Review

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Microbiota-gut-brain axis: enteroendocrine cells and the enteric nervous system form an interface between the microbiota and the central nervous system

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ABSTRACT

The microbiota-gut-brain axis transmits bidirectional communication between the gut and the central nervous system and links the emotional and cognitive centers of the brain with peripheral gut functions. This communication occurs along the axis via local, paracrine, and endocrine mechanisms involving a variety of gut-derived peptide/amine produced by enteroendocrine cells. Neural networks, such as the enteric nervous system, and the central nervous system, including the autonomic nervous system, also transmit information through the microbiota-gut-brain axis. Recent advances in research have described the importance of the gut microbiota in influencing normal physiology and contributing to disease. We are only beginning to understand this bidirectional communication system. In this review, we summarize the available data supporting the existence of these interactions, highlighting data related to the contribution of enteroendocrine cells and the enteric nervous system as an interface between the gut microbiota and brain.

INTRODUCTION

Humans have evolved through the influence of environmental factors. Among these factors, microbiota constitute the most important factor for the evolution of humans because bacteria appeared on Earth approximately 3.8 billion years ago, which was earlier than the appearance of human beings (Bordenstein *et al.* 2015). Thus, humans have evolved with the microbiota, and they have established a complex host-microbiota interaction since intestinal microbes confer numerous metabolic and biological functions that humans are unable to perform within their own cells. This symbiotic relationship may influence not only human health but also the risk of developing disease when the communication between the microbes and human organs is disordered (Fun *et al.* 2017; Liang *et al.* 2018; Valdes *et al.* 2018).

Recent advancements in the scientific knowledge have changed the prevailing thought of unidirectional communication from the brain to the gut. Recently, gut-brain crosstalk has been considered to be bidirectional and includes a complex communication system that not only ensures the proper maintenance of gut homeostasis but also likely has multiple effects on affect, motivation, and higher cognitive functions (Fun *et al.* 2017; Siva *et al.* 2020). Most individuals are made aware of such communication when alterations in gut function are transferred to the brain,

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eliciting perceptions of visceral events such as nausea, satiety, and pain or, in contrast, when stressful experiences lead to altered intestinal secretions and motility (Liang *et al.* 2018). These interactions are called based on the "gut-brain axis" or the "brain-gut axis". The role of the gut-brain axis is to monitor and integrate gut functions and to facilitate the communication of the energy, emotional and cognitive centers in the brain with peripheral intestinal functions and mechanisms. Recent studies have suggested that the gut-brain axis is influenced by the gut microbiota, together forming the "microbiota-gut-brain axis" (Montiel-Castro *et al.* 2013; Carabotti *et al.* 2015; Mayer *et al.* 2015; Cryan *et al.* 2019).

A number of different mechanisms have been proposed to explain how the intestinal microbiota might influence the nervous system including the enteric nervous system (ENS) and the central nervous system (CNS) and vice versa (Montiel-Castro *et al.* 2013; De Vadder *et al.* 2014; Dinan *et al.* 2015; Mukhtar *et al.* 2019). The immune system may also play an important role in many of the phenomena described below (Fagundes *et al.* 2012; Rooke *et al.* 2016; Reardon *et al.* 2018), but in this review, we will focus specifically on the nonimmune aspects of the communication between the gut microbiota and the nervous system. We will first provide descriptions of the gut microbiota, its metabolites, the enteroendocrine system and the ENS because they are essential components of the microbiota-gut-brain axis. Finally, we will discuss how these components contribute to the communication along the microbiota-gut-brain axis.

Gut microbiota

The gut microbiota is found in the gut lumen of vertebrates and invertebrates. It is a complex community consisting of more than 40,000 species, with *Firmicutes* and *Bacteroides* as the dominant phyla that contribute to the maintenance of a dynamic metabolic ecological balance (Lynch *et al.* 2016; Rinninella *et al.* 2019). The human gut contains 10^{13}–10^{14} microorganisms, many more than the number of nucleated cells in the human body, with 150-hold more genes than comprise the human genome (Marchesi and Shanahan 2007). The density of the microbiota exponentially increases from the proximal to the distal gastrointestinal (GI) tract, reaching its peak in the colon. The composition of the gut microbiota in the human stool is diverse, even among healthy individuals. However, the genes related to metabolic pathways are stable among individuals, regardless of bacterial composition (Human Microbiome Project Consortium 2012). Thus, the gut microbiota and its metabolic products are able to influence a variety of aspects of vertebrate physiology (Wikoff *et al.* 2009; Hills Jr *et al.* 2019). Commensal bacteria mediate the extraction, synthesis and absorption of a wide variety of metabolites (Nicholson *et al.* 2012). Imbalances in or disruptions to the microbiota are associated with various diseases including obesity, type 2 diabetes, nonalcoholic fatty liver disease, dyslipidemia and higher brain disorders (Fabbiano *et al.* 2017; Quesada-Vázquez *et al.* 2020).

Through the fermentation of undigested dietary fibers that reach the large intestine, the gut microbiota produces wide range of metabolites (Rooke and Garrett 2016). Among the quantitatively most important gut microbiota-derived metabolites are short-chain fatty acids (SCFAs). SCFAs are organic fatty acids, with from two to six carbon atoms, are produced in the caecum and the colon by the microbiota following the fermentation of indigestible dietary fibers, proteins, and glycoproteins (Wong *et al.* 2006). Acetate (C2), propionate (C3), and butyrate (C4) represent 95% of the SCFAs (Cummings *et al.* 1987). SCFAs locally modulate the gut function from the duodenum to the colon (Kaji *et al.* 2014; Akiba *et al.* 2015; Kaji *et al.* 2016) but they can also be absorbed (only 5%–10% are excreted in feces) and can control the metabolism of other organs—such as adipose, liver, muscle, and brain tissue, thus influencing the host's energetic homeostasis, including appetite regulation (Canfora *et al.* 2015; Silva *et al.* 2020; Xiao and Kang 2020). Under physiological conditions, luminal concentrations of total SCFAs reach up to 80–130 mM in the human colon (Cummings *et al.* 1987). SCFAs are absorbed by the host epithelium through the SCFAs transporter, sodium-dependent monocarboxylate transporter 1 (SMCT-1) or by passive diffusion (Fig. 1) (Cryan *et al.* 2019). Absorbed butyrate is used as an energy source for colonocytes. Propionate, acetate and the remaining butyrate are subsequently metabolized by hepatocytes resulting in 1–15 μM propionate and butyrate in the circulation while acetate is found in concentrations of approximately100–200 μM (Peters *et al.* 1992; Bloemen *et al.* 2009). In addition to serving as energy sources for the host, SCFAs function as signaling molecules (Silva *et al.* 2020). SCFAs are sensed by specific G protein-coupled receptors (GPCRs) and free fatty acid receptors 2 and 3 (FFA2 and FFA3) and they modulate a variety of physiological and hormonal processes that contribute to whole-body energy bal-

Fig. 1 Immunohistochemistry for slc5a8 in the distal colon of mouse. The SMCT-1 immunoreactivity in the distal colon is restricted to the luminal side of crypts (Iwanaga *et al.* 2006).

ance (Layden *et al.* 2013). FFA2 and FFA3 are activated by all three types SCFAs, with somewhat different affinities; FFA2 has a higher affinity for acetate than does FFA3. FFA2 and FFA3 are preferentially expressed in L-type enteroendocrine cells (EECs) in the distal ileum and colon of rats and humans (Fig. 2, Inset) (Karaki *et al.* 2006; Tazoe *et al.* 2008). This expression pattern at the natural site of their ligand production may indicate the close relationship between gut microbiota and the host. In addition to FFA2 and FFA3, other GPCRs modulated by SCFAs have been discovered namely, Olfr78 (OR51E2) and GPR109a (Thangaraju *et al.* 2009; Pluznick 2017). Compared to FFA2 and FFA3, the ligand profiles, expression pattern, and function of Olfr78 and GPR109a are less well characterized (Priyadarshini *et al.* 2018).

Bile acids also represent an important class of metabolites modulated by the gut microbiota (Staley *et al.* 2017; Wei *et al.* 2018). Bile acids affect a number of important processes including lipid, glucose and energy homeostasis through bile acid receptors (Fig. 2 Inset) (Wang *et al.* 1999; Lefebvre *et al.* 2009; Copple and Li 2016). Nearly 95% of bile acids can be reabsorbed in their conjugated form in the terminal ileum and recycled back to the liver via the portal vein in a process called enterohepatic circulation (Copple and Li 2016). Escaped primary bile acid is converted to secondary bile acids by colonic bacteria. Even in the small intestine, bile acids are subjected to biotransformation by the resident microbial community (Staley *et al.* 2017). Some conjugated bile acids are deconjugated by bacterial bile salt hydrolases (BSHs) to become free bile acids (Li and Chiang 2014). In the ileum, most conjugated bile acids are reabsorbed by the apical sodium-dependent bile salt transporter (ASBT) or the ileal bile acid transporter (IBAT) (Staley *et al.* 2017). Unconjugated bile acids can cross the epithelial cell membrane by passive diffusion, then function as signaling molecules and metabolic integrators (Li and Chiang 2014).

The gut microbiota produces a number of metabolites as explained above. Multiple bioactive molecules derived from the diet also undergo microbial modification. For example, indole, the most prevalent metabolite of tryptophan, is produced by many bacterial species (Lee and Lee 2010; Jaglin *et al.* 2018). Indole is reported to modulate the secretion of the incretin hormone, glucagon-like peptide 1 (GLP-1) (Fig. 2) (Chimerel *et al.* 2014). Indole presents in low millimolar concentrations in the colonic lumen and has an effect opposite that of GLP-1 release (Chimerel *et al.* 2014). The acute effect of indole is to raise intracellular Ca^{2+} levels and enhance GLP-1 secretion but it reduces GLP-1 secretion over longer periods. These effects are induced not via a specific indole receptor but by the inhibitory action of indole on voltage-gated potassium channels in primary mouse colonic L-cells. Blocking K^+ channels result in a widening of the action potentials and consequent voltage-gated Ca^{2+} entry, thereby acutely stimulating GLP-1 secretion. Thus, indole might also function as a chemical messenger through the release of GLP-1. However, it is not known whether GLP-1 secretion induced by indole paves a functional route for sending the luminal information to the ENS and/or CNS.

It has been suggested that at least some neuroactive compounds were conserved during the process of coevolution to serve as the "words" of a common language, thus allowing communication between the microbiota and the host. Indeed, the gut microbiota is able to produce a wide variety of neurochemicals including γ-aminobutyric acid (GABA), 5-hydroxytryptamine (5-HT), melatonin, histamine, acetylcholine (ACh), norepinephrine and dopamine (Iyer *et al.* 2004; Lyte 2011; Mazzoli and Pessione 2016). For example, multiple strains of *Lactobacillus* have been found to express glutamic acid decarboxylases (GADs) that convert glutamate into GABA (Lie and Cao 2010). In addition, GABA, produced by the gut microbiota is sufficient to influence neuronal activity in the ENS (Krantis 2000). Under physiological conditions, protein-sized molecules cannot pass through the intestinal epithelium. However, lipophilic and small hydrophilic compounds as large as 600 Da can

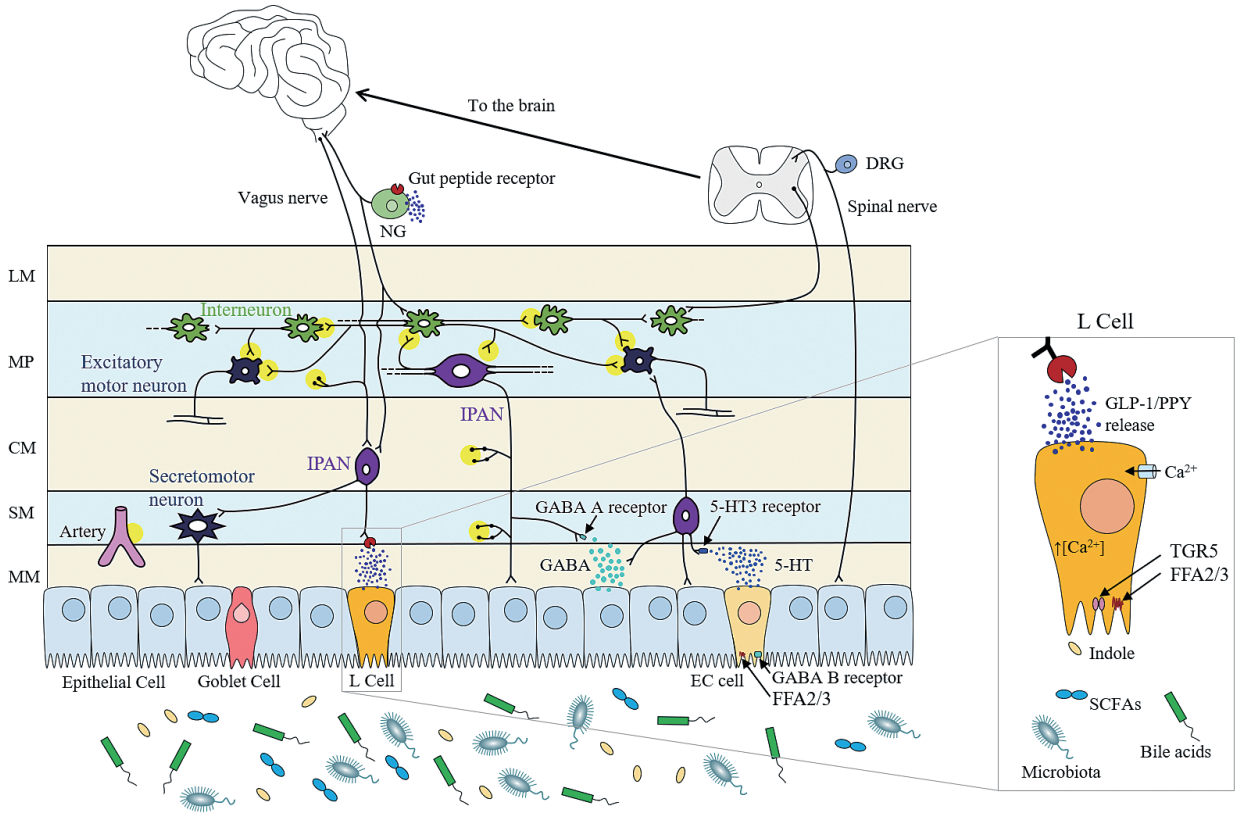

Fig. 2 Schematic drawing illustrating the putative communication pathways of the microbiota-gut-brain axis. There are numerous mechanisms through which the gut microbiota can signal to the CNS. Communication pathways of the microbiota-gut-brain axis include nervous system, enteroendocrine cells, microbiota and production of microbiota-derived metabolites. Through these elements, the microbiota-gut-brain axis controls central physiological processes. Dysregulation of the communication system subsequently leads to alterations in physiological processes in the brain and potentially contributes to stress-related disorders. The communication pathways include the release of gut hormones by enteroendocrine cells (EECs) where they activate chemosensory receptors and then, released hormones activate IPANs and extrinsic afferent nerve terminals in the gut. In addition, extrinsic afferent nerves are able to stimulate efferent nerve fibers through ANS. These actions of ANS can directly or indirectly affect local gut functions, thereby modulate gut local function including motility, secretion and thereby microbiota composition. Only a few examples of the microbiota-gut-brain axis pathways and gut peptides are represented in this figure.
Inset: Gut metabolites including SCFA, neuroactive compounds and small proteins exert their effects through direct or indirect interaction with chemoreceptors expressed on enteroendocrine cells or the IPANs and extrinsic afferent nerves. Stimulation of enteroendocrine cells by microbiota-derived metabolites resulted in the release of GLP-1/PYY and 5-HT into the basolateral side and then released hormones activate IPANs and/or extrinsic primary afferent neurons in spinal and vagus nerves to send luminal information to the CNS.
LM, longitudinal muscle; MP, myenteric plexus; CM, circular muscle; ICC, interstitial cells of Cajal; SM, submucosal plexus; MM, muscularis mucosa; IPAN, intrinsic primary afferent neuron; NG, nodose ganglion; DRG, dorsal root ganglion; EECs, enteroendocrine cells; EC, enterochromaffin cell; GABA, γ-aminobutyric acid; 5-HT, 5-hydroxytryptamine; SCFAs, short-chain fatty acids; FFA2/3, free fatty acid receptor 2/3; TGR5, Takeda G protein-coupled bile acid receptor 1; GLP-1, glucagon-like peptide 1; PYY, peptide YY.

cross the intestinal barrier through transcellular and paracellular routes (Keita and Söerholm 2010). Therefore, it is possible that small neuroactive compounds diffuse into the lamina propria, which is in contact with intrinsic and/or extrinsic afferent neurons to send the information from the gut lumen (Fig. 2). These neuroactive compounds essentially act locally on the ENS, as is discussed below (Lyte 2011; Sarkar *et al.* 2016). Finally, an important component of luminal signaling to the epithelium is accomplished by proteases. Both bacteria and host cells release proteases that can activate protease activated receptors (PARs) which are found not only on epithelial cells but also on enteric neurons and extrinsic nerves integrated into the gut (Steck *et al.* 2012). They are activated by the proteolytic cleavage of the N-terminus, which

Table 1 *Summary of enteroendocrine cells of the mammalian gastrointestinal tract*

Cell Types	Secretory products	Locations	Function
A (X-like) cells	Ghrelin	Stomach	Appetite control, growth hormone release
ECL cell	Histamine	Stomach	Regulation of gastric acid secretion
G cells	Gastrin	Stomach	Regulation of gastric acid secretion
D cells	Somatostatin	Entire gastrointestinal tract, Pancreas	Inhibition of gastrin release (stomach), modulation of insulin secretion (pancreas)
Enterochromaffin (EC) cells	5-HT, 5-HT is also contained in subgroups of I, K and L cells	Entire gastrointestinal tract	Facilitation of intestinal motility reflexes and secretion
I cells	CCK (5-HT)	Duodenum and jejunum	Gallbladder contraction, stimulation of pancreatic enzyme secretion, inhibition of food intake
K ells	GIP	Duodenum and jejunum	Regulation of insulin secretion
L cells	GLP-1, GLP-2, PYY, Oxyntomodulin (5-HT)	Ileum and colon	Stimulation of carbohydrate uptake, slowing intestinal transit, appetite regulation, stimulation of intestinal motility and secretion, inhibition of gastric acid secretion, enhancement of insulin secretion
M cells	Motilin	Duodenum and jejunum	Initiation of migrating motor complex in pig, dog, and human
N cells	Neurotensin	Small and large intestine	Inhibition of intestinal contractions
P cells	Leptin	Stomach	Appetite regulation, reduction of food intake
S cells	Secretin	Duodenum and jejunum	Reduction of acidity in duodenum and jejunum

ECL, Enterochromaffin-like cell; 5-HT, 5-hydroxytryptamine (serotonin); CCK, cholecystokinin; GIP, glucose-dependent insulinotropic polypeptide; GLP-1 and GLP-1, glucagon-like peptide 1 and 2; PYY, peptide YY.

allows the ligand domain to bind to the receptor. PAR1, PAR2 and PAR4 activate enteric neurons, enteric glia and extrinsic primary afferents (Vergnolle 2009). The next section will concentrate on the role of the EECs in the microbiota-gut-brain axis.

Enteroendocrine cells (EECs)

Understanding the function of EECs is essential because they establish neurological epithelial synapses with enteric afferent neurons and form a critical part of the reflex microcircuit mediating various GI functions (Fig. 2). The intestinal epithelium is one of the body's most important compartmentalized interfaces between the lumen and the host, and this single layer of epithelium forms a crucial barrier. EECs are found throughout the epithelium of the GI tract and are embedded in the majority of nonendocrine cells, including absorptive enterocytes, goblet cells, and Paneth cells (Fig. 2) (Gunawardene *et al.* 2011; Mace *et al.* 2015). EECs comprise only 1% of the epithelium but collectively form the largest endocrine system in mammals (Buffa *et al.* 1978; Sternini *et al.* 2008; Furness *et al.* 2013). The EEC population varies along the length of the GI tract with differing hormone production profiles (Roberts *et al.* 2019). L-cells are highly abundant and distinguishable by their production of GLP-1 and PYY which are known to suppress appetite and stimulate insulin secretion (Mace *et al.* 2015). EC cells are another subtype of EECs that secrete 5-HT and regulate secretory and peristaltic reflexes (Bertrand and Bertrand 2010; Gershon 2013; Mawe and Hofman 2013). EC cells produce approximately 95% of the 5-HT in the body and are the most numerous of the intestinal EECs (Diwakarla *et al.* 2017). EECs were originally believed to originate in the neural crest (Pearse and Polak 1971), but EECs have now been shown to develop from the same pluripotent stem cells as the other three intestinal epithelium cell lineages: absorptive enterocytes, goblet cells and Paneth cells (Barker *et al.* 2007). A summary of some of the different EEC subtypes, secreted hormones, receptors and their functions are provided in Table 1.

EECs establish transepithelial signal transduction routes that respond to luminal nutrients and microbiota metabolites by secreting gut peptides or binding specific receptors (Ralbould 2010; Begg and Woods 2013). The secretion of gut peptide/amine is considered to be a first step in sending a variety of messages from the gut lumen to the ENS and CNS since the most afferent nerve terminals innervating the gut mucosa cannot directly detect luminal chemicals but can detect them through EECs or immune cells (Mayer 2011; Gribble and Reimann 2016; Worthington *et al.* 2018). Indeed, certain luminal molecules activate intrinsic primary afferent neurons

(IPANs) and extrinsic afferent neurons through EECs, which release gut peptide/amine to stimulate axon terminals to trigger action potentials. These action potentials are then conducted orthodromically from the sensory neurons to the ENS and CNS. Therefore, EECs seem to function as chemical sensors and reside at an ideal position to send the luminal information to the host upon the release of gut hormones. This proposed action is supported by the observation suggesting that the structure and biochemical profiles of the EECs are similar to the taste cells of the lingual epithelia, expressing an array of chemical sensing proteins (Gershon 2013).

Specific gut microbiota profiles have been reported to play important roles in regulating the levels of EC cell-derived 5-HT because an analysis of the plasma metabolites in the germ-free mice shows a more than 2-fold decrease in 5-HT levels relative to the levels in conventionally colonized mice (Wikoff *et al.* 2009; O'Mahony *et al.* 2015). Indeed, human and mouse-derived gut microbiota promote colonic tryptophan hydroxylase 1 (TPH1) expression and 5-HT secretion from EC cells through the activation of SCFA receptors on EC cells (Reigstad *et al.* 2015; Yano *et al.* 2015). Furthermore, fluorescence-activated cell sorting (FACS)-purified EC cells from both the small intestine and colon express gut microbiota-derived metabolite receptors including SCFA receptors (Mawe and Hofman 2013). Therefore, 5-HT secretion from EC cells induced by SCFAs has local influence on neighboring cells as a major paracrine signal transmitted through 5-HT receptors including those on enteric nerves in the lamina propria, to modulate GI motility and send luminal information to the CNS (Mawe and Hofman 2013) (Fig. 2).

SCFAs stimulate GLP-1 and PYY release from L-cells through the activation of FFA2 and/or FFA3 (Tolhurst *et al.* 2012; Kaji *et al.* 2014; Tough *et al.* 2018). Released GLP-1 from L-cells is rapidly cleaved and inactivated by dipeptidyl peptidase 4 (DPP4) once it enters the circulation (Hansen *et al.* 1999). The rapid inactivation of GLP-1 by DPP4 raised the possibility that GLP-1 receptors (GLP-1Rs) located close to L-cells may act as local sensors of endogenous GLP-1 before GLP-1 is inactivated. Indeed, GLP-1R-positive neuronal cell bodies are reported to be located on the colonic submucosal plexus in mice and GLP-1R-positive nerve fibers are located close to L-cells (Amato *et al.* 2010; Richards *et al.* 2014). These results suggest that GLP-1R-expressing nerve terminals may function as sensory neurons to respond to SCFAs in the gut lumen. Moreover, GLP-1R fluorescent enteric neurons in primary culture have been shown to have increased their firing rates upon the application of GLP-1, and some of these neurons are considered to be IPANs because of their electrophysiological similarities with activated hyperpolarization (AH)/type II neurons (Richards *et al.* 2014). Thus, it is reasonable to speculate that L-cells expressing FFA2 and/or FFA3 may relay signals to other neurons in the ENS or send information to the CNS through extrinsic afferent neurons (Fig. 2, Inset). L-cells contain PYY in addition to GLP-1 (Cox 2007; Panaro *et al.* 2014) and SCFAs are reported to induce PYY secretion through the activation of FFA2 in the colon (Cherbut *et al.* 1998; Tough *et al.* 2018). Furthermore, the PYY receptor, Y1, is localized in the rat ENS (Jackerott and Larsson 1997). Recent study reported that FFA2 and FFA3 signaling differed and demonstrated that luminal propionate costimulates FFA2 and FFA3 pathways, reducing anion secretion and slowing colonic motility; FFA2 via PYY mediation and FFA3 signaling by activation of enteric sensory neurons (Tough *et al.* 2018). Thus, it is possible that PYY also conveys the luminal information by activating the FFA2 and FFA3 expressed on L-cells. Combined with these results, in one proposed signaling route for gut microbiota metabolites, SCFA-induced signaling is transduced from EECs to the ENS, which involves GLP-1 and PYY release and the activation of GLP-1R and Y1 receptor located on enteric neurons. In addition, FFA3 has recently been reported to be localized within the peripheral nervous system (Nøhr *et al.* 2013, 2015), further suggesting that SCFAs are important signaling molecules in the microbiota-gut-brain axis.

Bile acids activate farnesoid X receptor (FXR), and Takeda G protein-coupled receptor 5 (TGR5) (also known as the G protein-coupled bile acid receptor 1) (Schaap *et al.* 2014; Florucci and Distrutti 2015; Copple and Li 2016). TGR5 immunoreactivity is widely distributed throughout the GI tract of mice, with prominent expression in the ENS (Poole *et al.* 2010; Duboc *et al.* 2016). In the colon, bile acids are modified by the gut microbiota, which coverts primary bile acids into secondary more-hydrophobic bile acids via α-dehydroxylation (Li and Chiang 2014; Copple and Li 2016). The activation of apical membrane TGR5 on L-cells in the distal ileum and colon leads to portal release of the GLP-1 and PYY in both mice and humans (Fig. 2 Inset) (Harach *et al.* 2012; Wu *et al.* 2013). In addition, bile acid-activated TGR5 stimulates GLP-1 production in the STC-1, enteroendocrine cell line (Katsuma *et al.* 2005). The secondary bile acids can be pas-

sively reabsorbed through the epithelium (Dawson and Karpen 2015). Taken together, these data suggest that luminal secondary bile acids are directly detected by the TGR5 located on EECs to trigger the release of GLP-1 and PYY from L-cells. Then the released gut peptides affect the enteric neuronal circuit to send the information to the CNS (Poole *et al.* 2010; Duboc *et al.* 2016). Alternatively, absorbed secondary bile acids directly active TGR5 on enteric neurons to modulate neuronal activity and send the luminal information to the CNS. Evidence showing the localization of TGR5 on enteric neurons and the apical or basolateral membrane of colonocytes provides a neuroanatomical support for the concept of a microbiota-gut-brain axis. GLP-1 is known to promote insulin secretion and regulate glucose homeostasis. Therefore, it is possible that secondary bile acid can modulate energy homeostasis through the activation of the microbiota-gut-brain axis through the communication of EECs with the ENS. However, GLP-1 and PYY have been shown to be situated in separate storage vesicles in the same intestinal cells taken from a number of mammalian species including humans, pigs, rats and mice (Cho *et al.* 2014a, 2014b). These findings suggest the possibility that different hormones are selectively released from L-cells. However, further studies are needed to prove this hypothesis in more detail. On the other hand, FXR, a metabolic nuclear receptor, is highly expressed in hepatocytes and enterocytes (Makishima *et al.* 1999). FXR is mainly activated by both the free and conjugated primary bile acids, glycochenodeoxycholic acid (CDCA) and colic acid (CA) (Makishima *et al.* 1999). As a ligand-activated transcription factor, FXR binds to DNA (*i.e.*, FXR response element) to regulate the expression of the diverse genes involved in the metabolism of bile acids, lipids, and carbohydrates (Chan 2018; Shin and Wang 2019). Thus, FXR also contributes to the regulation of energy metabolism similar to that of TGR5 (Lynch *et al.* 2016; Kuhre *et al.* 2018). However, it is unclear whether FXR contributes to the transduction of luminal information to the ENS and CNS. Bile acids can also activate other bile acid receptors such as pregnane X receptor (PXR), constitutive androstane receptor (CAR) and vitamin D receptor (VDR) but the contribution degree of these receptors on microbiota-gut-brain axis is still unknown (Li and Chiang 2014).

In the GI tract, odorant receptors are functionally expressed by human EC cells and induce the 5-HT secretion which stimulates the submucosal sensory neurons (Braun *et al.* 2007). In addition, Olfr78 is expressed in mouse colonic L-cells (Fleischer *et al.* 2015). Moreover, Olfr78 and the human ortholog OR51E2 are activated by SCFAs, most notably propionate and acetate (Saito *et al.* 2009; Plznick *et al.* 2013). The functional implications of these receptors on microbiota-gut-brain axis are so far largely unknown but previous studies have indicated that odorant receptors expressed in nonolfactory tissues serve as receptors for small molecules and have roles similar to those in olfactory sensory neurons (Plznick *et al.* 2013). Moreover, odorant receptors expressed in the colonic intestinal epithelia are restricted to L-cells (Plznick *et al.* 2013). Thus, it is speculated that these receptors function as chemical sensors to monitor the luminal microenvironment and convey such information to the ENS and CNS, although further studies are needed to prove the hypothesis in more detail.

Gut microbiota can produce neuroactive compounds as described above. Among these compounds, some receptors and transporters critical for neuroactive compounds are found in enterocytes (Mazzoli and Pessione 2016). For example, high-affinity plasma membrane GABA transporters are present in the rat GI tract. Furthermore, in the rat mucosal epithelium, $GABA_B$ receptor-expressing EC cells are observed along the length of the GI tract from gastric corpus to the colon that is morphologically similar to EECs (Nakajima *et al.* 1996). Therefore, 5-HT-expressing EC cells can directly detect luminal GABA produced by the microbiota (Fig. 2). In the ENS, $GABA_B$ positive neuronal soma are found in both submucosal and myenteric ganglia throughout the entire GI tract (Hyland and Cryan 2010). Both $GABA_A$ and $GABA_B$ receptors have been shown to release EC cell-derived 5-HT from vasculary-perfused guinea-pig small intestine (Schworer *et al.* 1989). However, they appear to have opposite effects; baclofen ($GABA_B$ agonist)-induced inhibition of 5-HT release is TTX insensitive while $GABA_A$ receptor activation induces a predominant TTX-sensitive, muscarinic receptor-mediated release of 5-HT (Schworer *et al.* 1989). Therefore, 5-HT release by $GABA_B$ receptor activation may indirectly regulate ENS activity. These results indicate that neuroactive compounds derived from gut microbiota metabolism act as potential mediators of communication between the gut microbiota and the host.

EECs also detect signals from the microbiota through toll-like receptors (TLRs), which recognize bacterial products, such as lipopolysaccharide (LPS), and others (Bogunovic *et al.* 2007; Mayer 2011). A total of 10 TLRs are expressed in human whole

body (Frosali *et al.* 2015). In the intestine, TLR1, TLR2 and TLR4 are present on the apical surface of the EECs in human and adult mice (Bogunovic *et al.* 2007). In addition, 5-HT-containing EC cells in human and murine ileal and colonic epithelia were colocalized with TLRs, TLR2 and TLR4 (Bogunovic *et al.* 2007). LPS triggers a calcium influx in STC cells expressing TLR4, resulting in a rapid increase in cholecystokinin secretion (Bogunovic *et al.* 2007). Furthermore, the intestinal infusion of *Escherichia coli* proteins has been reported to increase the secretion of GLP-1 and PYY from L-cells (Breton *et al.* 2016). Taken together, these results indicate that TLRs expressed on EECs including EC cells can directly detect gut microbiota-derived metabolites. Therefore, it is possible that the information detected by TLRs on EECs is conveyed to IPANs and extrinsic primary afferent neurons. In other words, this route may function as an alternative pathway of communication between the gut microbiota and nervous system. Taken together, EECs are key players in the detection of luminal bacteria and their metabolites that can modulate microbiota-gut-brain axis through ENS activity.

Enteric nervous system

The ENS is a large, complex compartment of the peripheral nervous system that regulates many GI functions including motility and ion transport and abnormalities in its formation or functions cause several morbid or life-threatening human diseases. In the previous section, we have discussed how gut microbiota and/or its metabolites send their information to CNS through the activation of EECs in microbiota-gut-brain axis. The ENS is located upstream of the EECs in the microbiota-gut-brain axis. Therefore, in this section, we discuss how the gut microbiota and/or its metabolites send information to the ENS by the release of gut peptides from EECs and how the ENS sends the received information further upstream through microbiota-gut-brain axis. Several mechanisms have been proposed for the communication of microbiota with enteric neurons (Hyland and Cryan 2016).

The ENS is derived from neural crest progenitors that colonize the gut during fetal development to form two interconnected ganglionated plexuses (Fig. 2) (Rao and Gershon 2018). The ENS is sometimes called the "second brain" because of the diversity of its neuronal cell types and the complex, integrated circuits that are similar to those of the CNS (Furness *et al.* 2013). The ENS exhibits a columnar topology along the radial axis of the GI tract similar to that of the CNS (Lasrado *et al.* 2017). The ENS is composed of more than 600 million neurons and glia approximately the same number of neurons as in the spinal cord. The ENS runs along the GI tract and is organized into two main plexuses. The submucosal plexus (or Meissner's plexus) which itself is subdivided into two smaller plexuses (the inner and outer submucosal plexuses) in larger mammals lies in the submucosa, and the myenteric plexus (Auerbach's plexus) lies between the longitudinal and circular muscle layers in the intestinal wall (Fig. 2) (Furness 2012). These plexuses integrate a variety of signals from the CNS via connections with the parasympathetic and sympathetic branches of the ANS and vice versa. Nerve fiber bundles connect to the ganglia within the plexuses and between different plexuses. Myenteric and submucosal neurons are composed of discrete populations of neurons that can be classified based on their function and morphology. These include intrinsic primary afferent neurons (IPANs) that allow them to regulate GI motility and secretion without CNS input, motor neurons (muscle, secretomotor and vasodilator neurons) and interneurons. The majority of the sensory nerve fibers innervating the intestinal mucosa is derived from IPANs of the ENS (Ekblad *et al.* 1987). IPANs project directly to motor and interneurons through which they send luminal information to other enteric neurons and to the CNS. Therefore, IPANs are a second target for gut microbiota metabolites or the microbiota itself in the microbiota-gut-brain axis. In addition to paracrine or hormonal signaling mechanisms, a synapse may form between the EECs and enteric nerves projecting to the epithelium (Bohórquez *et al.* 2015). This synaptic structure may also be important to pave an alternative route to send the luminal information to the ENS and CNS. Neurons in the ENS are also divided into two subtypes based on their electrophysiological properties, which correlate with their morphologies (Furness 2012). AH (after hyperpolarizing) neurons have multiple long processes and a large oval soma (AH/Dogiel Type II neurons) and S (synaptic) neurons (Brookes *et al.* 1995; Clerc *et al.* 1998; Nurgali *et al.* 2004). Morphology of S neuron is flattened, slightly elongated with stellate or angular forms. The characteristic belongs to Dogiel Type I neurons. S neurons function as muscular motors, secretomotors and interneurons. On the other hand, AH neurons are chemo- and mechanosensitive IPANs. Both types of neurons are located in close proximity to and in contact with spinal and vagal afferent nerves that send intestinal information to the CNS, and

they also communicate in a bidirectional manner with the CNS via both vagal parasympathetic and sympathetic nerve pathways. Glia constitutes another component of ENS. The primary roles of glia in GI function remains incompletely understood but enteric glia participate in bidirectional communication with enteric neurons to regulate motility (Turco *et al.* 2014; Rao *et al.* 2017).

The ENS components are separated from the contents of the intestinal lumen including the gut microbiota, by the epithelial cell barrier and mucous layer. Direct interactions require the gut microbiota or its metabolites to pass the epithelial barrier to access the nerve endings of IPANs but under physiological conditions, the gut microbiota usually cannot penetrate into the subepithelial space (Ashida *et al.* 2012; Sharon *et al.* 2014). Previous studies demonstrated that the gut microbiota and their metabolites originating in the gut lumen influence the ENS through direct or indirect mechanisms and changes in ENS activity contribute to afferent signals transduced to the brain (Kunze *et al.* 2009; Forsythe *et al.* 2012). For example, luminal puff application of *Lactobacillus rhamnosus (JB-1)*, *Bacteroides fragilis* or the capsular exopolysaccharide polysaccharide A (PSA) to the epithelia evoked brief low-frequency bursts of orthodromic action potentials in *ex vivo* mouse small intestinal preparations (Mao *et al.* 2013). This close relationship between IPANs and the gut microbiota has been supported by the observation revealing that the absence of the microbiota results in a decrease in gut IPAN excitability in the mouse (McVey *et al.* 2013). The report also demonstrated that PSA is necessary and sufficient to evoke action potential in IPANs. From these results, the authors speculated that specific luminal bacteria and their carbohydrate components interact with cells in the epithelium through C-type lectin or TLRs as these are present on epithelial cells and EC cells (Sharma *et al.* 2010). Furthermore, considering the latency of neuronal responses, these authors speculate that PSA may first act on EECs, which then activate IPANs through the release of intermediate mediators including gut peptides (Mao *et al.* 2013). Therefore, the luminal gut microbiota may be able to send the luminal information through epithelial cells to the ENS. The mechanisms whereby gut microbiota signals to enteric neurons (AH and S cells) to alter their excitability include many plausible possible routes including EECs as mentioned above. Thus, further studies are needed to identify the molecular mechanism since it is still unknown what kind of molecules are involved in the activation of IPANs but this pathway composes important intrinsic afferent pathway in the microbiota-gut-brain axis.

SCFAs are important gut microbiota-derived mediators in the microbiota-gut-brain axis as mentioned in the previous section. The mucosal application of 5×10^{-3} M butyrate also induced a non-synaptic burst of action potential in rat colonic myenteric AH neurons (Kunze *et al.* 2009). In addition to the activation of FFAs expressed on EECs, SCFAs are also transported by the SCFA transporter, SMCT-1 into the subepithelial space, where they affect various aspects of ENS activity (Iwanaga *et al.* 2006). Indeed, direct application of 10^{-1} M sodium butyrate into the myenteric AH neurons of the guinea pig proximal colon induced transient depolarization associated with increased excitability (Neunlist *et al.* 1999). In contrast to a previous study in which an action potential was observed in rat colonic myenteric AH neurons stimulated by butyrate, the mucosal application of butyrate on AH neuron somata was ineffective when applied directly onto the mucosa (Mayer 2000). The reasons for these discrepancies remain speculative but might result from species differences or segmental differences. Butyrate but not acetate or propionate is reported to enhance the expression of choline acetyltransferase in rat colonic myenteric neurons through the activation of FFA3 (Soret *et al.* 2010). This result suggests that butyrate may modulate microbiota-gut-brain axis signaling pathway through the activation of neurotransmitter synthesis mechanisms. However, to prove this molecular mechanism in this pathway, further study is needed.

The ENS expresses multiple 5-HT receptors; three excitatory receptors and one inhibitory receptor for 5-HT have been identified using electrophysiological methods (Galligan 1996). 5-HT activates IPANs and extrinsic sensory terminals in the lamina propria mainly via 5-HT_3 receptors (Bertrand *et al.* 2000). Furthermore, 5-HT_3 receptor-expressing nerve fibers are in contact with the basolateral side of 5-HT-expressing EC cells (Bellono *et al.* 2017). Thus, EC cells in close proximity with 5-HT_3 receptor-expressing nerve fibers appear to form synaptic-like structures for transmitting signals from EC cells to the ENS (Fig. 2). Based on these observations, the gut microbiota can directly or indirectly influence 5-HT production in EC cells and released 5-HT modulates the neuronal activity of the ENS. The cholera toxin also releases 5-HT from EC cells, and the released 5-HT acts on 5-HT_3 receptors on sensory nerves (Fung *et al.* 2010). Therefore, it is possible that microbiota-modulated 5-HT release in the mucosa may participate in sending luminal informa-

tion to the CNS through the modulation of ENS neuronal activity. Furthermore, dysregulation in the serotonergic system has been related to chronic inflammatory diseases such as intestinal bowel disease (IBD) and diarrhea (Monocha *et al.* 2012). Together, these findings suggest that 5-HT functions as a chemical language with which it communicates with the gut microbiota and the ENS. This communication route is important to maintain microbiota-EEC-ENS interactions. In the ENS, GABA acting on $GABA_A$ receptors evoked the depolarization of AH neurons in the intact myenteric plexus in the guinea pig ileum (Bertrand and Galligan 1992; Auteri *et al.* 2015). Therefore, the endogenous source of GABA produced by the microbiota also functions as a chemical language of the gut microbiota and the ENS. This supposition is supported by the observation indicating that the concentrations of most neuroactive compounds found in the GI tract are equal to or higher than those in the brain (Sampson and Mazmanian 2015).

Despite the separation between the microbiota and the ENS, enteric neurons in TLR2-knockout mice showed altered ENS architecture and neurochemical profiles; a reduction in the number of ileal neurons and glial cells and reduced myenteric ganglion area (Brun *et al.* 2013). They also presented with structural abnormalities in the submucosal plexus, which manifested functionally as a decrease in nerve-driven secretory responses to cholinergic stimulation and induced intestinal dysmotility (Brun *et al.* 2013). Similar alterations were observed in the TLR4-knockout, which showed a reduction in *in vivo* transit coupled with important changes in neurochemistry (Anitha *et al.* 2012). Therefore, the collective evidence suggests that both TLR2 and TLR4 influence both the ENS and the function of the small intestine, with similar neurochemical changes observed in the myenteric neurons in the proximal colon of TLR4-deficient mice. The TLR 3 and 7 proteins have been identified in the murine and human ENS, respectively, and on neural elements innervating Peyer's patches, which may provide a pathway for microbes to access the ENS (Barajon *et al.* 2009). Thus, TLR3 and 7 may also function as messengers of the communication system formed by the microbiota-gut-brain axis. Enteric glia also expresses TLRs (Turco *et al.* 2014). Using immunohistochemistry studies, Kabouridis *et al.* recently demonstrated that the microbiota is required for both the initial establishment (*i.e.*, migration) and for the postnatal development of enteric glial cells in the intestine (Kabouridis *et al.* 2015). Therefore, the microbiota may affect the microbiota-gut-brain axis through TLRs expressed on glial cells. The gut microbiota also activates resident immune cells of the GI tract, which may signal to the ENS. Briefly, molecular mediators secreted by gut-resident immune cells can be detected by corresponding receptors in the ENS and affect enteric function. For example, muscularis macrophages of the gut secrete bone morphogenetic protein 2 (BMP2), which activates BMP receptors on enteric neurons to regulate motility (Muller *et al.* 2014).

Overall, the ENS combined with EECs is considered to function as an intermediate transducer between the gut microbiota and the CNS that signals through the microbiota-gut-brain axis. Furthermore, information may be transferred to the CNS through changes in the excitability status of enteric neurons and then, the CNS modulates peripheral gut functions including motility and/or ion secretion, in addition to brain function through sympathetic and parasympathetic pathways. Bacterial signaling to the CNS through visceral afferent neurons of the GI tract is discussed in the following section.

Microbiota-gut-brain axis: Communication between the CNS and ENS

Neural communication between the GI tract and CNS occurs through the innervation of the ANS. Incoming visceral information from the GI tract is processed by the CNS, which then evokes responses essential for survival. From an anatomical perspective, in general, there are two distinct neuroanatomical routes of neural communication from the intestine to the CNS; nonpainful homeostatic functions, including satiety, distention, and motility, are mediated predominantly through vagal/pelvic nerves and painful sensory stimuli evoke the transmission of information through splanchnic nerves (Fig. 2) (Vermeulen *et al.* 2014). Furthermore, these signals can also be transmitted through spinal splanchnic nerves via the *nucleus tractus solitarii* (NTS) of the brainstem to higher centers of the brain (Christianson *et al.* 2009). Three afferent pathways connect the GI tract to the CNS: vagal afferents, pelvic afferents and splanchnic afferents (Beyak *et al.* 2006). These visceral afferent nerve fibers transmit sensory information from the GI tract to the CNS, including to the brainstem and sensorimotor brain circuits (Berthoud *et al.* 2004; Brookes *et al.* 2013; Furness *et al.* 2014; Keightley *et al.* 2015). Several structurally distinct types of sensory endings are reported to be present in the gut wall. Each of these major types of ending structures seems to be associated with distinctive

combinations of physiological responses (Brookes *et al.* 2013). Among these types, "mucosal" endings are located in the subepithelial layer, thus these endings can detect intestinal molecules such as bacterial metabolites, gut peptides and neurotransmitters (Beyak *et al.* 2006; Brookes *et al.* 2013). Cell bodies of vagal and spinal afferents are located in nodose ganglion (NG) which project to the brainstem, and dorsal root ganglia (DRG), which project to the dorsal horn of the spinal cord and the dorsal column nuclei. Histological and electrophysiological evidence indicates that visceral afferent endings in the GI tract express a diverse array of chemical and mechanosensitive receptors which are the main targets of the gut peptides released from EECs (Beyak *et al.* 2006; Blackshaw *et al.* 2007; Egerod *et al.* 2018). For example, the GPCRs for gut peptides expressed on vagal unmyelinated afferents are neurotensin receptor 1 (NTSR1), neuropeptide YY receptor 2 (NPY2R), CCK-1R and GLP-1R (Egerod *et al.* 2018). Furthermore, both vagal and spinal afferents have collateral branches that innervate the ENS (Fig. 2) (Blackshaw *et al.* 2007). These findings suggest that changes in the neuronal activity of the ENS are easily transmitted to the CNS through these collateral branches. Thus, visceral afferent fibers may indirectly sense microbiota signals through the interplay between the EEC and ENS, and the corresponding gut information is thus transferred to the CNS to exert various reactions; CNS function and, subsequently human and animal behavior, is influenced by microbiota and their metabolites (Mayer *et al.* 2015; Sampson and Mazmanian 2015).

As described above, microbiota metabolites are able to signal both locally and to distant organs, including the brain (Sharon *et al.* 2014; Schroder *et al.* 2016). For example, butyrate directly activates rat jejunal vagal afferent nerve fibers (Lal *et al.* 2001). As FFA3 was found to be expressed in the mouse NG but no expression was observed in the brain or spinal cord (Nøhr *et al.* 2015), it is possible that this effect was mediated by FFA3 expressed on vagal sensory neurons. Furthermore, the oral administration of butyrate to fasting mice resulted in decreased neuronal activity in the NTS and dorsal vagal complex and decreased activity of orexigenic NPY-positive neurons in the hypothalamus (Li *et al.* 2018). Therefore, these results indicate that SCFAs modulate satiety activity at the CNS level through the activation of vagal nerve signaling. This supposition is further supported by observations indicating that the butyrate concentration of peripheral blood is relatively low (~18 μmol/L in the portal vein in a fasting human and in the lower μmol range in peripheral blood) and radiolabelled butyrate uptake in primate brain was less than 0.006% and subjected to higher turnover, as indicated by positron emission tomography study (Kim *et al.* 2013). Thus, it can be expected that butyrate levels in the brain tissue or cerebrospinal fluid are extremely low. In another example, acetate was implicated in the regulation of weight gain and appetite based on the parasympathetic nervous system; an increase in acetate production by the gut microbiota in response to a high fat diet promoted glucose-stimulated insulin and ghrelin secretion by activating the parasympathetic nervous system (Perry *et al.* 2016). Furthermore, acetate derived from the colon has been reported to directly induce anorectic signals in the hypothalamic *arcuate nucleus* (ARC) (Frost *et al.* 2014). Therefore, gut-derived SCFAs may actively regulate satiety homeostasis through neural circuits involved in the brain both by visceral sensory afferent activation and EEC-ENS interplay. The visceral afferent nerve in the microbiota-gut-brain axis also contributes to the energy homeostasis affected by propionate. Propionate enhances glucose homeostasis by modulating the microbiota-gut-brain neural circuitry; that is, propionate acts as an agonist of FFA3 in the vagal afferent innervating the portal vein to induce intestinal gluconeogenesis via the activation of the dorsal vagal complex which receives input from the vagal afferent pathway (De Vadder *et al.* 2014). Taken together, these results indicate that the mechanisms of energy homeostasis controlled by SCFAs somewhat differ by the types of SCFAs. More research is needed to clarify which specific neuronal circuits are activated by a specific type of SCFAs to induce signaling in the brain and how this specificity affects satiety-related behavior.

Another microbiota-derived metabolite, indole influences higher brain function through the microbiota-gut-brain axis; direct administration of indole into the cecum of rats induced the activation of the vagal afferent fibers, which was confirmed by *c-fos* expression in the *dorsal vagal complex* (DVC), and this activation induced a dramatic increase in the number of eye blinks (Jaglin *et al.* 2018). The results also suggest that the vagus nerve is a major modulatory communication pathway between the gut microbiota and the brain. However, the underlying molecular mechanisms are still unknown.

The microbiota itself also affects the microbiota-gut-brain axis through the modulation of visceral afferent nerves. For example, the luminal application of *Lactobacillus rhamnosus (JB-1)* into the jejunal

segment of male Swiss Webster mice increased vagal afferent discharge within minutes but the application of *Lactobacillus salivarius* (a negative control) was ineffective (Perez-Burgos *et al.* 2013). The increase in vagal discharge was abolished by vagotomy. Chronic treatment with *JB-1* also led to region-dependent alterations in central GABA receptor expression, accompanied by reduced anxiety- and depression-like behaviors and attenuation of the stress-induced corticosterone response, and these effects also required an intact vagus nerve (Bravo *et al.* 2011). Thus, the results suggest that the gut microbiota is an important factor influencing stress-induced behavior and that the intact vagus nerve is particularly linked to microbiota-induced behavioral changes. Another gut microbiota, *Bifidobacterium longum* NCC3001, was also reported to normalize anxiety-like behavior and hippocampal brain-derived neurotrophic factor (BDNF) in mice with infectious colitis, and the anxiolytic effect of NCC3001 required vagal integrity but did not involve gut immunomodulation or production of BDNF by neurons (Bercik *et al.* 2011; Bravo *et al.* 2011). Furthermore, the results suggest that signals transmitted to the CNS by activating vagal pathways are transduced at the ENS level since the products of bacterial fermentation decreased the excitability of enteric neurons. From these results, the vagus nerve and the ENS are considered to be important to the neural pathway modulation of the constitutive communication between the gut microbiota and the brain. However, to identify the nerves affected by the microbiota, further study is needed. Furthermore, to determine whether the effects are due to the direct stimulation of vagal afferent nerve terminals or are secondary to the effects on the ENS, more study is required. In addition, these findings suggest that certain microbiota may be involved in stress-related disorders such as anxiety and depression.

In the previous section, we have discussed the contribution of TLRs in the microbiota-gut-brain axis. Recently, electrophysiological recordings from the vagal afferent pathway stimulated by TLRs in the intestine have been described; the application of peptidoglycan (a ligand for TLR2), a major component of the wall of gram-positive bacteria, to rat distal colonic mucosa resulted in increased nerve firing in the vagus (Buckley *et al.* 2018). However, LPS (a ligand for TLR4) had no effect on vagal nerve activity, although TLR4, a selective receptor for LPS, was expressed in the vagal afferent neurons in the NG (Hosoi *et al.* 2005; Reardon *et al.* 2018). Therefore, TLR2 may contribute to the afferent input pathway in the microbiota-gut-brain axis at the peripheral level since enteric neurons express TLR2 (Brun *et al.* 2013). On the other hand, LPS may affect afferent neuronal activity of NG through circulation.

Spinal afferents are generally associated with pain (Vermeulen *et al.* 2014), but they are also equipped to convey information on physiological events (Harrington *et al.* 2018). These neurons form the afferent limb of spinal and brainstem reflexes (Brookes *et al.* 2013). Spinal afferent signaling is also evoked by bacterial cell products (Kamiya *et al.* 2006; Ma *et al.* 2009; Ochoa-Cortes *et al.* 2010). Recent studies have shown that extrinsic sensory neurons respond directly to several bacterial products such as cell wall components and toxins. For example, *Citrobacter rodentium* infection increased the hyperexcitability of mouse colonic DRG neurons and resulted in enhanced pain associated with colorectal distension (Ibeakanma *et al.* 2009). This hyperexcitability of colonic DRG neurons is caused by bacterial LPS; LPS enhanced the excitability and firing rate of the DRG neurons and increased the production of pro-inflammatory TNF-α and IL-β transcripts and cytokines (Ochoa-Cortes *et al.* 2010). TLR3, 4, 7 and 9 are expressed on spinal afferent neurons and in DRG (Reardon *et al.* 2018). Taken together, these results suggest that the microbiota itself also sends information to the CNS through the activation of TLRs expressed in the ENS and spinal afferent neurons.

Under normal circumstances, the majority of visceral afferent signals are not consciously perceived, with only salient (*e.g.*, hunger) or potentially noxious stimuli (*i.e.,* pain) eliciting a behavioral response reaching consciousness (Mayer 2011). However, dysfunction at any level of the microbiota-gut-brain axis may disrupt this delicate balance. Irritable bowel syndrome (IBD) is related to stress-related psychiatric disorders, including major depression and anxiety and is recognized as a disorder of the gut-brain-axis (Collins 2014; Quigley 2018). Gut microbiota treatment has been shown to ameliorate stress-related GI diseases. Indeed, *Bifidobacterium* and *Lactobacillus* have been reported to improve mood and reduce anxiety symptoms in patients with IBD and chronic fatigue syndrome (Bravo *et al.* 2011; Collins 2014; Quigley 2018). Furthermore, the consumption of *Lactobacillus acidophilus Rosell-52* and *Bifidobacterium longum Rosell-175* has been shown to be effective in ameliorating stress-related GI complaints such as abdominal pain and nausea/vomiting (Diop *et al.* 2008). These results, taken together, show that certain microbiota strains can modulate brain function and behavior, some of which are vagus dependent.

However, it is still unknown how the microbiota affects higher behavioral response through the microbiota-gut-brain axis and what kinds of mechanisms are involved. Therefore, further studies are needed to a better understand the mechanism by which visceral information transmitted through the microbiota-gut-brain axis elicits behavioral responses.

The efferent part of the ANS and the central circuits also affect the microbiota-gut-brain axis signaling pathway (Cryan *et al.* 2012). Most efferent pathways contain pre-enteric neurons that end within the ENS and control or modify the activities of enteric neurons (Holst *et al.* 1997; Gunawrdene *et al.* 2011). The sympathetic and parasympathetic efferent branches of the ANS directly connect emotional arousal and central autonomic brain circuits within the ENS, which in turn innervates visceral smooth muscles and mucosal epithelial cells to induce endocrine and immune functions. Preganglionic neurons of the vagal efferent originate from the motor neurons of the dorsal motor nucleus and synapse with postganglionic neurons within the myenteric plexus. Physiological and psychological stressors increase sympathetic tone and decrease parasympathetic tone in the ANS, which is a key regulator of the ENS (Chang 2011). At the gut level, these stress effector systems influence various gut functions, including motility and secretion, mucosal permeability, local and circulating immune cell functions, inflammatory reactions and the microbiota composition and its function (Bailey *et al.* 2011; Bischoff *et al.* 2014). For example, four motility patterns in the small intestine have been classified: the interdigestive motor complex (MMC), segmentation, power propulsion and neutrally programmed musculomotor quiescence (Grundy *et al.* 2006). The program for each motility pattern is controlled by the ENS, but the CNS modulates the ENS activity to change motility patterns. These motility patterns easily change the composition of the gut microbiota. For example, impaired intestinal transit caused by acute pancreatitis is associated with bacterial overgrowth in the small intestine (Van Felius *et al.* 2003). Thus, the brain can influence the gut microbiota through changes in GI motility, secretion and intestinal permeability (Mayer 2000).

Under healthy conditions, the GI tract is densely inhabited by commensal bacteria, which are active producers of metabolites. Therefore, the gut chemosensory system, including the EEC and ENS continuously monitors chemical signatures in the gut lumen by the microbiota-gut-brain axis to maintain body homeostasis. Alterations in this bidirectional communication cause impaired gut function and brain function, such as stress responses, anxiety and altered memory functions, as described above. Thus, more work is required to understand how microbiota-gut-brain axis contributes to maintain our body homeostasis.

Conclusion

In this review, we have outlined recent developments in microbiota-gut-brain axis research especially the involvement of EECs and the ENS. The ENS expresses the molecular machinery required to respond directly or indirectly to the microbiota and its metabolites, and this feature also characterizes EECs and extrinsic sensory nerves. The information transfer by EECs and the ENS seems to be an important route of communication along the microbiota-gut-brain axis. An understanding of the interaction between EECs and ENS may provide new insights into gut-brain communication to help explain how the gut microbiota may modulate pathophysiological processes relevant to brain disorders, such as anxiety and depression in addition to physiological processes. Furthermore, understanding the microbiota-gut-brain axis can lead to the development of new therapeutics for disorders. Thus, future studies are needed to fully understand this complex network.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

REVIEW CRITERIA

Literature searches have mainly concentrated on publication in the past 10 years. Combinations of the following search terms were used: “gut microbiota”, “gut-brain axis”, “brain-gut axis” “enteric neurons”, “autonomic nervous system” “enteroendocrine cells” “chemosensing”, “nutrient sensing”, “microbiota metabolites”, “gut hormone”. Searches were conducted using Pubmed and Google Scholar.

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