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The Microbiota-Gut-Brain Axis: From Motility to Mood

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Abstract

The gut-brain axis plays an important role in maintaining homeostasis. Many intrinsic and extrinsic factors influence signaling along this axis, modulating the function of both the enteric and central nervous systems. More recently the role of the microbiome as an important factor in modulating gut-brain signaling has emerged and the concept of a microbiota-gut-brain axis has been established. In this review, we highlight the role of this axis in modulating enteric and central nervous system function and how this may impact disorders such as Irritable Bowel Syndrome and disorders of mood and affect. We examine the overlapping biological constructs that underpin these disorders with a special emphasis on the neurotransmitter serotonin, which plays a key role in both the gastrointestinal tract and in the brain. Overall, it is clear that although animal studies have shown much promise, more progress is necessary before these findings can be translated for diagnostic and therapeutic benefit in patient populations.

Lay Summary:

25–30 words and very briefly summarize the article's fundamental components: In this comprehensive review, the authors have united the known and most recent evidence of how the

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microbiota-gut-brain axis modulates enteric and central nervous system development and function to impact disorders such as Irritable Bowel Syndrome and those affecting mood and affect

Introduction: Microbiota-gut-brain axis

The understanding that the brain and gut participate in continuous, bidirectional communication was recognized as remotely as in Ancient Greece where philosophers such as Hippocrates, Plato and Aristotle postulated that the brain and the rest of the body are intrinsically connected. This notion led to the understanding that in order to study disease processes, the whole person must be considered rather than an isolated organ system[1]. It wasn't until the 1840s, however, that William Beaumont experimentally showed that emotional status affected the rate of digestion and, thus, that the brain affects the gut and that there is a brain-gut axis. Although this concept was subsequently recognized by the greats of modern biology including Darwin, Pavlov, James, Bernard and Cannon[2], it took until the early to mid 20th century for the first scientifically recorded observations to be made that correlated gut physiology changes with changes in emotion. These studies were limited, however, by simple techniques and the lack of study of the reciprocal effects of changes in gut physiology on mental function[1]. Emerging data has confirmed connections between brain and gut health and has further suggested several mechanistic underpinnings. Alterations in gastrointestinal (GI) function and GI symptoms have been reported to accompany an increasing number of central nervous system (CNS) disorders and, as in the case of Parkinson's disease, GI dysfunction might occur even before central neurological symptoms become evident[3]. Similarly, GI symptoms are an important component of disorders of brain-gut interactions such as IBS, which are commonly associated with psychological symptoms and psychiatric diagnoses. Moreover, with the advent of brain imaging, the reciprocal interactions can be visualized for the first time, demonstrating that gut stimuli can activate key brain regions involved in emotion regulation[2].

Most aspects of GI physiology are under neural control, which is exerted via a vast network of intrinsic enteric neurons and glia that span throughout the enteric nervous system (ENS), GI smooth muscle and the lamina propria of the mucosa, as well as extrinsic innervation from primary afferent and autonomic fibers that connect the intestine to the spinal cord and the brain[4, 5]. Although the ENS can regulate GI peristalsis largely independent of CNS input, GI motility is also modulated by factors extrinsic to the ENS, including the brain[6] and other divisions of the autonomic nervous system (ANS), the gut associated immune system and the gut microbiome. The influence on the gut is not unidirectional, as the gut also sends information to these various systems through complex pathways that function as bidirectional conduits for homeostasis, and alterations in this communication are associated with disease. Adequate gut function is thus critical for not only long-term survival but also for brain-gut homeostasis. Precisely how gut-brain communication occurs in health and disease in humans, however, remains an active area of investigation.

More recently, the microbiome (the trillions of microrganisms that reside in the gut) has emerged as an integral player in gut-brain communication and a microbiome-gut-brain axis has been proposed[7–10]. Although mechanistic studies on how this expansive

community of microorganisms influences human ENS and CNS development[11], GI motility[12], mood[10], cognition and learning[13] are still in their infancy, it offers itself as a potentially important site for future therapeutic interventions (Figure 1). Gut microbes communicate to the CNS through neuronal, endocrine, and immune signaling channels. Conversely, the CNS can affect the gut microbiota directly via stress mediator-induced virulence gene expression and indirectly through ANS-mediated control of gut function (e.g., motility, immune modulation and secretion)[14] (Figure 1). In addition, the ENS can directly modulate microbial composition through changes in secretion, motility, permeability and immunological defense. These parallel and interacting pathways are thus emerging to investigators as a complex communication matrix which also has been referred to as the gut connectome[15].

In addition to the contributions of the microbiome, studies in animal models have provided evidence that some GI dysfunction in neurological conditions may also be due to genetic defects and/or environmental influences that can simultaneously impact gut and brain development and/or function. Supportive of this notion are the demonstrations that the ENS, often described as a “second brain,” shares many likenesses with the CNS. Their shared structure, developmental patterns and neurochemistry have formed the basis for research in understanding how pathogenic mechanisms that give rise to CNS disorders might also lead to ENS dysfunction and vice versa. For example, one of the key transmitters in the CNS and the intestine, serotonin (5-HT), can act in neuroendocrine, endocrine and/or paracrine fashions to impact the development and long-term functions of both the ENS and CNS.

Given the critical involvement of the ENS, CNS and the microbiome in brain and gut development and function, a greater understanding of the relationships between these systems is likely to enable the development of novel therapeutic targets for some of the most common yet poorly understood medical conditions. The current state of research, while impressive, leaves many vital unanswered questions that need to be addressed in order to facilitate novel, effective therapeutic development. In this review, we address current evidence supporting the ways in which the brain, the gut and the gut microbiota interact and the emerging data supporting its contribution to human disease.

The Microbiota-Gut-Brain (MGB) Axis

Driven by the development of next-generation sequencing technologies in tandem with large cohort studies, the past decade has seen a dramatic increase in our understanding of the microbiome in many aspects of health and disease[16]. In humans, the greatest abundance of microbes is found in the distal gut that hosts approximately $3{\cdot}10^{13}$ microbes from more than 60 genera[17]. Although bacteria are the most abundant and best-studied gut microorganisms, the multitude of archaea, yeasts, single-celled eukaryotes, helminth parasites and viruses are also more recently being considered, though the role that these other microorganisms play in MGB interactions is currently unknown. There are large inter-individual differences in the microbial composition and we are only beginning to understand the factors that influence this in health and disease [16]. Moreover, there is a growing appreciation from cross sectional human studies that changes in the diversity and relative abundances of the microbiota and microbial metabolites are associated with a wide array of neurological and

psychiatric disorders, including Parkinson's disease, Alzheimer's disease, Autism spectrum disorders and depression[18]. Results from these studies, however, have been inconsistent and without evidence establishing causality for the gut microbiome. Further, examination of the MGB axis in clinical populations has been mostly restricted to cross-sectional studies demonstrating associations of gut microbes with brain architecture in healthy subjects or disease states [19]. There is thus a large gap in our understanding of the underlying mechanisms involved in MGB dialogue. Based largely on results obtained in preclinical animal models, the more well-studied routes of communication thus far include the immune system[20], metabolites and neurotransmitters and vagal nerve activation [21, 22].

Microbiome-Gut-Brain Axis in early Life

Whether microbial colonization occurs *in utero* is not yet fully understood[23]. Maternal diet [24] and maternal stress exposure during pregnancy[25, 26], however, have been shown to influence the infant microbiome, and it is clear that the maternal microbiome can play a key role in shaping infant host development and physiology [27, 2829, 30]. Intriguingly, the periods of major change in the developing microbiota overlap partially with the time-frames for development of other bodily systems and particularly the brain[31] and the enteric nervous system[11]. This parallel development is likely to be biologically relevant, and these periods may correspond to sensitive periods in the development of the MGB axis that will be critical for establishing appropriate communication along the axis throughout the lifespan.

The postnatal microbiota is relatively volatile, gaining stability and diversity across maturation[32]. Most colonization of the infant gut starts at birth when delivery exposes the infant to a complex microbiota that are dependent on many elements, including mode of delivery, breastfeeding, prematurity, the environment, host genetics, antibiotic exposure, and maternal factors such as infection status, stress and/or obesity.

After the first several days of life, there is a shift towards a microbiota population focused on extracting nutrients in order to support the rapid development of the brain and body of the host[33]. A key component of gut microbiota differences may be dependent on whether an infant is breast or formula fed. While heterogeneity exists among the population characteristics and study techniques, most studies show that both diversity and richness of the microbiome are lower in breastfed than formula fed infants, with higher levels of Proteobacteria and Bifidobacteria, and lower levels Bacteroidetes and Firmicutes found in breast fed infants compared to those who are formula fed[34, 35]. However, these differences do not appear to be linked to infant behavioral distinctions such as those associated with colic[35].

The last major change takes place at weaning, as the infant shifts from breastmilk or formula to a solid diet with a pattern observed across species, including humans and rodents[36, 37]. Although there are continuous changes well into adolescence [32], these alterations are more gradual and geared towards an adult-like profile [38]. In the adult, diet has the greatest life-long influence on microbiota composition[39] although antibiotic usage is also a key factor in disrupting the microbiota across the lifespan[40].

Microbiota and CNS Development

Overall, fundamental central neural processes including development, myelination, neurogenesis and microglia activation have shown to be dependent on the composition of the microbiota [4142–4849, 50]. The strongest evidence for a role of the microbiota in neurodevelopment comes from research in germ-free (GF) mice [41, 51]. Studies where GF rodents have been recolonized with “normal” microbiota (i.e., from specific pathogen-free animals) at different ages have shown that post-weaning re-colonization is more effective at restoring GF deficits than re-colonization later in life, at least for specific aspects of brain or immune function and behavior[52–56]. Still other functions in GF animals, such as those affecting CNS serotonergic neurotransmission, cannot be restored by re-colonization by the age of weaning, suggesting that the window for microbial influence on these functions is already closed[56].

Although these studies in GF mice have been important in providing evidence supporting the concept that the microbiome is involved in brain processes involved in stress hormone signaling, neural function and neuroprotection [51], there are significant limitations to human translation of these findings including, but not limited to, the presence of defects in the immune system development, ENS formation and CNS maturation of GF animals [574142–4849, 50]. The mechanistic underpinnings of these relationships are also little understood.

The human studies that have sought to evaluate the relationship between the microbiota and CNS development remain limited; most have been conducted in infants and are largely cross-sectional. Studies that have extended follow-up to two years of age, however, have continued to show connections. Antibiotic exposure in infancy has been reported to have a negative impact on cognitive development [58]. Another study linked cognitive function at two years of age with microbiota composition assessed one year earlier [59]. More recently, the same research group demonstrated that microbiota alpha diversity was also related to cognitive outcomes at two years of age and, further, was associated with functional connectivity between the supplementary motor area and the inferior parietal lobule in infancy. Importantly, this connectivity was also related to cognitive outcomes at two years of age [60].

Enteric Nervous System Development

In the early postnatal period, enteric neuro- and glio-genesis is accompanied by a functional maturation of intestinal neural circuits. [33, 61]. This evolution has been shown to continue beyond the postnatal period in preclinical models; enteric gliogenesis is maintained at low levels throughout life [62–64], enteric neuronal turnover may occur even more rapidly than that of glial cells [65] and changes in synaptic contacts within the enteric circuitry are seen in mice through adolescence [66].

To date, ENS development has been examined primarily from its molecular and genetic origins [67]. An increase in the understanding of the importance of postnatal ENS development, however, has led to an emergence of literature focusing on factors that are

contained within the postnatal gut microenvironment, including the presence of a complex gut microbiota and immune system[11, 68–70].

Gut microbiota-driven effects on ENS development and function have been exemplified in studies on GF mice. These mice harbor reduced numbers and subtype distributions of enteric neurons that are associated with deficits in gut motility[68–70] as well as attenuated excitability of intrinsic primary afferent neurons, a key component of gut-brain neural pathways[71, 72]. Conventionalization of adult GF mice reduces the deficit in intestinal transit time[69, 73], restores neuronal excitability[71], alters the chemical coding of enteric neurons and normalizes enteric glial cell density and gut physiology[64, 69] demonstrating an important role for the microbiota in ENS plasticity. Similar effects have been noted after bacterial exposure through probiotics or specific bacterial strains[74–76]. Moreover, studies have also provided insight as to which microbial mechanisms may affect enteric nerve activity[77, 7878, 79]. These include G-protein-coupled receptor-mediated signaling pathways [80], 5-HT, short chain fatty acids (SCFAs) [78], microbial-epithelial interactions [79] and the transcription factor, aryl hydrocarbon receptor (Ahr).

Ahr is a recognized biosensor for intestinal epithelial- and immune-cell homeostasis in the gut. As such, enteric neuron specific Ahr may serve as a node that integrates signals from the luminal microbiota environment with the physiological output of intestinal neural circuits to maintain gut homeostasis[81]. Interestingly, in a recent study it was shown that neuron-specific deletion of Ahr, or constitutive overexpression of its negative feedback regulator, CYP1A1, results in reduced peristaltic activity of the colon, similar to that observed in microbiota-depleted mice [76]. Moreover, expression of Ahr in the enteric neurons of mice treated with antibiotics partially restores intestinal motility. These studies suggest that the ENS possesses an ability to monitor the luminal microbial environment and adjust neuronal activity and motility accordingly and thus provides a further basis for studies that examine the microbial detection mechanisms used to alter GI and or gut-brain physiology. Overall, more research is needed to understand the mechanisms by which the microenvironment of the gut lumen influences ENS plasticity.

Finally, reverse regulation in which the ENS contributes to the shaping of the microbiome is also possible and has been addressed by several preclinical studies, including several in which alterations in the composition of colonic and/or fecal microbiota were observed in murine or zebrafish models of congenital aganglionosis[8283]. It still needs to be determined, however, whether these abnormalities represent direct effects of ENS circuits on microbiota or are merely the consequences of abnormal peristalsis.

Mechanisms of Microbiota to Gut-Brain Signaling

Vagus Nerve—As a major bidirectional highway of brain-gut connection, the afferent branch of the vagus nerve has been the focus of multiple studies examining its effects on brain-gut communication in health and disease [14, 84]. While the sensory vagus nerve and ENS are intrinsically linked, the mechanisms underpinning this interaction and the role of vagal signalling from the ENS to the brain remain incompletely understood.

The afferent branch of the vagus nerve is the main neural conduit connecting the gastrointestinal tract to the nucleus of the solitary tract and higher emotion-regulating networks in the mammalian brain [85]. Although it does not appear to interact with the gut microbiota directly, evidence suggests that the vagus nerve can sense microbial signals in the form of bacterial metabolites, or be influenced via microbiota-mediated modulation of enteroendocrine (EECs) and enterochromaffin (ECCs) cells in the gut epithelium [86] (Figure 2). For example, gut bacteria produce SCFA metabolites (e.g., butyrate, propionate, acetate and valerate) that regulate physiological intestinal functions, including those involving motility, secretion and inflammation (see below), through their cognate free fatty acid receptors (FFARs)[87]. Further, other receptors on vagal nerve fibers such as those for serotonin (5-HT_3, 5-HT_4) and other gut peptide receptors may also facilitate these messenger pathways[86, 88]. Vagotomy studies in mice also highlight possible roles for the vagus nerve in CNS-microbiota communication which may translate to human mood and neurobehavioral disorders. For example, in mice, vagotomy has been shown to block central signaling of *Lactobacillus and Bifidobacterium* species, resulting in the besiegement of their mood-modifying effects[55, 89, 90].

A bidirectional communication system between diet, the gut microbiome, ECCs and the vagus nerve has recently been reported. ECCs contain more than 90% of the body's serotonin (5-HT) and 5-HT synthesis and release in ECCs is modulated by SCFAs and 2BAs produced by spore-forming *Clostridiales*[12, 91]. These microbes increase their stimulatory actions on ECCs with increased dietary tryptophan availability[92]. ECCs also communicate with afferent nerve fibers through synaptic connections of neuropod-like extensions of ECCs[93]. On the other hand, the ANS can activate ECCs to release 5-HT into the gut lumen, where it can both be taken up by serotonin transporter-like mechanisms and influence gut microbial function [94].

Immune Mechanisms for microbiota to gut-brain signaling—In the gut, an intact immune system is critical for maintaining the careful balance between homeostatic tolerance of commensal organisms and the simultaneous protection of the body against pathogenic microbial invasion. In addition,immunity also serves a critical role in mediating communication between the gut microbiota, the ENS and the brain. Toll-like receptors (TLRs) and peptidoglycans (PGNs) mediate the immune response to microbes by acting as sensors of microbial components[95, 96].An intact gut barrier also prevents the inappropriate activation of immune cells and the development of systemic immune activation.

Bacteria can release immune agonists, such as lipopolysaccharide (LPS) and PGN, into the circulation where they can gain access to the brain. TLRs have been found in the brain of mouse disease models, and especially the microglia, where they have been studied in the development of Alzheimer's Disease[97], Parkinson's disease [98], visceral pain[99] and depression[100]. GF and antibiotic-treated mice also both display a reduction in the expression of several of the receptors that detect PGN in the striatum, which suggests that gene expression in the brain is sensitive to microbiota manipulation. [101]. Moreover, knockdown of a PGN sensing receptor, PGN-recognition protein 2, resulted in an increase in sociability in mice, indicating that loss of the ability to sense peptidoglycan results in host behavioral

changes[101]. More research is needed, however, to unravel the functional consequences of such immune signaling across the lifespan in health and disease

Diet-induced changes in the gut microbiome can lead to a compromised mucus layer, allowing access of luminal microbes to extensions of dendritic cells, resulting in activation of these cells by both pathogens and commensals. This local immune activation can lead to increased permeability of the epithelial tight junctions that further compromises the intestinal barrier. The diet-induced release of immune mediators into the systemic circulation is referred to as metabolic endotoxemia, which can lead to immune activation in different organs, including the brain[102]. This low grade immune activation has been implicated in the pathophysiology of some forms of depression and neurodegenerative disorders such as Alzheimer's and Parkinson's disease.

Immune Signaling and the ENS—TLRs and other components of the innate immune system (e.g., macrophages) may serve as sensors of gut microbial presence and deliver messages to the ENS that result in changes in gut nervous system development and function. Enteric neurons and glia have the machinery necessary to detect the gut microbiota; they both express TLR2 and TLR4[68, 103]. Further, antibiotic depletion of the microbiota alters TLR expression in mice and also results in concomitant alterations in GI motility and sensitivity to acetycholine [104].These effects may, at least in part, may be mediated by TLR4 and/or TLR2. TLR4 deficient mice have decreased numbers of nitrergic neurons and reduced motility, a similar phenotype to that observed in GF and antibiotic-treated mice[68]. Mice deficient in TLR2 signaling exhibit abnormalities in the neurochemical coding of the ENS that is accompanied by gut dysmotility and attenuated chloride production in intestinal explants [103]. Further, antibiotic treatment of wild-type mice leads to ENS abnormalities that can be reversed after supplementation with a TLR2 agonist, further confirming the idea that the gut microbiota-TLR2 axis is important for ENS morphology and function[103].

These data suggest that the ENS has the capacity to respond to stimuli from distinct types of microbes affecting its physiology. Precisely how microbe-TLR communication affects ENS structure and function, and how these changes relate to gut-brain signaling, have yet to be determined. For example, it would be important to determine how alterations in TLR activation during early-life, including those induced by infections and/or antibiotics, can affect ENS development and long-term function of brain-gut interactions. The plasticity of the ENS demonstrated in these studies and others make this likely to be high-yielding area of therapeutic investigation.

Macrophages are present throughout the gut where they play an essential role in the reparative response to intestinal injury [105, 106]. Monocyte-derived and tissue-resident macrophages are decreased in quantity in GF mice or in mice that that are microbiota-depleted with antibiotics, implying a role for the microbiota in intestinal macrophage recruitment and differentiation[107]. Further, muscularis macrophages (MMs) engage in a bidirectional relationship with enteric neurons that appears to be regulated by the gut microbiota; MM activation by the cytokine, bone morphogenetic protein 2 (BMP-2) results in alterations in GI motility and production of macrophage colony-stimulating factor 1 (CSF1), a critical mediator of MM development, and both CSF1 and BMP2 production are

decreased after antibiotic treatment [107]. Microbiota-neuron-macrophage interactions have also been exemplified in studies showing that sympathetic ganglia activation elicited by *S. typhimurium* may influence macrophages to protect the ENS by stimulating processes that both limit enteric neuronal damage and enhance gut motility[108]. The ENS may also protect itself from invasive *S. typhimurium* infection by producing IL-18, a cytokine that both drives goblet cell antimicrobial peptide production and maintains the mucosal barrier [109]. It has most recently been demonstrated that the gut microbiota may influence gut-extrinsic sympathetic activation through a gut-brain circuit[110].

Microbial Metabolites—The gut microbiota generate metabolites that are implicated in the modulation of both CNS and ENS physiology and behavior. Although *in vitro* studies have shown that specific bacteria can produce neurotransmitters (e.g., noradrenaline, dopamine and GABA)[111, 112], whether these neurotransmitters are capable of reaching specific targets within the CNS, and/or in sufficient concentrations, is unknow. The short half-lives of most neurotransmitters and their limited ability to cross the blood brain barrier, however, make this possibility unlikely. The way this gut-brain communication may happen has been studied most extensively for serotonin and other tryptophan metabolites (*see below*) which have been shown to exert major influences on ENS and CNS development and functions including GI motility, mood and behavior[113–118].

Short Chain Fatty Acids: Gut bacteria produce SCFA metabolites that can regulate motility, secretion, and gut-brain signaling by acting through FFARs on epithelial cells, EECs, ECCs, immune cells, and intrinsic and extrinsic neurons[12, 91] (Figure 2) [119]. Centrally, administration of acetate, propionate and butyrate is capable of restoring morphological deficits of microglia in GF mice [120] and reversing the behavioral and physiological effects of chronic stress [121]. Moreover, SCFAs may influence the production of neurotransmitters in the brain through regulating the expression of enzymes involved in their biosynthesis; administration of propionate and butyrate to PC12 cells (neuroblastic cells which differentiate to neuron-like cells) *in vitro* increased the expression of tyrosine hydroxylase, the rate-limiting enzyme involved in noradrenaline and dopamine synthesis [122]. Whether these microbial metabolites are capable of regulating neurotransmission *in vivo*, however, is not clear. More evidence is also needed to determine the extent to which physiologically relevant concentrations of SCFAs are capable of reaching the brain given their relatively short half-life (25 minutes to three hours). To date, studies investigating the effect of exogenous SCFA administration upon brain physiology and behavior have typically used concentrations that far exceed what is microbially derived [123].

Serotonin: A key regulator of the MGB Axis in the Gut and Brain—There is growing appreciation that the neurotransmitter 5-HT is one of the key players in MGB axis signaling in both the brain, the gut and in gut to brain communication. Indeed, recent studies have shown that specific spore-forming bacteria from both humans and mice increase colonic and serum 5-HT levels in GF mice and ameliorate GF-associated gut dysmotility by producing SCFAs[124], which increase 5-HT production by up-regulating Tph1 expression in ECCs[12, 91] (Figure 2). Recent evidence suggests that 5-HT released from ECCs communicates with the gut microbiota, *Turicibacter sanguinis*, that possesses

serotonin uptake mechanisms which are involved in its colonization and host physiology[125]. This may account for some of the bidirectional influences that occur between some psychotropic drugs, including SSRIs, and the intestinal microbiota[126]. Gut microbiota has also been shown to induce maturation of the adult ENS via activation of 5-HT_4 receptors (5-HT4R); GF mice retain a higher degree of nestin-expressing neuronal stem cells and exhibit slower intestinal transit and both factors normalize following bacterial colonization that is dependent on 5-HT4R signaling[73]. Finally, the gut microbiota may also act through neurotransmitter precursors; it can influence serotonergic neurotransmission through regulating the availability of the 5-HT precursor, tryptophan. Circulating tryptophan concentrations are significantly higher in male GF mice relative to conventional controls [56] and these altered tryptophan levels correspond with an increase in hippocampal serotonin, and its metabolite, 5-hydroxy-indole acetic acid [56]. Whether this has any bearing on the social deficits observed in these animals, however, requires further investigation.

Tryptophan Metabolism: Beyond Serotonin—The main physiological pathway for tryptophan metabolism is along the kynurenine pathway. GF mouse studies have shown an increased availability of tryptophan in GF animals as a consequence of a reduction in peripheral kynurenine pathway activation[56]. Moreover, in a rodent model of chronic variable stress, a stress induced reduction of Lactobacilli reduced hydrogen peroxide (H_20_2)-mediated inhibition of Indoleamine 2, 3-Dioxygenase 1 (IDO1). This inhibition resulted in an increase in the conversion of tryptophan to kynurenine, a feature that was linked to depression-like behavioral alterations in mice exposed to chronic stress[127]. In contrast to serotonin, kynurenine can traverse the blood brain barrier and negatively impact brain health by inducing neuroinflammation and neurodegeneration[128].

Indole production which is limited to the gut microbiota, is catalyzed by the microbial enzyme, tryptophan hydroxylase and indole has been detected in blood, brain and the GI tract [92]. There is a growing literature supporting the concept that the microbial processing of tryptophan to indole affects gut-brain axis function[129]. Indoles exert many beneficial actions on intestinal and systemic homeostasis[130].Yet, adverse effects on the gut-brain axis are also evident following absorption from the gut and host processing, where some indole derivatives, have been shown to exert neurodepressive-like affects on behavior, at least in preclinical studies[131].

Tryptamine—Bacteria express tryptophan decarboxylase and are capable of producing tryptamine from dietary tryptophan. It has recently been demonstrated that bacterial-derived tryptamine can act via the 5-HT4R to impact gastrointestinal motility[88]. The capacity of the gut microbiota to produce metabolites like tryptamine, that can influence host physiology by activation of G protein-coupled receptors, is an important facet of host-microbe interactions that warrants increased attention[132,133]. It is currently unclear, however, if microbially derived tryptamine reaches the CNS and whether it acts there to control behavior.

The Microbiota-Gut-Brain axis in Healthy subjects

There are a number of studies that demonstrate the effects of microbiota-targeted interventions in healthy individuals [19]. Four weeks of probiotic consumption by healthy

females led to changes in the functional connectivity of an emotion recognition network in the brain[134]. In a different cohort of female healthy control subjects, white and gray matter imaging parameters were associated with two bacterial genus-based clusters that each differed in the structures associated with emotional, attentional, and sensory processing and also in fMRI-measured emotional reactivity [135]. Several trials involving the administration of a *Lactobacillus rhamnosus* (JB-1) supplement to healthy males showed inconsistent outcomes in stress-related behaviors with either a positive[136] or no[137] difference in those receiving the supplement. In another examination of healthy subjects, however, probiotics improved emotional decision-making and affect relative to placebo with concomitant changes in specific taxa[138]. In contrast to preclinical models, there is currently no solid evidence that probiotic ingestion in healthy individuals can modulate behavior, as reported studies on probiotic consumption in small clinical studies have produced contradictory outcomes[139–143].

Disorders of brain-gut interactions: IBS

IBS is the most common disorder of brain-gut interaction, occurring in up to 4.8 % of the population worldwide[144]. Based on the current symptom criteria,[145] IBS is defined by chronically recurring abdominal pain associated with altered bowel habits in the absence of detectable organic disease. This gut-restricted definition overlooks the findings that up to 50% of individuals who meet diagnostic criteria for an anxiety disorder have IBS, and that individuals with IBS have a greater than three-fold risk of meeting diagnostic criteria for an anxiety disorder[146]. While in the majority of IBS patients, CNS-related precipitants in early and adult life (e.g., psychological trauma, stress, abuse, maternal neglect) [147] have been identified, about half of IBS patients present after an intestinal trigger [148]. The bidirectional nature of brain-gut involvement in IBS was illustrated in a one-year population-based prospective study that evaluated individuals with anxiety +/− depression and IBS as well as control individuals with neither condition. At the conclusion of the study, it was found that individuals with higher baseline levels of anxiety and depression were significantly more likely to develop IBS and, conversely, that those subjects with baseline IBS reported significantly higher levels of anxiety or depression. Interestingly, in 2/3 of these co-morbid cases, an IBS diagnosis preceded the mood disorder, implying that in some patients primary gut dysfunction might serve as a driver for mood disorders[149].

Alterations in fMRI-detected brain activity are linked to abdominal pain[150]. Connections have been shown between brain networks that mediate anxiety and ANS output, like the amygdala, with mechanisms that modulate colonic sensitivity and gut motility. Increased and decreased activation of endogenous pain excitatory and inhibitory pathways, respectively, have also been observed in CNS locations that have been associated with visceral afferent processing and emotional arousal[151–153]. Interestingly, these pathways share significant homology to a stress circuit in rodents that implicates the involvement of corticotropin releasing factor in the central and peripheral regulation of brain-gut interactions in IBS[154].

IBS and the Microbiome—A causal role for altered gut microbiota in IBS symptoms remains to be determined, even though a number of cross sectional studies have reported

alterations in fecal microbial community composition in IBS subjects, based on disease subtype (IBS-D, IBS-C, IBS-M), age (pediatric vs. adult), and/or compartment (mucosa vs. stool)[155]. Recent evidence suggests the presence of IBS subgroups based on gut microbial community structure, with groups not differing from healthy controls despite GI symptoms[156, 157]. In one study, a dysbiotic IBS subgroup differed in regional brain volumes from a group with normal gut microbiota[157], suggesting a relationship between microbial community composition and brain structure. However, as both microbiota-defined subgroups met the IBS diagnostic criteria and did not differ in any clinical parameters, these findings put into question a causative role for dysbiosis in IBS symptoms. The absence of group differences in the microbial composition between healthy controls and individuals with IBS has been reproduced elsewhere, though IBS symptom severity was found to be correlated with dysbiosis[158]. A more recent cross-sectional study revealed significant differences among IBS subtypes in the distribution of Clostridiales. Relative Clostridiales abundance was correlated with significant differences in the level of fecal SCFAs, which together were associated with altered fecal cytokine levels[159]. Even though this study aimed to identify mechanistic pathways in gut microbe-host interactions, the findings need to be confirmed in a study with a control population and a larger sample size.

The diverse findings from the IBS microbiota studies have been attributed to the extensive range of technologies employed for microbiota study, sample source, differences in IBS subtype, differential effects of the ANS on other aspects of physiology (e.g., mucus secretion, intestinal permeability and mucosal immunity; reviewed in[160]) as well as the many other influences that affect microbial composition and function (e.g., age, diet, antibiotic exposure, geography, probiotic intake, medication exposure)[155, 161]. Further complicating this dynamic is the need to factor in the CNS-mediated aspects of motility and gut physiology, including sleep quality and stress. The examination of much larger cohorts in longitudinal studies that also integrate clinical phenotypes and diet are thus needed for a more comprehensive understanding of these populations.

IBS and serotonin—Serotonin is one of most highly studied neurotransmitters in IBS physiology. As a major determinant of ENS and CNS development and a modulator of IBS-related symptoms (e.g., motility, secretion and visceral hypersensitivity) as well as mood [113], serotonin may thus be an important developmental modulator of the comorbid mood and IBS diagnoses made in some affected patients. Alterations in enteric mucosal and blood serotonin signaling have also been demonstrated in adults and children with IBS, potentially indicative of GI-initiated serotonergic dysregulation[162, 163]. Although serotonin can activate more than 15 receptors/receptor subtypes in the brain and intestine, the majority of IBS research has focused on the 5-HT_3 and 5-HT_4 receptors, as both have been shown to have effects on mood, motility and abdominal pain (reviewed previously[113, 164–167]).

Altogether, these data suggest a possible role of the gut microbiome in altered brain gut interactions in IBS. They also provide the basis for larger, longitudinal interventional studies, both clinical and functional, to identify the roles of specific microbiota in behavioral and gut dysfunction in IBS and also the utility of serotonin-based modulators as potential therapeutic targets (Figure 2).

Gut Microbiome in Depression

In recent years, there have been an increasing number of studies showing that patients with Major Depressive disorder (MDD) have an altered gut microbiome composition when compared to healthy controls, although the nature of the alterations in each study are diverse[168–170]. This variation in outcomes is likely due to similar reasons as those noted for IBS. It is worth noting that studies have also shown that transferring the microbiome of a depressed individual into a healthy rodent can induce depressive-like behaviors in the murine recipient suggesting the possibility of a causal role for the microbiota in pathophysiology of depression and opening up the concept of targeting the microbiome for mental health benefit[19, 169].

Mood and IBS: Targeting the Microbiota-Gut-Brain Axis

The effects of enteric microbial manipulations in controlled clinical trials in patients with depression and/or IBS have been evaluated with probiotics, antibiotics and the low fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) diet. Several studies have demonstrated the effectiveness of a low FODMAP diet in the short-term treatment of IBS symptoms, and diet induced changes in the gut microbiome have been implicated as an underlying mechanism. The low FODMAP diet results in a decreased production of gas and osmotically active metabolites, as a result of decreased microbial fermentation, that is thought to lead to improvements in bloating, flatulence, and pain[171]. In line with this theory, adult RCTs demonstrate that intake of a low FODMAP diet improves IBS symptoms, regardless of subtype, as well as health-related quality of life (QOL), anxiety, and activity impairment in adults with IBS-D[171]. However, even though these study results indirectly support a role for the gut microbiome in some IBS symptoms and may be useful for short term treatment of some IBS symptoms, the value of a low FODMAP diet for long term treatment for IBS has been questioned[171]. This reduction efficacy may be because of the reduction of oligosaccharides important for the diversity and abundance of the gut microbiota, and/or because of the low compliance rate associated with long-term adherence[171].

A different dietary approach has been taken in the treatment of depression which comes under the umbrella of Nutritional Psychiatry. There is clinical evidence from both epidemiological and several interventional studies that a largely plant based diet, such as the traditional Mediterranean diet, has benefits in the adjuvant treatment of depression[172, 173]. There are many open questions that remain, especially in regard to the mechanisms of how diet may impact mood and which components are the most beneficial.

Supplementing the Gut Microbiome to improve MGB Axis—Apart from dietary intervention, probiotics have also been tried as a treatment meant to target the microbiome. Although numerous preclinical and some clinical studies have reported beneficial effects of specific probiotics on mood and emotional behaviors, clinically significant effects of probiotics in the treatment of psychiatric disorders have not been demonstrated[174]. There is thus a need for high quality randomized controlled clinical studies in human subjects to demonstrate that the beneficial effects observed in preclinical models can be translated and confirmed in human settings.

Studies on probiotics, including strains of *Bifidobacterium* and/or *Lactobacillus,* as well as VSL#3, have been shown to improve symptom severity in adults and children with IBS[175–177]. Due to the insufficient quality of these studies, however, the recently published AGA Guidelines on probiotics do not recommend their use in IBS, other than in controlled studies[176, 177].

Interestingly, although some probiotic supplementation in humans changes the gut microbiota composition, as judged by 16S rRNA sequencing, others show no or only transient modification of the collective microbiome transcriptional state. These findings suggest that measurement of probiotic intervention on gut microbial profiles must be accompanied by technologies assessing microbial function, such as metagenomics or metabolomics.

Fecal microbiome transplantation as a therapy for IBS & mood disturbances— Clinical studies on fecal microbiota transplantation (FMT) remain limited and systematic review fails to show any overall benefit[178, 179]. Two recent RCT studies showed alterations in gut microbial composition in the group receiving a FMT. However, while one study showed a significant IBS symptom reduction three months after FMT, the other study demonstrated greater symptom improvement in the placebo-treated group[178–180]. High quality FMT studies in patients with depression are in the pipeline.

Conclusions and Future Directions

Considerable progress has been made in the understanding of the MGB axis in preclinical models of human brain disorders and in the potential translation of these findings to humans. A growing body of research has confirmed that disorders of brain gut interactions like IBS have a strong brain component and that many brain disorders have a GI facet to their manifestations or even their origins. A causal role of the gut microbiome in these interactions remains to be determined. This valuable knowledge will inform the development of cross-disciplinary therapeutic approaches well into the future.

Key areas that have not yet been examined extensively are the roles that sex and race play in microbiome-gut-brain-axis development and disease. Accumulating evidence has shown, however, that both sex and race may exert important influences over the gut microbiota. The microbiome may alter brain development in a sex-specific manner and females are significantly more likely to suffer from stress-related and functional GI- disorders[56, 181, 182]. On the other hand, at least in animal models, the male brain appears to be more susceptible to microbial disturbances in early life than the female brain[183]. These sex-specific effects have given rise to justified calls for more intensive study of the “microgenderome”[181, 184]. Although racial diversity of the gut microbiome has been explored somewhat in cancer and other medical conditions, there has been no in-depth exploration of how race impacts microbial diversity and its relationship with brain-gut axis conditions[185]. The current knowledge, however, emphasizes the need to understand how the sexual dimorphism and racial diversity impact the microbiome and contribute to brain-gut axis disorders.

It is important to reiterate that translation of preclinical findings into more effective therapies for human brain disorders has largely been unsuccessful to date (Figure 3). Moving forward, the development of live biotherapeutics or substances whose beneficial effects on the brain are bacteria-mediated (i.e., psychobiotics) are currently being investigated as direct and/or adjunctive therapies for brain disorders, but this field is very much in its infancy[19]. Until identification of gut microbiome-related patient subtypes becomes possible and new pharmacologic and specific microbiome targeted approaches emerge, the most effective treatments for IBS and other disorders of MGB interactions remain a combination of personalized diet approaches, behavioral therapies and a limited number of pharmacologic treatments aimed at improvement of bowel function[186].

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What You Need to Know

BACKGROUND AND CONTEXT:

A growing body of research has confirmed that disorders of brain gut interactions (e.g., IBS) have a strong brain component and that many brain disorders have a GI facet to their manifestations or even their origins. The gut-brain axis plays an important role in maintaining homeostasis. Hence, when this system goes awry, disease can develop. More recently, the role of the microbiome as an important factor in modulating gut-brain signaling has emerged and the concept of a microbiota-gut-brain (MGB) axis has been established.

NEW FINDINGS:

In this review, we highlight the role of the MGB axis in modulating enteric and central nervous system function and how this modulation may impact disorders such as Irritable Bowel Syndrome and disorders of mood and affect. We also examine the overlapping biological constructs that underpin these disorders with a special emphasis on the neurotransmitter serotonin, which plays a key role in both the gastrointestinal tract and in the brain.

LIMITATIONS:

Although considerable progress has been made in the understanding of the MGB axis in preclinical models of disease and in the potential translation of these findings to humans, several limitations remain. These limitations include a lack of understanding of whether there is a causal role of the gut microbiome in these interactions and the identification of gut microbiome-related patient subtypes.

IMPACT:

Overall, it is clear that although animal studies have shown much promise, more progress is necessary before these findings can be translated for diagnostic and therapeutic benefit. Although in its infancy, the development of live biotherapeutics or substances whose beneficial effects on the brain or gut are bacteria-mediated may ultimately serve as novel, effective therapies for brain-gut disorders.

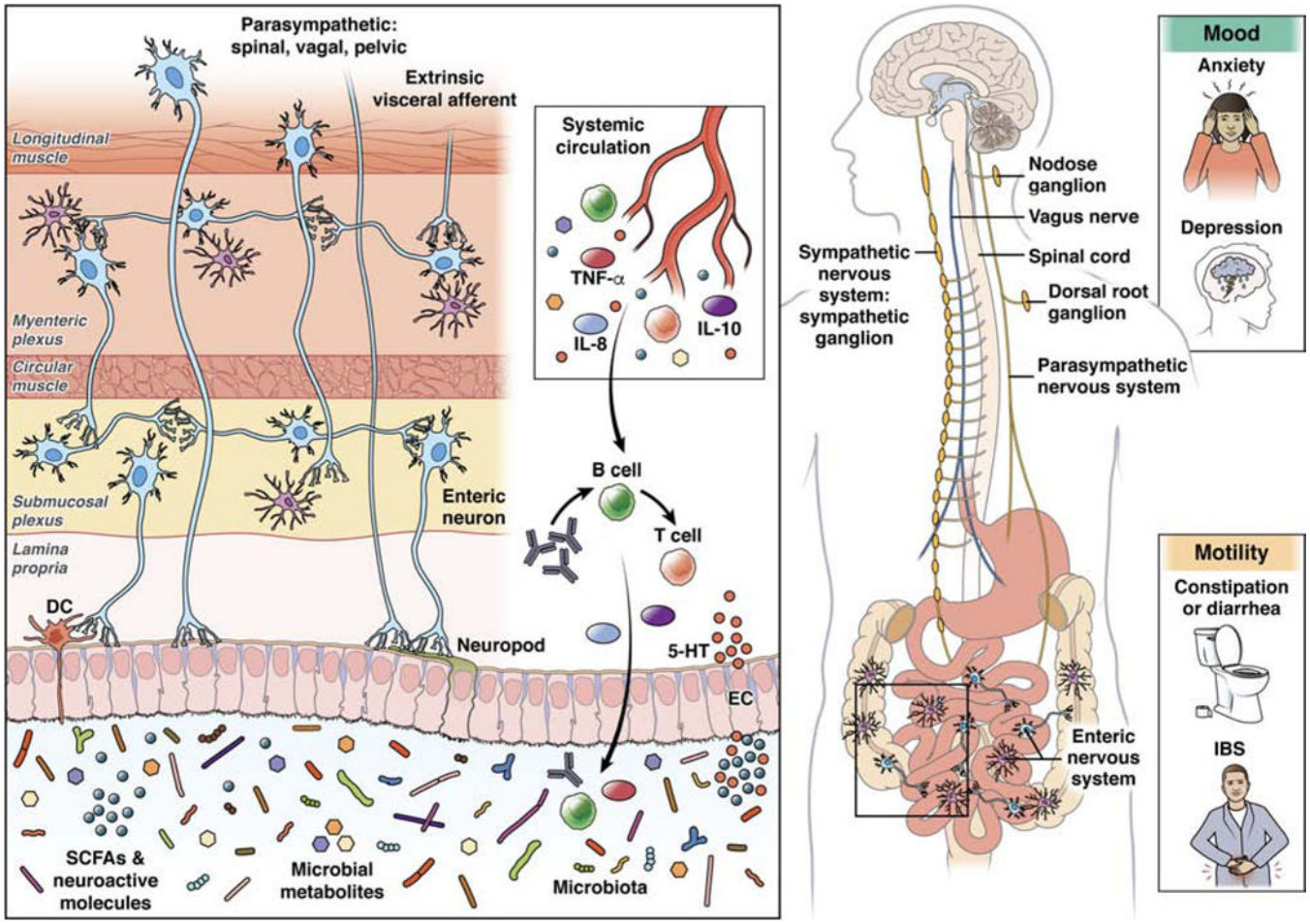

Figure 1: Pathways of communication between microbiota & brain

A growing body of research is implicating different pathways of communication between the microbiome and brain in disorders of both mood and motility. Multiple direct and indirect (via systemic circulation) pathways exist through which the gut microbiota can modulate the gut-brain axis. They include endocrine (cortisol), immune (cytokines) and neural (vagus, enteric nervous system and spinal nerves) pathways. Several gut microbes are capable of synthesizing neurotransmitters (i.e., γ-amino butyric acid (GABA), noradrenaline, and dopamine) locally, which can act on target cells in the gut and act as an important avenue of communication. Neuroactive microbial metabolites can modulate brain and behavior through a number of ways that are still being elucidated. These include affecting epithelial cells to impact gut barrier function and enteroendocrine cells (EECs) to release GI hormones, as well as dendritic cells (DCs) to modulate immune function. Specialized structures on EECs and ECCs, known as neuropods have been shown to transduce sensory signals from the intestinal milieu to the brain through forming synapse-like connections to afferent nerves, including the vagus nerve. The enteric nervous system is perfectly poised to be an integral hub for microbial signals and can communicate with the brain via vagal and spinal pathways. However, the exact molecular signaling pathways of all these pathways involved remain to be defined.

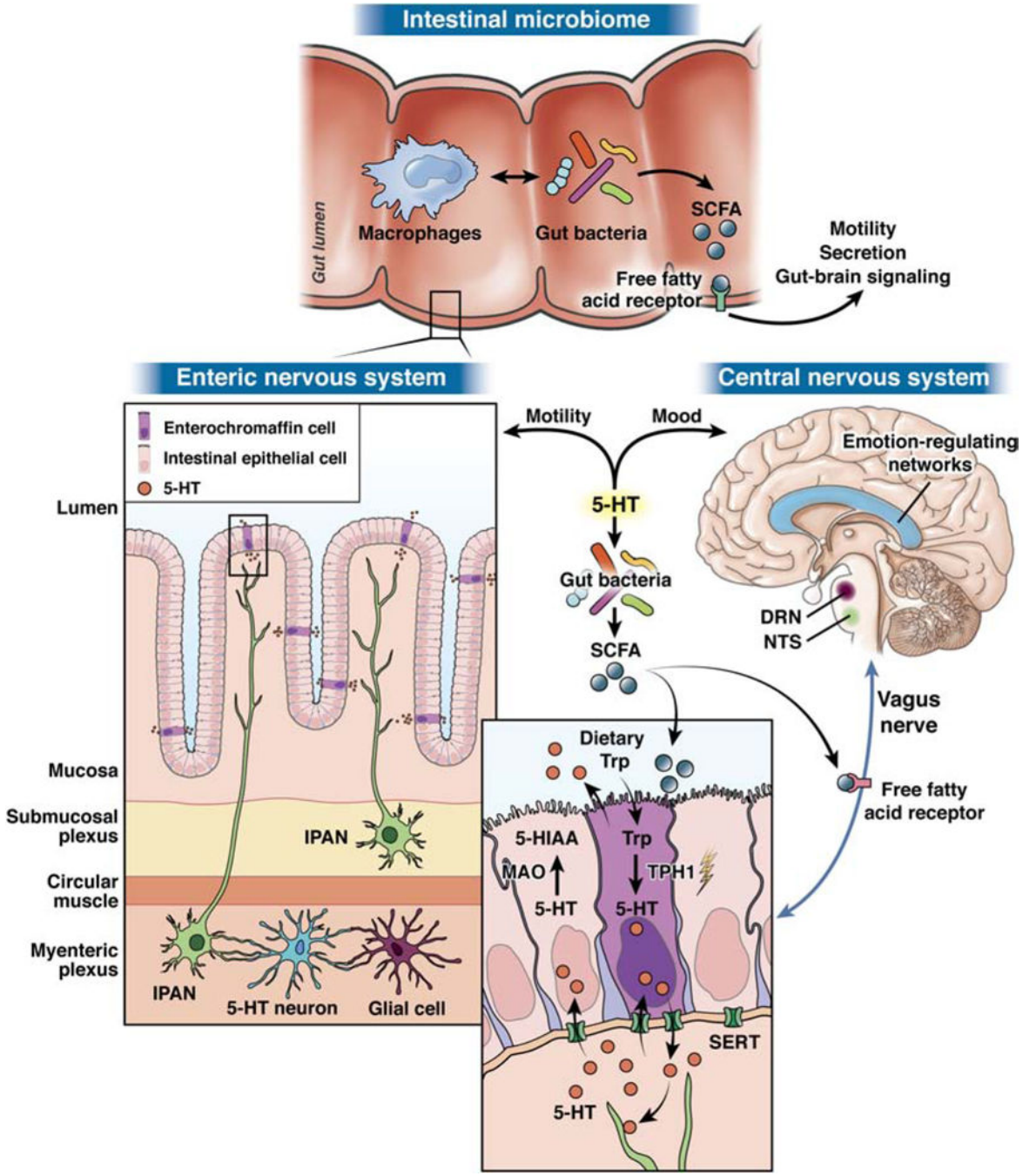

Figure 2: Serotonin (5-HT) as a critical regulator of gut-brain-microbiome axis signaling.

Gut bacteria in the intestinal microbiome produce short-chain fatty acids (SCFAs) that directly stimulate tryptophan hydroxylase 1 (TPH1), resulting in 5-HT synthesis in and secretion from intestinal enterochromaffin (ECC) cells. 5-HT released from the basal membrane of intestinal EC cells then interacts with receptors from neurons in the enteric nervous system to modulate motility and, during development, neuronal development and differentiation. Vagal afferents signal to the nucleus of the solitary tract (NTS) and the dorsal raphe nucleus (DRN), the latter of which houses the majority of the brain's 5-HT neurons.

These areas then interact with emotion-regulating brain networks that influence mood. Of note, SCFAs produced by gut bacteria can also directly stimulate free fatty acid receptors on multiple cell types, including epithelial cells, ECCs, immune cells, and nerve cells, including the vagus nerve and primary afferent neurons. This signaling can also modulate downstream regulation of motility, secretion, and gut-brain signaling. Abbreviations: Trp: tryptophan; SERT: serotonin reuptake transporter; MAO: monoamine oxidase.

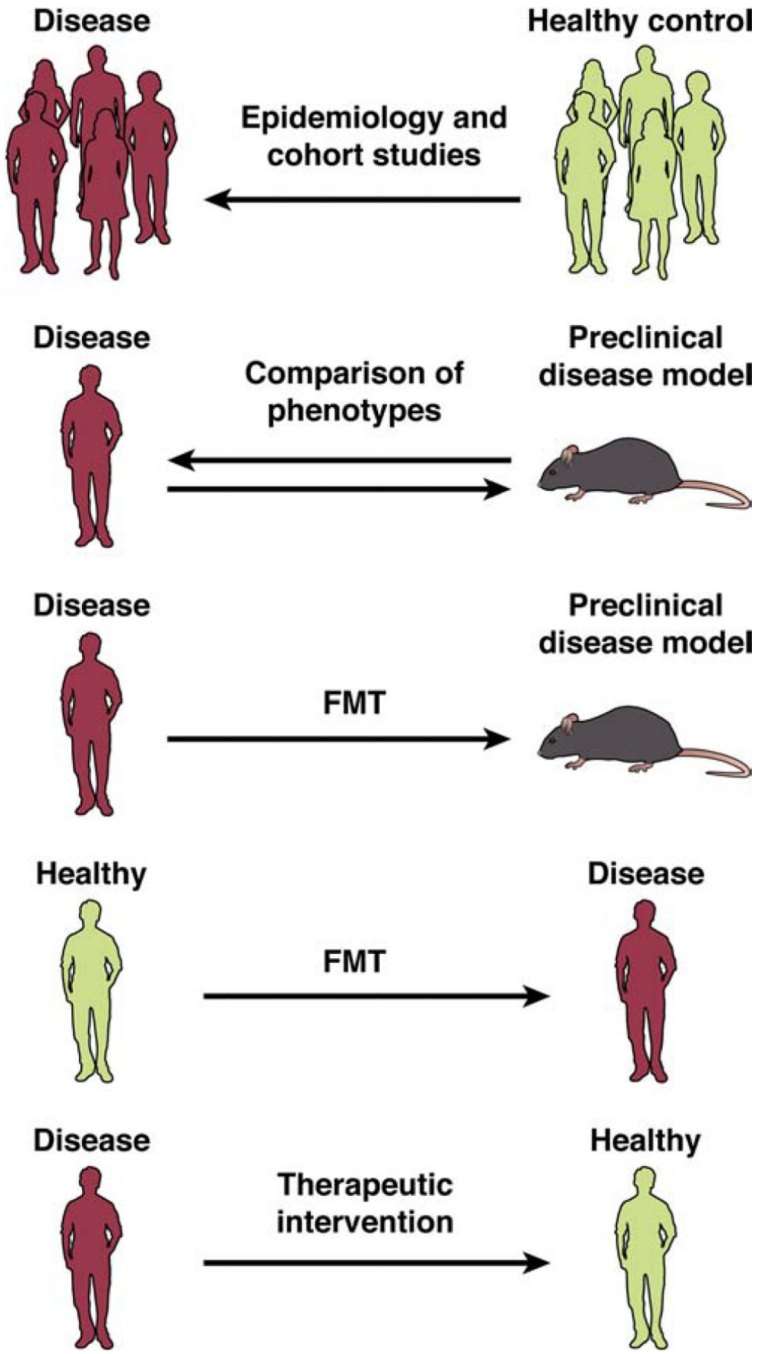

Figure 3: Challenges in Translational Research.

Schematic representation of research approaches aimed to identify a causal role of the gut microbiome in human brain and brain gut disorders. There is extensive evidence for cross sectional differences in the gut microbial composition between defined disease populations and healthy control populations (top row). A number of rodent models of human brain diseases have been developed that mimic certain disease aspects (second row from top). More recently, studies have been reported in which fecal microbial transplants from patients with certain brain diseases into germ free mice have resulted in altered mouse behaviors,

mimicking some aspects of the human phenotype (middle row). Fecal microbial transplants from healthy human subjects into individuals with brain disorders have not resulted in consistent improvement in respective symptoms to date (second row from bottom). To date, there is limited evidence for the effectiveness of therapeutic interventions targeted at the microbiome (bottom row).