

Modulation of Gut Microbiota–Brain Axis by Probiotics, Prebiotics, and Diet

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ABSTRACT: There exists a bidirectional communication system between the gastrointestinal tract and the brain. Increasing evidence shows that gut microbiota can play a critical role in this communication; thus, the concept of a gut microbiota and brain axis is emerging. Here, we review recent findings in the relationship between intestinal microbes and brain function, such as anxiety, depression, stress, autism, learning, and memory. We highlight the advances in modulating brain development and behavior by probiotics, prebiotics, and diet through the gut microbiota–brain axis. A variety of mechanisms including immune, neural, and metabolic pathways may be involved in modulation of the gut microbiota–brain axis. We also discuss some future challenges. A deeper understanding of the relationship between the gut bacteria and their hosts is implicated in developing microbial-based therapeutic strategies for brain disorders.

KEYWORDS: *gut microbiota, brain function, probiotics, prebiotics, diet*

■ INTRODUCTION

The gut is closely connected to the brain via 200–600 million neurons.¹ Bidirectional communication between the gut and the brain has long been recognized; that is, signals from the brain can influence the motor, sensory, and secretory modalities of the gastrointestinal (GI) tract and, in turn, visceral messages from the gut can influence brain function.² Recently, there is expanding evidence for the view rethinking the gut–brain axis as the concept of a gut microbiota–brain axis due to the crucial role of gut microbiota in the bidirectional gut–brain axis,³ for example, germ-free mice showing reduced anxiety-like behavior,⁴ memory lapse even in the absence of stress,⁵ a distinct perturbation of the composition of gut microbiota in animal models of depression and chronic stress,⁶ and the elevation of some mucosa-associated bacteria (*Ruminococcus gnavus* and *Ruminococcus torques*) in gastrointestinal biopsies taken from children with autism spectrum disorder (ASD).⁷ However, the routes of the communication between gut microbiota and brain are not fully elucidated, possibly through neural, endocrine, and immune pathways, which could be affected by gut microbiota or microbiota-generated metabolites.⁸

Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host”.⁹ The probiotic strains used for human consumption must survive gastrointestinal transit with human origin and nonpathogenic feature.¹⁰ Prebiotics refer to nondigestible (by the host) food ingredients that have a beneficial effect through their selective metabolism in the intestinal tract:¹¹ natural or processed “functional foods” that contain biologically active compounds (e.g., indigestible dietary fiber, carbohydrate), which also have a health benefit on the host through the metabolic products from anaerobic fermentation of gut bacteria. Synbiotics are simply the combination of probiotics and prebiotics. Accumulating data reveal that the bidirectional interaction between gut microbiota and brain can be modulated

by probiotics, prebiotics, synbiotics, and diets, which exert beneficial impacts on brain activity and behavior.¹²

In this review, we first give an overview of the relationship between the composition of gut microbiota and brain disorders, including anxiety, depression, stress, autism, etc. Next, we discuss the modulation of the gut microbiota–brain axis with probiotics, prebiotics, and diet and potential mechanisms of action. Then, we delineate some challenges for the future.

■ COMPOSITION OF GUT MICROBIOTA ASSOCIATED WITH BRAIN FUNCTION

Gut Microbiota and Depression and Anxiety. Numerous works focus on the impact of the microbiota on behaviors such as depression or anxiety. Compared with specific pathogen free (SPF) mice, germ-free (GF) mice exhibited reduced anxiety and increased motor activity.^{4,13} Colonization of germ-free NIH Swiss mice with BALB/c microbiota reduced exploratory behavior, whereas colonization of germ-free BALB/c mice with microbiota from NIH Swiss mice increased exploratory behavior.¹⁴ This could be attributed to the established difference of the two mouse strains in their microbiota and behaviors.^{15,16} Such a microbiota transfer effect is similar to that observed in obesity,¹⁷ confirming the ability of the microbiota to influence behavior. However, there is very limited information in the literature on the effects of depression/anxiety on the intestinal microbiota. In a recent study using chronic depression- and anxiety-like mouse model induced by bilateral olfactory bulbectomy (OBx), Park et al.¹⁸

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revealed that depression leads to an altered intestinal microbiota profile in OBx mice.

It is noted that anxiety and depression are generally considered to be correlated with stress. Increasing data show that stress has also been associated with changes in the GI microbiota via the sympathetic nervous system and adrenergic response. By deprivation of food, water, and bedding in mice inoculated with *Salmonella*, it was shown that environmental and dietary stresses significantly alter the gut microbiota in mice: stressed mice had lower levels of lactobacilli compared with control mice.¹⁹ Maternal separation in rodents is a well-studied model of early-life stress. O'Mahony et al.²⁰ demonstrated that maternal separation stress causes marked population-based alterations in the fecal microbiota of separated animals, and such a disrupted microbiota can persist into adulthood. Bailey et al.²¹ demonstrated that a prolonged restraint stressor significantly reduced microbial richness and diversity, such as a reduction in the family Porphyromonadaceae. Another study from this laboratory also showed that social disruption significantly changed the community structure of the microbiota, specifically, increasing the relative abundance of the genus *Clostridium* and decreasing the relative abundance of the genus *Bacteroides*.²² In addition, it has been found that grid floor-induced stress significantly increased the relative abundance of *Odoribacter*, *Alistipes*, and an unclassified genus from the Coriobacteriaceae family.²³ These findings suggest that stress leads to anxiety/depression by altering the gut microbiota (Table 1) and that anxiety/depression influences the gut microbiota, which also affects anxiety and depression.

Table 1. Link between Microbiota and Brain Function

brain function	altered microbes	refs
stress	Porphyromonadaceae ↓	21
stress	<i>Clostridium</i> ↑, <i>Bacteroides</i> ↓	22
stress and anxiety	<i>Odoribacter</i> ↑, <i>Alistipes</i> ↑, Coriobacteriaceae ↑	23
autism	<i>Clostridium</i> ↑	27–29
	<i>Akkermansia muciniphila</i> ↓	30
	<i>Sutterella</i> ↑, <i>Ruminococcus torques</i> ↑	7, 31, 32
	<i>Desulfovibrio</i> ↑, <i>Bifidobacterium</i> ↓	
	<i>Prevotella</i> ↓, <i>Coprococcus</i> ↓, Veillonellaceae ↓	33

Gut Microbiota and Autism. Autism spectrum disorders (ASD) comprise a complex neurobiological disorder; its characteristics include impairment in social interaction and communication and restricted, repetitive, and stereotyped patterns of behavior, interests, and activities.²⁴ Genetic and environmental factors are involved in the pathogenesis of ASD. In particular, one potentially important environmental factor is abnormal intestinal microbiota, as GI disturbances are frequently reported in infants with ASD,²⁵ and there is a strong positive correlation between GI problems and ASD severity.²⁶ Indeed, altered compositions of the intestinal microbiota have been reported in several studies. Specifically, the *Clostridium* species were higher in the ASD patients compared to control.^{27–29} Moreover, lower levels of the mucolytic bacterium *Akkermansia muciniphila*³⁰ and *Bifidobacterium* species,³¹ higher levels of *Sutterella*, *Ruminococcus torques*, and *Desulfovibrio* species,^{7,32} and significantly lower abundances of the genera *Prevotella*, *Coprococcus*, and unclassified Veillonellaceae³³ were found in ASD patients (Table 1).

Modulation of Gut Microbiota–Brain Axis for the Healthy Brain. The reported link of gut microbiota to brain health and disorders raises the question of how to modulate the microbiota for re-equilibration of the gut microbiota compositions altered by brain disorders. There is a growing body of evidence documenting the ability of probiotics, prebiotics, synbiotics, and other diets to normalize the dysbiotic microbiota associated with psychological disorders.³⁴

Probiotics-Based Modulation. Although a complex relationship between gut microbiota and anxiety/depression exists, it is possible to improve anxiety and depression by probiotics modulation (Table 2). For example, Bercik et al.³⁵ indicated that *Bifidobacterium longum* normalized anxiety-like behavior induced by the noninvasive parasite *Trichuris muris* infection; Bravo et al.³⁶ showed that chronic treatment with *Lactobacillus rhamnosus* (JB-1) reduced the anxiety- and depression-related behavior in the *Trichuris muris*-infected mice.

Recently, using the maternal immune activation (MIA) mouse model of ASD in mouse offspring, Hsiao et al.³⁷ showed that oral administration of the human commensal *Bacteroides fragilis* reversed the abnormalities in gut permeability and ASD-related behaviors. Interestingly, these behavioral effects are not specific to *Bacteroides fragilis*, as *Bacteroides thetaiotaomicron* also significantly improves anxiety-like behavior, although *Enterococcus faecalis* does not exhibit anxiolytic action in MIA offspring.

Especially, ingestion of selected probiotics also exhibits effects on brain activity in humans. After 2 months of administration of *Lactobacillus casei* strain Shirota in chronic fatigue syndrome patients, there were significant rises in both *Lactobacillus* and *Bifidobacterium* and also a significant decrease in anxiety symptoms among those taking the probiotic versus controls.³⁸ The oral administration of *L. helveticus* R0052 and *B. longum* R0175 for 2 weeks was shown to alleviate anxiety and depressive symptoms in healthy volunteers, as measured by the Hopkins Symptom Checklist (HSCL-90), the Hospital Anxiety and Depression Scale (HADS), the Perceived Stress Scale, the Coping Checklist (CCL), and 24 h urinary free cortisol (UFC).³⁹ Similarly, after 4 weeks of consumption of a fermented milk product with probiotics (FMPP) (containing *Bifidobacterium animalis* subsp. *lactis*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *Lactococcus lactis* subsp. *lactis*) by healthy women, Tillisch et al.⁴⁰ found that FMPP intake affected the activity of brain regions that control central processing of emotion and sensation, including affective, viscerosensory, and somatosensory cortices.

Several studies have spurred better understanding of the acting mechanisms of probiotics involved in gut–brain axis signaling. After exposure to *Bifidobacterium infantis*, the levels of brain-derived neurotrophic factor (BDNF) and *N*-methyl-D-aspartate (NMDA) receptor subunit 2a (NR2a) in the cortex and hippocampus were reduced in GF animals compared to SPF controls.⁴¹ Likewise, the induced anxiety-like behavior in *Trichuris muris*-infected mice was still present after vagotomy, which was also associated with decreased hippocampal BDNF mRNA and increased circulating tumor necrosis factor- α and interferon- γ . When *Bifidobacterium longum* reversed the anxiety-like behavior in infected mice, the hippocampal BDNF expression was also restored, although the cytokines were not affected.³⁵ This means that visceral signals derived from bacterial colonization can be transmitted to the brain, and the anxiolytic actions of *Bifidobacterium infantis* and *Bifido-*

Table 2. Modulation of Brain Behaviors by Probiotics and Prebiotics

model	behavior tests	intervention	duration	effects	refs
<i>Trichuris muris</i> -infected mice	light/dark preference and step-down tests	<i>Bifidobacterium longum</i>	14 days	normalized anxiety behavior	35
<i>Trichuris muris</i> -infected BALB/c mice	open field, elevated plus maze, fear conditioning, stress-induced hyperthermia	<i>Lactobacillus rhamnosus</i> (JB-1)	28 days	reduced anxiety- and depression-related behavior	36
maternal immune activation C57BL/6N mice	open field, marble burying, social interaction, and adult ultrasonic vocalizations	<i>Bacteroides fragilis</i> , <i>Bacteroides thetaiotaomicron</i>	6–9 weeks	reversed autism spectrum disorder, improved anxiety-like behavior	37
chronic fatigue syndrome patients	Beck Depression and Beck Anxiety Inventories	<i>Lactobacillus casei</i>	8 weeks	decreased anxiety symptoms	38
Wistar rats and humans	conditioned defensive burying test, Hopkins Symptom Checklist, Hospital Anxiety and Depression Scale, Perceived Stress Scale, Coping Checklist	<i>Lactobacillus helveticus</i> R0052, <i>Bifidobacterium longum</i> R0175	14 days/30 days	reduced anxiety-like behavior in rats, alleviated psychological distress in humans	39
humans	brain response to an emotional faces attention task and resting brain activity	fermented milk product with probiotics (FMPP) (containing <i>Bifidobacterium animalis</i> subsp. <i>lactis</i> , <i>Streptococcus thermophilus</i> , <i>Lactobacillus bulgaricus</i> , and <i>Lactococcus lactis</i> subsp. <i>lactis</i>)	4 weeks	reduced task-related response of a distributed functional network containing affective, viscerosensory, and somatosensory cortices	40
humans	stress hormone, cortisol, and emotional processing	galactooligosaccharides	3 weeks	lowered cortisol awakening reactivity and increased attentional vigilance to positive versus negative stimuli	59

bacterium longum were mediated by a BDNF-dependent and cytokine-independent pathway.

However, in Sprague–Dawley rats treated for 14 days with *Bifidobacterium infantis*, Desbonnet et al.⁴² showed that there are significant decreases of cytokines (IFN- γ , TNF- α , and IL-6), 5-HIAA concentration in the frontal cortex, and DOPAC in the amygdaloid cortex, but remarkable increases in plasma concentrations of tryptophan and kynurenic acid, in the *Bifidobacteria*-treated rats relative to controls. Moreover, the same team assessed the potential benefits of *Bifidobacterium infantis* in the rat maternal separation (MS) model of depression.⁴³ The results showed that *Bifidobacteria* treatment reversed behavioral deficits, restored basal noradrenaline (NA) concentrations, and normalized immune response; especially peripheral interleukin (IL)-6 release and amygdala corticotrophin-releasing factor mRNA levels were enhanced. These findings point to immune response-dependent antidepressant properties of *Bifidobacterium infantis*.

Wall et al.⁴⁴ reported that the administration of a *Bifidobacterium* strain to mice had a significant impact on the fatty acid composition of the brain. Significantly higher concentrations of arachidonic acid (ARA) and docosahexaenoic acid (DHA) were observed in mice receiving the bacteria for 8 weeks, compared to control mice. These fatty acids play a critical role in neurotransmission and protection against oxidative stress^{45,46} and are also involved in the improvement of cognitive functions such as memory and learning.^{47,48} These data indicate that probiotics are able to enhance brain function by modulating the metabolism of the host.

Bravo et al.³⁶ indicated that the reduced anxiety/depression effects in *Lactobacillus rhamnosus*-treated mice were accompanied by the reduction of corticosterone, the region-dependent alterations of γ -aminobutyric acid (GABA) and its receptors in the brain. Specifically, GABAB1b was up-regulated in cortical regions and down-regulated in the hippocampus, amygdala, and locus coeruleus; GABA(A α 2) was increased in the hippocampus but reduced in the prefrontal cortex and amygdala, compared to the control mice. However, these neurochemical and behavioral changes caused by *Lactobacillus rhamnosus* were not observed in vagotomized mice. This

highlights that the vagus nerve is involved in the direct communication between the bacteria and the brain, supporting the important role of GABA-mediated signaling pathway in the bidirectional communication of the gut microbiota–brain axis.

Except for GABA secreted by certain strains of *Lactobacillus* and *Bifidobacterium*,⁴⁹ other important bacterial metabolites include norepinephrine produced by *Saccharomyces*, *Bacillus*, and *Escherichia*; serotonin produced by *Enterococcus*, *Streptococcus*, *Escherichia*, and *Candida* and dopamine from *Bacillus* and *Serratia*.⁵⁰ All of these neurotransmitters play a major role in the action of antidepressants.

The administration of *L. helveticus* for 21 days can prevent high fat diet-induced anxiety-like behavior in wild type mice; however, in immunocompromised (IL-10 deficient) mice, high fat diet-induced anxiety-like behavior was not prevented by *L. helveticus* administration, demonstrating the importance of IL-10 signaling in the microbiota–brain communication pathway.⁵¹

In *Citrobacter rodentium*-infected mice, Gareau et al.⁵ demonstrated that treatment with a combination of probiotics of *Lactobacillus rhamnosus* R0011 and *Lactobacillus helveticus* R0052 improved learning and memory behaviors; normalized the population of *Firmicutes* and *Eubacterium rectale* but elevated the levels of *Bacteroides*; restored hippocampal c-Fos and BDNF expression; and ameliorated serum corticosterone and colonic IFN- γ , but not TNF- α , levels. This reflects a BDNF- and IFN-dependent mechanism of action for probiotics to normalize the memory dysfunction occurring in *Citrobacter rodentium*-infected mice exposed to acute stress.

Hsiao et al.³⁷ reported that oral administration of *Bacteroides fragilis* and *Bacteroides thetaiotaomicron* significantly restored the relative abundance of the Lachnospiraceae family as well as unclassified Bacteroidales and normalized ASD-like behavior in MIA offspring. Importantly, on the basis of an altered serum metabolomic profile in MIA offspring, they found that *Bacteroides fragilis* changed back the abundance of 34% of the plasma metabolites, including 4-ethylphenylsulfate (4EPS), indolepyruvate, serotonin, glycolate, imidazole propionate, and *N*-acetylserine. Interestingly, the systemic administration of the single metabolite 4EPS (with a 46-fold increase) to

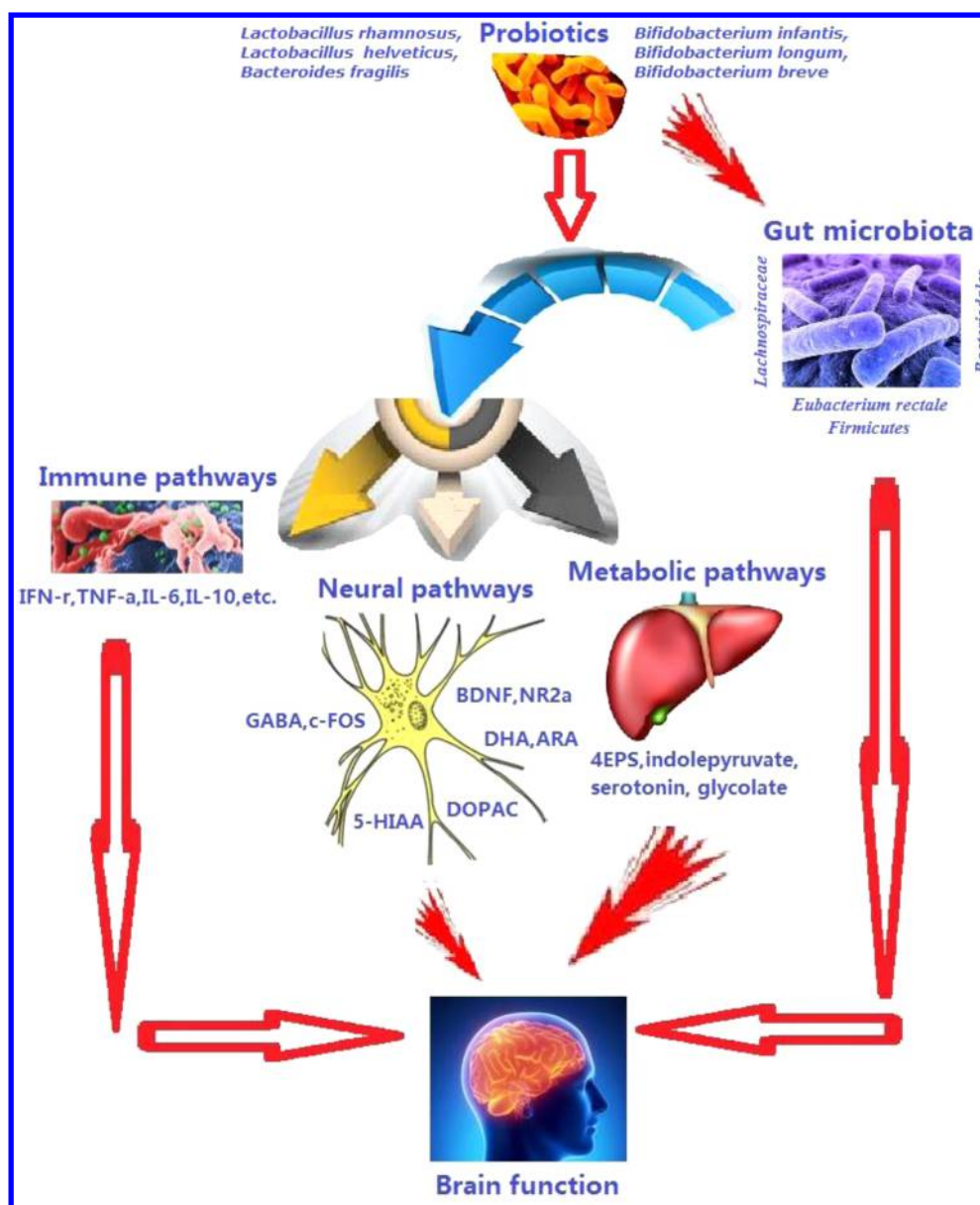


Figure 1. Proposed mechanism for probiotics-based modulation in the gut microbiota–brain axis. Probiotics modulate brain function by altering the composition of gut microbiota or regulating multiple signaling pathways including neural pathways, immune pathways, and metabolic pathways. INF- γ , interferon-gamma; TNF- α , (umor necrosis factor-alpha; IL-6, interleukin-6; IL-10, interleukin-10; BDNF, brain-derived neurotrophic factor; NMDA, N-methyl-D-aspartate; NR2a, receptor subunit 2a; GABA, gamma-aminobutyric acid; 5-HIAA, 5-hydroxyindoleacetic acid; DOPAC, dihydroxyphenylacetic acid; DHA, docosahexaenoic acid; ARA, arachidonic acid; 4EPS, 4-ethylphenylsulfate.

healthy mice is sufficient to induce anxiety-like behavior. It has been shown that commensal microbes are required for the production of serum 4EPS in mice; the precursor 4-ethylphenol is believed to be produced by several species of *Clostridium*;⁵² the putative ASD biomarker *p*-cresol with a structure similar to that of 4EPS also derives from *Clostridium* species.⁵³ In addition, the elevated indolepyruvate in MIA offspring, which is also corrected by *Bacteroides fragilis* treatment, is reported to be produced by bacterial metabolism.⁵⁴ These findings suggest the critical role of the microbially modulated metabolism signaling pathway in modulating neurodevelopmental disorders.

Overall, as discussed above, the mechanisms of action are largely speculative and are not well-defined. On the basis of current knowledge, probiotics treatment alters the composition of gut microbiota, and multiple pathways may be involved in the conversation between gut microbiota and brain (Figure 1):

vagus nerve-mediated pathways,^{5,35,36,41} immune response-mediated pathways,^{5,20–23} and metabolite-mediated pathways.³⁷

Prebiotics-Based Modulation. It has been shown that varieties of polysaccharides can improve brain function in controlled human, animal, and in vitro studies after oral, systemic, and localized administration.⁵⁵ For example, ingestion of isolichenan, an α -glucan from the lichen *Cetrariella islandica*, reversed ethanol-induced memory impairment in mice.⁵⁶ Supplementation with a mixed polysaccharide product (Ambrotose complex) significantly improved cognitive function and mood in healthy middle-aged adults.⁵⁷ The oral administration of arabinoxylan from the yeast *Triticum aestivum* and β -glucan from barley were able to preserve memory in a mouse model of vascular dementia.⁵⁸

A recent study explored the neuroendocrine and affective effects of two prebiotics (fructooligosaccharides, FOS, or

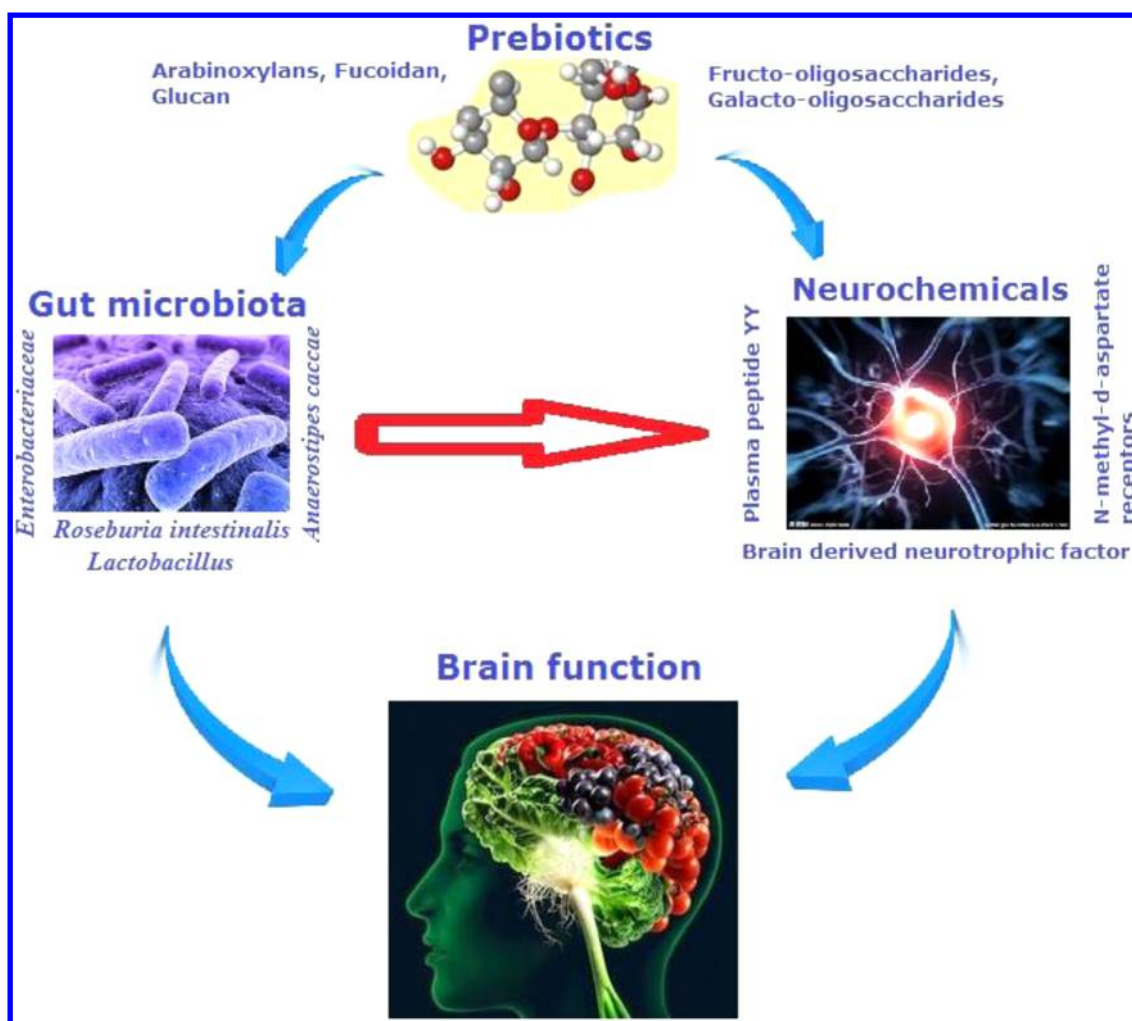


Figure 2. Proposed mechanism for prebiotics-based modulation in the gut microbiota–brain axis. Prebiotics modulate brain function by altering the composition of gut microbiota or influencing the production of neurochemicals.

Bimuno-galactooligosaccharides, B-GOS) in healthy human volunteers (Table 2).⁵⁹ Results revealed that no effects were found after the administration of FOS. In contrast, B-GOS lowered cortisol awakening reactivity and increased attentional vigilance to positive versus negative stimuli on the dot-probe task compared to the placebo group. The cortisol-awakening response is a reliable marker of HPA axis activity, which was increased in individuals at high risk of depression.⁶⁰ This indicates that B-GOS administration may have an antidepressant effect by modulating the HPA axis.

Then, how do prebiotics act on the gut microbiota–brain axis? On the one hand, plant polysaccharides have major influences on gut microbiota. For example, the above-mentioned arabinoxylan was found to stimulate the growth of known butyrate producers (*Roseburia intestinalis*, *Eubacterium rectale*, *Anaerostipes caccae*);⁶¹ fucoidan was reported to reduce the Enterobacteriaceae population in the newly weaned pig;⁶² glucan, which is highly fermentable by the intestinal microbiota in the cecum and colon, can enhance the growth of *Lactobacillus* strains in the human intestine.⁶³ On the other hand, prebiotics directly influence signaling molecules in brain. In a recent study, Savignac et al.⁶⁴ revealed that prebiotics (FOS and galacto-oligosaccharides (GOS)) increased hippocampal BDNF and NR1 subunit expression, but did not alter amino acids associated with glutamate neurotransmission

compared to controls. The intake of GOS also increased hippocampal NR2A subunits, frontal cortex NR1, and plasma peptide YY (PYY). This suggests that the prebiotics-mediated effect of the gut microbiota on brain chemistry is similar to that of probiotics, increasing brain BDNF expression probably through the involvement of gut hormones (Figure 2). However, the detailed mechanisms of prebiotic action, including how alterations in gut bacteria can affect these brain functions and behaviors, are not yet explored in these studies. Additional research is needed to determine whether these prebiotics-induced changes in brain physiology convincingly lead to cognitive and mood outcomes.

Moreover, compared with probiotics-based modulation, prebiotics could be advantageous to some extent, due to the presence of a survival problem in the GI tract for probiotics. In particular, genetic factors could have major impacts. For example, a single host gene, FUT2, is strongly associated with the diversity and composition of the human bifidobacterial population.⁶⁵ Specifically, several genotypes, including *Bifidobacterium bifidum*, *Bifidobacterium adolescentis*, and *Bifidobacterium catenulatum/pseudocatenulatum*, were absent or rarely colonized in the individuals without the FUT2 gene. On the other hand, probiotics are usually supplemented by one or several species at one time, whereas prebiotics supplementation

could stimulate a number of beneficial species simultaneously, which is good for their synergistic actions.

Diet-Based Modulation. Except for probiotics and pre-biotics, diet can affect neural activity in the central nervous system. Hanstock et al.⁶⁶ reported that a fermentable carbohydrates-based diet increased anxiety and aggression behaviors in rats, and these effects were not related to the concentrations of dopamine and 5-HT in the brain, suggesting the mechanism of action could be neurotransmitter-independent. Haghpanah et al.⁶⁷ showed that oral administration of kefir (a fermented product of milk) can significantly improve spatial learning and consolidation of memory in rat. However, these studies did not examine the alterations in the bacterial populations. In fact, diet not only affects behavior but also alters the composition of the GI microbiota. For example, using male CF1 mice fed standard rodent chow (PP diet) or chow containing 50% lean ground beef (BD diet) for 3 months, Li et al.⁶⁸ showed that significantly higher bacterial diversity was observed in the BD group compared with the PP group. Three genera (*Atopobium*, *Bacteroidales*, and *Erysipelothrix*) were exclusively present in the PP group, whereas 12 genera (*Alistipes*, *Allobaculum*, *Chthoniobacter*, *Dorea*, *Eggerthella*, *Gemella*, *Leuconostoc*, *Proteus*, *Sarcina*, *Serratia*, *Staphylococcus*, and *Turicibacter*) were unique to the BP group. Furthermore, BD diet mice also displayed increased learning and memory behaviors as well as decreased levels of anxiety-like behavior compared to PP-fed animals. Notably, the nutritional components for both the PP and BD diets were approximately equal with the exception of taurine and fat, which were present at 18 and 1.5 times the concentrations in BD compared to PP diet, respectively. Although taurine improved learning behavior in aged mice⁶⁹ and high fat fed C57Bl/6ByJ mice increased learning and memory behaviors compared to regular chow fed mates,⁷⁰ it is not clear how BD diet-induced alterations in bacterial diversity lead to altered learning and memory behaviors.

■ FUTURE CHALLENGES

Selection of a Suitable Model or Strain for a Specific Behavior or Mood Is Essential. Except for the intrinsic complex in the gut microbiota–brain communication, one major impact factor is the use of different research methods, such as the selection of a suitable animal model or strain. For example, when using the bacterial infection method to induce anxiety-like behavior in animals, different infections will certainly lead to different anxiety models due to the different central sensing of GI infections. Indeed, acute infection with *Campylobacter jejuni* results in anxiety-like behavior by nonimmune responsive activation of vagal pathways,⁷¹ whereas a recent study³⁶ reported that chronic *Trichuris muris*-infected mice exhibit anxiety-like behavior (present even after vagotomy), which is associated with decreased hippocampal BDNF and increased circulating tumor necrosis factor- α and interferon- γ , as well as the kynurenine, suggesting an inflammation-dependent and vagus nerve-independent pathway. Moreover, it is also possible to generate different anxiety models, even using the same bacterial species. For example, *Citrobacter rodentium* infection, an animal model of inflammatory bowel disease (IBD), induces anxiety-like behavior in mice during the early phase of infection (7–8 h post-infection), which is accompanied by unchanged plasma levels of the cytokines IFN- γ , TNF- α , and IL-12 and elevated c-Fos protein in vagal sensory ganglia.⁷² These findings suggest that the

behavioral effect is likely mediated by vagal sensory neurons, not by inflammation-related stress.⁷² In another study,⁵ *Citrobacter rodentium*-infected mice exhibit stress-induced memory dysfunction but no anxiety-like behavior during the late phase of infection (10 and 30 days post-infection), with decreased serum corticosterone, hippocampus BDNF, and c-Fos expressions; increased colonic IFN- γ and TNF- α levels; and altered gut microbiota (significantly elevated *Firmicutes*, *Enterobacteriaceae*, *Eubacteria rectale*, and *Bacteroides*), suggesting a neural and cytokine-mediated sensing pathway and highlighting the pronounced impacts of measuring behaviors at different time points.

On the other hand, the difference in the commonly used strains of mice (CD-1, MF1, NIH Swiss, C57BL6/J, and BALB/C) should be considered when using them to establish behavioral models. That is, BALB/c mice express more anxiety-like behavior and timidity and higher levels of serotonin and dopamine (may be genetically determined) in the colon than NIH Swiss mice, which are generally more gregarious and less timid.^{13–15} It is imaginable that the results should be variable when investigating the effect of environmental factors on their behaviors, even under the same experimental conditions. Therefore, it is very important to identify the most suitable model or strain for a specific brain disorder model.

Strain–Strain Differences Are Important Factors for Modulating the Gut Microbiota–Brain Axis. Studies have supported a role of probiotics in modulating gut microbiota and improving brain behaviors, but the beneficial effects are divergent and are dependent on the strain. For example, Wall et al.⁴⁴ showed that reduced proportions of *Eubacteriaceae* (3 vs 12%) and increased proportions of *Clostridiaceae* (25 vs 12%) were observed in mice supplemented with *Bifidobacterium breve* DPC 6330 compared to mice supplemented with *Bifidobacterium breve* NCIMB 702258. Interestingly, administration of *Bifidobacterium breve* NCIMB 702258 was found to have a greater effect on the fatty acid composition of the brain; for instance, there were significantly higher concentrations of arachidonic acid (ARA) and docosahexaenoic acid (DHA) in the brain of mice supplemented with *B. breve* NCIMB 702258 than in the brain of mice supplemented with *B. breve* DPC 6330. Studies have indicated that ARA and DHA play important roles in neurogenesis and neurotransmission,^{45,46} and their concentrations in the brain influence cognitive processes such as learning and memory.^{47,48} Therefore, it is important to perform extensive studies on whether or not the strain-dependent differences in the compositions of gut microbiota and brain fatty acids or other neurochemicals result in remarkably functional consequences for the host.

Communication among the Gut, Gut Microbiota, and Brain. Although the existence of multiple signaling pathways including immune, neural, and metabolic pathways in the modulation of the gut microbiota–brain axis is discussed above, it is still challenging to fully understand how the gut, gut microbiota, and brain cooperate to maintain the functioning of the host.

The gut possesses its own nervous system, named the enteric nervous system (ENS). It is well-known that the ENS maintains constant and direct communication with the central nervous system (CNS) via nerves.¹ Therefore, the remaining issue is the communication between ENS or CNS and gut microbiota. Notably, the ENS is separated from gut microbiota by mucosal cell layers, which makes the intestinal commensal microbes inaccessible to ENS. Thus, there are two possibilities to realize

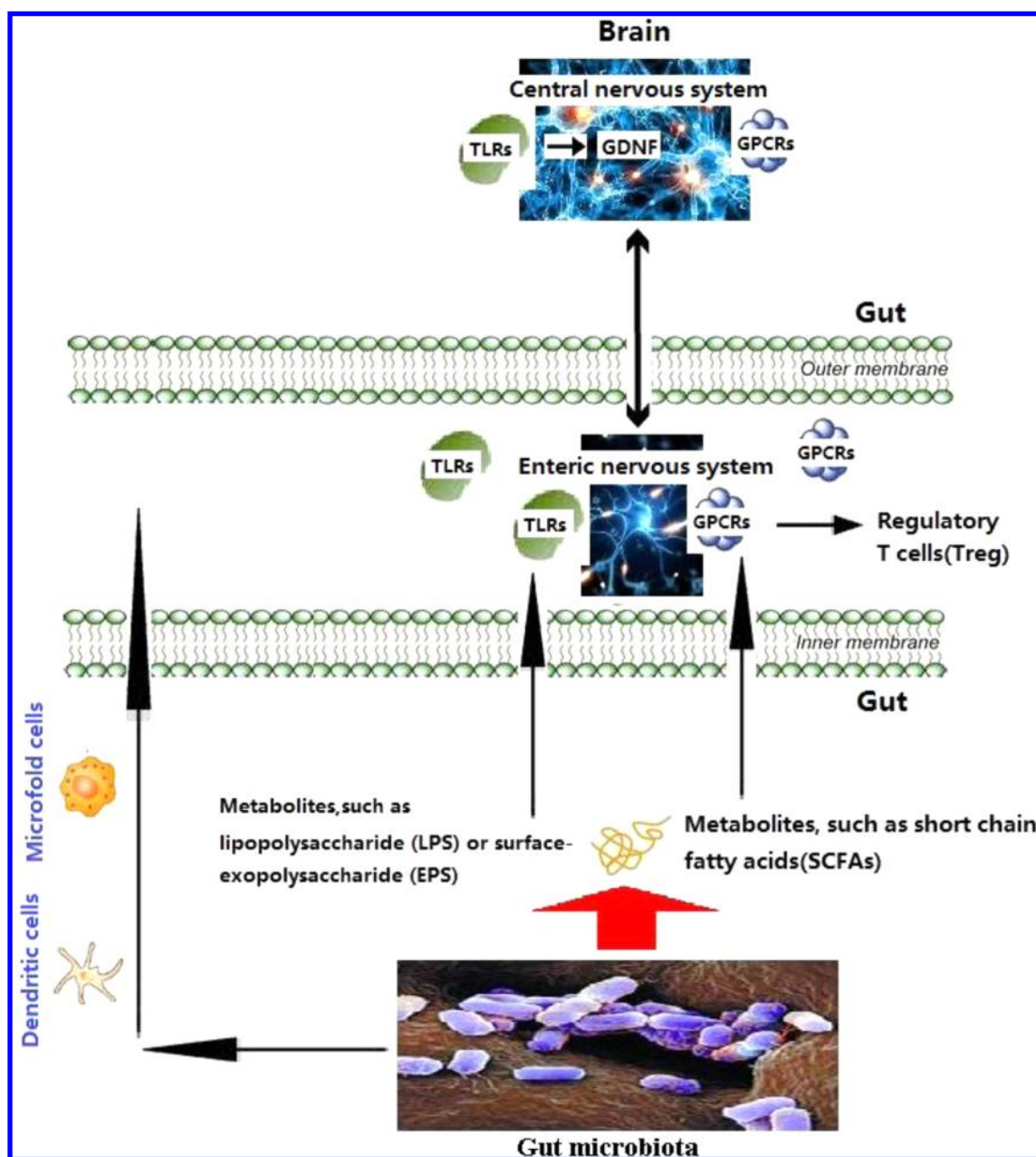


Figure 3. Communication among the gut, gut microbiota, and brain. The enteric nervous system (ENS) directly communicates with the central nervous system (CNS). The resident microbes translocate to the lamina propria from the intestinal lumen by the microfold cells and/or by dendritic cells. The microbiota-generated components, such as bacterial lipopolysaccharide (LPS), surface exopolysaccharide (EPS), and short-chain fatty acids (SCFAs), cross or bypass the epithelium to act directly on the ENS. The resident microbes or their metabolites are able to interact with some specific receptors such as Toll-like receptors (TLRs) and G-protein coupled receptors (GPCRs). The gut microbiota regulates the ENS by TLR signaling, stimulates the expression of a glial cell line-derived neurotrophic factor (GDNF), and increases the number of enteric neurons and glial cells. SCFAs regulate colonic regulatory T cell (cTreg) homeostasis by acting on GPCRs.

communication between gut microbiota and ENS: (1) the resident microbes translocate to the lamina propria from the intestinal lumen by microfold cells and/or dendritic cells, probably by direct entrance in disease state due to “leaky” intestinal wall; (2) the microbiota-generated components access the lamina propria. Indeed, bacterial lipopolysaccharide (LPS) or polysaccharide A (PSA) or surface-exopolysaccharide (EPS), short-chain fatty acids, glutamate, and so on, are able to cross or bypass the epithelium to act directly on the ENS.⁷³ After the resident microbes (probably secreted products or metabolites) or their products/metabolites translocate to the lamina propria, they are able to interact with some specific

receptors, such as pattern recognition receptors including Toll-like receptors (TLRs) and G-protein coupled receptors (GPCRs).

TLRs are universally distributed in macrophages, endothelial cells, glia, and neurons.⁷⁴ In the immune system, TLRs recognize the invading microorganisms, and in the nervous system, TLRs recognize endogenous ligands released by undifferentiated or necrotic/injured cells; their activities are associated with brain disorders.⁷⁵ After interaction, the ENS and immune system are activated to release cytokines and neurohumoral mediators.⁷⁶ In fact, Brun et al.⁷⁷ revealed that gut microbiota regulate ENS integrity by TLR-2 signaling,

stimulate the expression of a glial cell line-derived neurotrophic factor (GDNF), and increase the number of enteric neurons and glial cells, hence facilitating the development and survival of many types of neurons. Depletion of gut microbiota significantly reduced the expression of GDNF in mice; TLR-2-deficient mice increased susceptibility to inflammation, whereas the severity of inflammation was reduced by administration of GDNF. This highlights the importance of the gut microbiota–TLR-2–GDNF axis in modulating the ENS and inflammation.

GPCRs are also universally expressed in many sites, including in the CNS, especially in the striatum,⁷⁸ and these receptors play a critical role in the regulation of metabolism, inflammation, neurological/psychiatric disorders, and other diseases.^{79,80} Short-chain fatty acids (SCFAs) are produced by the gut microbiota during the fermentation of partially digestible or nondigestible polysaccharides. Except for inhibition of histone deacetylases (HDACs), another major SCFA signaling mechanism is activation of GPCRs. Especially, GPR43, GPR41, and GPR109A have been identified as receptors of SCFAs.⁸¹ Smith et al.⁸² reported that acetic acid, propionic acid, and butyric acid, the three most abundant microbial-derived SCFA, display an exciting role in regulating colonic regulatory T cell (cTreg) homeostasis and protecting against colitis by acting on GPR43. This is supported by Atarashi et al.,⁸³ who found that a rationally selected mixture of *Clostridia* strains from the human microbiota enhances Treg induction. In particular, De Vadder et al.⁸⁴ showed that SCFAs such as propionate from fermentation of gut microbiota on prebiotic GOS promote metabolic benefits on body weight and glucose control via a gut–brain neural circuit involving the fatty acid receptor GPR41. This highlights the importance of the gut microbiota–GPCRs–brain axis in regulating host functions.

Thus, although the exact mechanism describing the communication among gut, gut microbiota, and brain still warrants further study, it can be speculated that various axes of gut microbiota–receptors–brain could be responsible for the realization of many functions between the gut and brain (Figure 3), such as the gut microbiota–TLRs–brain axis for probiotics modulation and the gut microbiota–GPCRs–brain axis for prebiotics modulation.

Novel Technologies in the Gut Microbiota–Brain Axis. To extensively explore the detailed mechanism underlying the gut microbiota–brain axis, novel techniques are highly needed. One technology is functional magnetic resonance imaging (fMRI), which has shown the potential for studies of gut–brain signaling in humans over the past few years. For example, using fMRI, Van Oudenhove et al.⁸⁵ found that there was a significant fat–emotion interaction effect on the blood oxygen level-dependent signal (BOLD signal) in multiple regions of interest, including medulla/pons, midbrain, hypothalamus, thalamus, caudate head, putamen, cerebellum, and right hippocampus, suggesting that fatty acid infusion attenuated the behavioral and neural responses to sad emotion induction in humans. By physiological/pharmacological MRI (phMRI), Jones et al.⁸⁶ revealed that intragastric dodecanoate caused strong bilateral activation of a matrix of CNS areas, including the brain stem, hypothalamus, and limbic areas, and ghrelin bolus decreased the BOLD signal in feeding-activated areas of the CNS. Very recently, Tillisch et al.⁴⁰ demonstrated that the reactivity of a widely distributed network of brain regions to an emotional attention task was reduced in women who had ingested FMPP, including primary interoceptive and

somatosensory regions, prefrontal cortex, precuneus, basal ganglia, and the parahippocampal gyrus. This provides important clues for further exploring the mechanism of action.

Another technology is omics analysis (i.e., transcriptomics, proteomics, metabolomics, and systems biology). For example, transcriptomics, which is the study of the complete set of RNA transcripts produced in cells or tissues, was applied to explore in vivo mucosal responses of healthy adults to probiotics.^{87,88} The results revealed that gene-regulatory networks and pathways were differentially induced in the human mucosa and were dependent on the preparations of living and heat-killed *Lactobacillus plantarum* bacteria and the specific species (*L. acidophilus*, *L. casei*, and *L. rhamnosus*). Transcriptomic comparisons of two breast milk-derived *Lactobacillus* (*L. reuteri* ATCC 55730 and ATCC PTA 6475) identified candidate genes involved in the survival and persistence in the gut, and genes responsible for immunomodulation and health-promoting factors such as antimicrobial agents and vitamins especially predicted a complete pathway for thiamin biosynthesis in strain 55730 for the first time.⁸⁹

Proteomics is the large-scale study of the entire set of proteins produced or modified by an organism or system, which is largely an underexploited approach at the interface of gut microbiota and brain physiology.⁹⁰ In a *Trichuris muris*-infected mice model, Bercik et al.³⁶ showed that anxiety-like behavior was induced and CNS biochemistry was changed, and several proteins related to inflammation and neural function were identified by proteomic analysis. Using comparative proteomics, Kim et al.⁹¹ investigated the response of *Bifidobacterium longum* subsp. *infantis* to different prebiotics. ATP-dependent sugar transport systems related to the consumption of different prebiotics was determined, providing molecular foundations for different prebiotics consumption in *B. infantis* and evidence for differential impacts on brain health.

Metabolomics is simultaneously analyzes hundreds to thousands of small-molecule metabolites in biological samples by an array of analytical techniques including mass spectrometry and high-resolution nuclear magnetic resonance spectroscopy. Real-time analysis of the small-molecule metabolites derived from the gut, microbes, and brain is increasingly useful for deciphering inherent and intimate gut microbiota–brain relationships.⁹² Using capillary electrophoresis with time-of-flight mass spectrometry (CE-TOFMS), Matsumoto et al.⁹³ identified 196 metabolites from the cerebral metabolome in both GF and Ex-GF mice (inoculated with suspension of feces obtained from specific pathogen-free mice); among them, 38 metabolites differed significantly, and 10 of these metabolites are known to be involved in brain function. A novel relationship between gut microbiota and cerebral glycolytic metabolism was found. These findings suggest that the intestinal microbiota is closely connected with brain health and disease through the gut microbiota–brain axis. Indeed, an excellent study conducted by Hsiao et al.³⁷ supported this observation. In this study, MIA-associated changes in serum metabolites were profiled using gas chromatography–liquid chromatography with mass spectrometry (GC-LC/MS)-based metabolomics technology, demonstrating that 8% of all serum metabolites were significantly altered by MIA induction and 34% of all metabolites were significantly affected by *B. fragilis* treatment. Among them, 4-ethylphenylsulfate (4EPS) displayed a 46-fold increase in serum levels of MIA offspring and is completely restored by *B. fragilis* treatment. Strikingly, systemic administration of the single metabolite, 4EPS, to healthy mice is sufficient to induce

anxiety-like behavior. This highlights an important role of a single metabolite regulated by gut microbes in the molecular connections between the gut and the brain. It is believable that more metabolic signatures in the gut microbiota–brain axis can be identified when this method is applied to other neurodevelopmental disorders.

In summary, abnormalities of brain function are associated with the altered composition of the gut microbiota. The use of probiotics, prebiotics, and functional foods can partly or completely reverse the dysbiosis in the microbiota caused by some brain disorders. Multiple pathways are involved in the modulation of the gut microbiota–brain axis: vagus nerve-mediated pathways, immune response-mediated pathways, and metabolite-mediated pathways. The availability of novel techniques such as functional magnetic resonance imaging, metatranscriptomes, metaproteomics, and metabolomics will rapidly expand our knowledge on the complex mechanisms of action underlying the interactions between microbiota components and the host. A comprehensive understanding of the gut microbiota–brain axis may provide novel approaches for the prevention and treatment of brain dysfunctions.

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Notes

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