dysfunctional or maladaptive response and/or survival of glial cells; might be primary and result from genetic or acquired dysfunction emanating from glia, or secondary whereby glial dysfunction results from effects in the surrounding tissue; exerts detrimental effects that contribute to disease	Glial support of epithelial barrier homeostasis is impaired in Crohn's disease in vitro[122,181]; enteric glial networks are impaired and display dysfunctional responses in patients with Crohn's disease[77,90]

Table 3 |

Targeting glia for translational benefit

Glial mechanism	Potential benefit	Available drugs
Neuron–glia communication in acute inflammation	Reduce neuroinflammation, neuroprotection and visceral hypersensitivity (pain and dysmotility)	P2X7R antagonists: AZD9056, CE-224,535, GSK1482160 (REFs[147,177,182,183]) Pannexin 1 antagonists: spironolactone[178] Neurokinin 2 receptor antagonists: ibodutant[177]
Pathways triggering gliosis	Inflammation, inflammatory postoperative ileus, acute inflammation, inflammation, bacterial infections or in gastrointestinal diseases involving bacterial translocation, and neuroinflammation	Anakinra: IL-1R antagonist[152] Proliferator-activated receptor-α agonist: palmitoylethanolamide[62] Toll-like receptor, receptor for advanced glycation endproducts or nitric oxide synthase antagonists: L-NMMA, PF-04494700 (REFs[13,61,148,174,175,184]) Peroxisome proliferator-activated receptor-γ agonist: cannabidiol[135] S100β inhibitor: pentamidine[148,173,184,185]
Gliotransmission	Motility disorders such as postoperative ileus, chronic intestinal pseudo-obstruction or chronic constipation	None