

Relationship between the gut microbiome and brain function

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It has become increasingly evident in recent years that the gut microbiome and the brain communicate in a bidirectional manner, with each possibly affecting the other's functions. Substantial research has aimed to understand the mechanisms of this interaction and to outline strategies for preventing or treating nervous system-related disturbances. This review explores the evidence demonstrating how the gut microbiome may affect brain function in adults, thereby having an impact on stress, anxiety, depression, and cognition. In vitro, in vivo, and human studies reporting an association between a change in the gut microbiome and functional changes in the brain are highlighted, as are studies outlining the mechanisms by which the brain affects the microbiome and the gastrointestinal tract. Possible modes of action to explain how the gut microbiome and the brain functionally affect each other are proposed. Supplemental probiotics to combat brain-related dysfunction offer a promising approach, provided future research elucidates their mode of action and possible side effects. Further studies are warranted to establish how pre- and probiotic interventions may help to balance brain function in healthy and diseased individuals.

INTRODUCTION

According to a statement by the World Health Organization, probiotics, when consumed in appropriate amounts, are beneficial to human health and well-being.¹ The benefits of probiotics include, but are not limited to, improved skin health, enhanced resistance to allergens, immune system support, reduction of pathogenic microorganisms, and protection of macromolecules (DNA, proteins, lipids) from oxidative damage.²⁻⁵

Human health can be both positively and negatively affected by the microorganisms living in the gut, known collectively as the gut microbiota,⁶ which consists of bacteria, bacteriophages, viruses, fungi, protozoa, and archaea.⁷⁻⁹ There is increasing evidence that the intestinal microbiota resembles a remarkably densely populated and

diverse microbial community that plays a critical role in both the maintenance of human health and the pathogenesis of disease. The gastrointestinal (GI) tract is home to various microorganisms, whose collective genome is termed the gut microbiome.¹⁰ Advances in DNA sequencing technology combined with novel bioinformatics tools have enabled scientists to describe the gut microbiome with unprecedented precision. It is estimated that the number of bacteria inhabiting the healthy human GI tract reaches up to 50 different phyla, 1000 different bacterial species, and 10^{14} viable bacteria per gram of luminal content.^{11,12} The density of the human microbiome is highest in the colon, where Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria^{11,13} are the most abundant organisms, constituting approximately 64%, 23%, 8%, and 3% of the population, respectively.⁷⁻⁹

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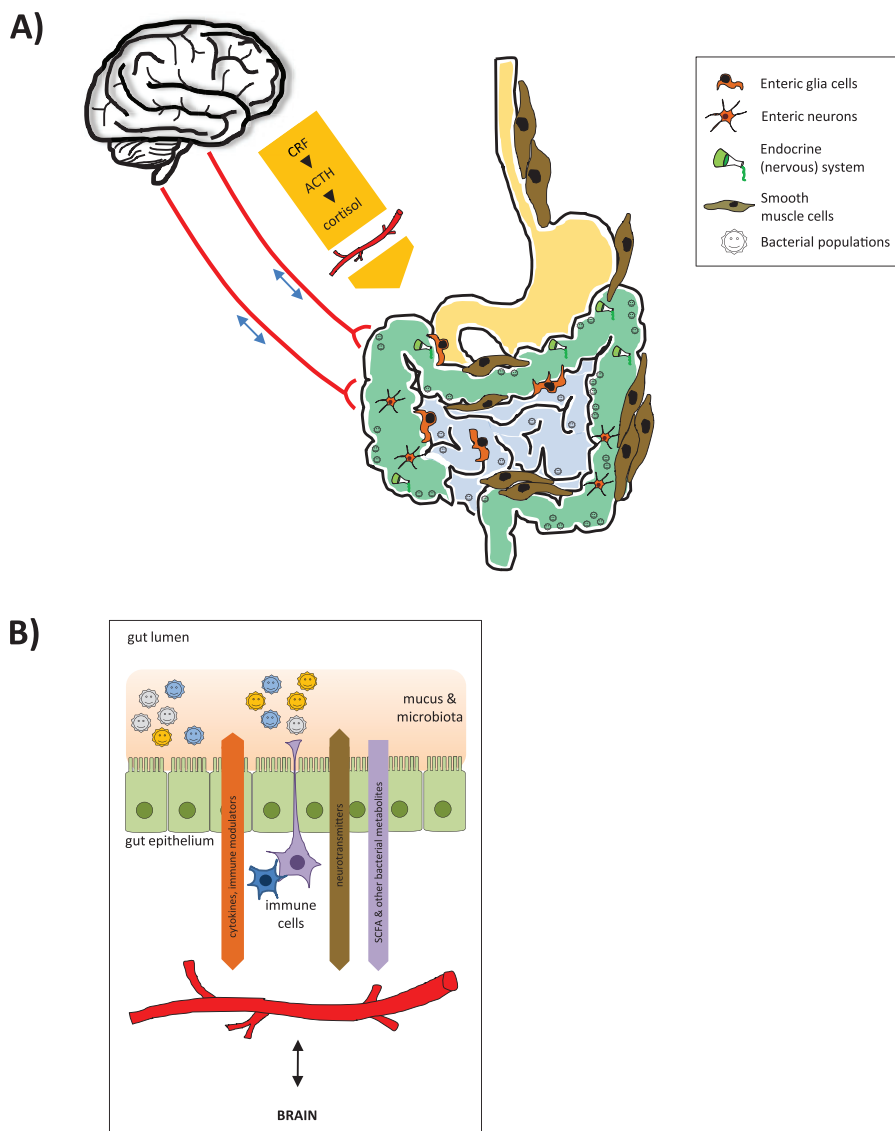


Figure 1 Schematic presentation of the gut–brain axis. (A) The upper part of the gastrointestinal (GI) tract, shown in yellow, includes the esophagus and the stomach. The small intestine (duodenum, jejunum, ileum) is shown in light blue; the large intestine (cecum and the ascending, transverse, and descending colon) is shown in green. The interactions between the GI tract and the autonomic and central nervous system are indicated by red lines. The short bidirectional blue arrows indicate afferences and efferences. The hypothalamic–pituitary–adrenal (HPA) axis is shown in dark yellow. **(B)** Simplified representation of the crosstalk between the microbiota, the brain, and the immune system. The gut microbiota and the immune system affect each other by releasing immunomodulators and/or cytokines, with potential systemic effects on the host. Short-chain fatty acids and other microbial metabolites are produced by the GI microbiota and may influence brain function, whereas several neurotransmitters are involved in the bidirectional communication between the host and the microbiota (see text for details). *Abbreviations:* ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing hormone; SCFA, short-chain fatty acids.

Diet is an important factor to influence the gut microbiome. For example, short-term consumption of diets composed entirely of animal or plant products rapidly changes structures of the microbial community, overwhelming interindividual differences in microbial gene expression.¹⁴

Scientific evidence accumulated in recent years suggests that the gut microbiota affects some aspects of brain function and behavior, including emotional behavior and related brain systems.¹⁵ Figure 1 schematically outlines routes of communication between the gut and the brain. This review will examine the mechanisms

of action of this communication and explore the implications for human health and daily living.

THE GUT MICROBIOME: ALTERATIONS THROUGHOUT LIFE

In healthy individuals, the gut microbiome is highly variable because the taxonomic variability within the GI tract depends on many factors, including genetic, physiological, psychological, and environmental determinants.^{16–18} Despite the notion that each person's microbiota is unique, it is thought that humans might share a core microbiome and have a similar colonization of the GI tract by microbiota throughout life.¹⁹ It has been recently shown that bacteria can be found in amniotic fluid, placenta, and the meconium of newborns,²⁰ which may help to explain the similarity of the microbiome in infants after a period of adaptation. Notably, developing embryos are exposed to bacteria in utero.²⁰ While infants born vaginally receive a seed of their microbiota during passage through the birth canal via exposure to maternal vaginal and perhaps fecal microbes, infants born by cesarean delivery receive their first major exposure to bacteria from their mother's skin and the hospital environment.²¹ *Bifidobacterium*, *Lactobacillus*, Enterobacteriaceae, and *Staphylococcus* are the most populous organisms in the GI of the healthy, vaginally delivered infant GI tract, followed by *Veillonella* and Lachnospiraceae.²² The composition of the infant's gut microbiota is unstable until approximately 2 years of age, ie, until the child begins to eat solid food.²¹ Breastfed infants have a different microbiome than formula-fed infants, and by age of 3 years, the microbiota of most infants stabilizes and develops toward what becomes the adult microbial composition.²⁰

In healthy adults, the gut microbiota is dominated by only a few phyla, as noted above,^{7–9} and is characterized by a wide diversity of bacterial species.¹⁷ The human microbiome changes with age, normally becoming less diverse in the elderly as a result of higher numbers of *Bacteroides* species and reduced numbers of *Clostridium* groups.²³ Even if the microbiome of adults is relatively stable when compared with that of infants or elderly, several factors can dramatically influence its composition over a relatively short period of time.²⁴ Such factors include antibiotic treatment, stress, infection, host genetics, and diet.¹⁹

THE MICROBIOTA–GUT–BRAIN AXIS

The commensal bacteria benefit from a nutritionally rich and protected habitat in the human GI tract, while they in turn benefit the host by making indigestible

nutrients available to the body. In addition to producing energy, vitamins, and other metabolites, some beneficial bacteria also help restrict the access of pathogenic microorganisms to the gut tissue by building a protective biofilm.²⁵

It is now known that the benefits of human–microbe symbiosis can be extended to human mental health, and in recent years evidence has shown that the gut–brain axis, or the bidirectional communication between the resident microbes of the GI tract and the brain,¹⁵ plays a key role in maintaining brain health. The GI microbiota influences human behavior and may affect the pathophysiology of mental illnesses.²⁶ The knowledge gained in recent years about the function and importance of the microbiome has broadened the concept of the gut–brain axis to the “microbiota–gut–brain axis,” emphasizing the importance of the microbiome in the regulation of gut–brain communication.^{27–29}

Several systems are at work to ensure the efficient functioning of the microbiota–gut–brain axis, including the central, autonomic, and enteric nervous systems, the immune system, and the endocrine system.^{16,26,30,31} The central nervous system (CNS), the enteric nervous system (ENS), the sympathetic and parasympathetic branches of the autonomic nervous system, and neuroendocrine and neuroimmune pathways are all involved in communication with the gut microbes.¹⁶ The neuronal interaction between the GI tract and the brain is facilitated by efferent and afferent nerves.³² As a consequence, the CNS regulates the secretory and sensory functions as well as the mobility of the GI tract.³³

The microbiota has the potential to affect neuronal function directly or indirectly through vitamins, neurotransmitters, and neuroactive microbial metabolites such as short-chain fatty acids.^{16,33} How these metabolites affect brain function is difficult to ascertain, as the presence of the blood–brain barrier and various feedback mechanisms impede a direct access to the brain. Experimental data suggest that the microbiota may send signals to the brain by activating afferent sensory neurons of the vagus nerve via neuroimmune and neuroendocrine pathways.¹⁹

The study of germ-free animals shows that brain development is abnormal when the gut microbiome is missing.^{21,34} The gut microbiome influences the inflammatory reactions within the brain by modulating the activation of microglial cells³⁵ and affecting myelination³⁶ and neurogenesis in adult brains.³⁷ Fecal transplantation between mouse strains with different levels of anxiety has demonstrated that the microbiota can even change behavioral characteristics of mammals by altering brain chemistry.³⁸

Irritable bowel syndrome (IBS) and inflammatory bowel disease in humans are 2 conditions that exemplify the consequences of a faulty gut–brain communication.^{39,40} The involvement of the gut microbiota in the pathophysiology of IBS has been shown repeatedly, as symptoms of IBS develop after the disruption of the microbiome due to acute gastroenteritis (ie, postinfectious IBS)^{41,42} or following the use of antibiotics.⁴³ In addition, gastrointestinal dysfunction such as bowel diseases are frequently accompanied by comorbid psychiatric conditions.^{44,45}

The ENS, which is part of the automatic nervous system, innervates the wall of the GI tract, covering the entire length from the esophagus to the anus. In addition, the gut also receives input from the vagus nerve and from central spinal and sacral afferent terminals.^{32,46} An important feature of the ENS is that it can operate independently of the spinal cord and brain despite being connected to the CNS.^{47,48} Apart from the ENS, the vagus nerve is instrumental for the flow of information from the gut to the brain.^{32,49} Vagotomy experiments underline the importance of the vagus nerve for microbiota–gut–brain communication,^{50–52} even though this connection does not seem to be necessary for all microbes.^{27,50} There is great interest in clarifying how probiotic species modulate neuronal pathways, thereby affecting neuronal function and behavior. The neuronal population affected varies, depending on the bacteria used and the experimental paradigm employed. Recent data provide evidence that related bacterial species can interact specifically with a variety of different neuronal populations. For example, *Lactobacillus helveticus* R0052 affects the functioning of CNS neurons in the hippocampus and amygdala,⁵³ whereas *Lactococcus lactis* subsp *cremoris* H61 modulates the activity of auditory brain stem neurons⁵⁴ and *Lactobacillus reuteri* (DSM 17938) is implicated in the function of visceral nociceptive neurons of the gut.⁵⁵ This diverse specificity of microorganisms to interact with specific neural circuitries suggests great potential to design dedicated interventions targeted to affect specific neuronal functions. The ability of the ENS system to adapt to altering microbial populations in the GI tract has been known for over 30 years.⁵⁶ Indeed, the ENS responds to changing bacterial populations by adapting the neuronal physiology and by changing gene expression. The intracellular recordings of afterhyperpolarization neurons and of sensory neurons residing in the gut wall are different in germ-free mice than in normal mice. Afterhyperpolarization neurons are less excitable in germ-free mice, an abnormality that is normalized after conventionalization with gut microbiota.^{55,57,58} In addition, expression of the calcium-binding protein calbindin in the enteric neurons in the

gut of conventionalized germ-free mice was similar to that in controls, whereas expression in germ-free animals was significantly less than that in either the conventionalized mice or the controls.^{59,60} Calbindin expression is linked to nutritional status because it depends on vitamin D concentrations in the nerve and intestinal cells.^{61,62} These findings may indicate that the ENS is plastic, ie, it can sense and react to changes in GI tract microbes. Since the sensory neurons in the ENS are connected to the brain via the vagus nerve, there may be an avenue of communication whereby information about the bacterial contents of the gut can be conveyed to the brain.

The hypothalamic–pituitary–adrenal (HPA) axis, which regulates the body’s response to stress, represents another route of gut–brain crosstalk. It is a complex set of involuntary influences and feedback interactions between 3 endocrine glands: the hypothalamus, the pituitary gland, and the adrenal glands. The HPA axis is directly and indirectly controlled by neural activity throughout the forebrain and brainstem.⁶³ It not only controls the body’s reaction to stress but is also implicated in controlling digestion, the immune system, mood and emotional status, sexuality, and energy storage and expenditure. Dysregulation of HPA activity is associated with mental health disorders such as depression and schizophrenia, both of which are known to affect the microbiota composition.^{63–65} Stress response by HPA activity involves the secretion of corticotrophin-releasing factor by neurons in the medial parvocellular portion of the hypothalamic paraventricular nucleus, causing the endocrine cells (corticotrophs) in the anterior pituitary to secrete adrenocorticotrophic hormone. Adrenocorticotrophic hormone, in turn, stimulates the endocrine cells, primarily in the zona fasciculata of the adrenal cortex, to secrete the glucocorticoid hormones cortisol and/or corticosterone (reviewed by Spencer and Deak⁶⁶). Cortisol is released in response to stress, and low blood-glucose concentration affects the response to stress in addition to other metabolic and immune-related functions.⁶⁶

Finally, the role of the immune system in microbiota–gut–brain communication seems to be species-specific. Germ-free mice lacking all gut bacteria exhibit specific abnormalities in immune, neuronal, GI tract, and metabolic function,⁶⁷ and infection of mice with a pathogen, *Citrobacter rodentium*, induced anxiety-like behavior.⁵² Moreover, the abnormal gut and neuronal function in B- and T-cell-deficient *Rag1* knockout mice was partially normalized by probiotic treatment, providing evidence of a role for the adaptive immune system in maintaining intestinal and brain health.⁶⁸

A number of recent studies provide evidence of the interplay between the microbiome and brain function, which may affect mammalian behavior (Table 1).^{27,34,51,52,68–84} Germ-free mice exhibit learning deficits⁸⁰ and show anxiolytic-like behavior^{85–88} and reduced sociability.^{88,89} In addition, they also demonstrate an exaggerated HPA stress response.⁶⁹ Importantly, the enhanced HPA response of germ-free mice could be partially corrected by reconstitution with pathogen-free feces of normal animals at an early age, but not at a later age, demonstrating that exposure to microbes at an early developmental stage is required for the HPA system to become fully susceptible to inhibitory neural regulation. These results suggest that commensal microbiota can affect the postnatal development of the HPA stress response in mice.⁶⁹

Recent work in germ-free mice demonstrated hypermyelinated areas in the prefrontal cortex and defective microglial cells with reduced capacity for activation after bacterial or viral challenge.³⁶ This suggests that germ-free mice have a compromised ability to mount appropriate immune responses in the CNS.³⁵ The same authors showed that limited diversity in the microbiota composition, achieved by antibiotic treatment, resulted in defective microglia and that recolonization with a complex microbiota partially restored microglial features.³⁵ In addition, mice deficient for the short-chain fatty acids receptor FFAR2 had the same microglial defects found in germ-free mice.

Taken together, these findings suggest that host bacteria are crucial for regulating microglial maturation and function and that microglial impairment can be ameliorated to some extent by the microbiota.³⁵ Moreover, the consequences of antibiotic treatment resemble the findings in germ-free animals, such as deficits in social and cognitive behaviors, increased anxiety, and reduced microglial activation and expression of brain-derived neurotrophic factor.^{27,34,35}

The role of the microbiome in influencing the crosstalk between periphery and the brain was studied further in a murine model of experimentally induced sickness behavior: mice exhibited elevated levels of inflammatory cytokines such as tumor-necrosis factor- α (TNF- α) and interleukin 6.⁷² Sickness behaviors are debilitating symptoms in patients with systemic inflammatory diseases such as irritable bowel disease, rheumatoid arthritis, or chronic liver disease. In a rodent model, an oral gavage of a mixture of 8 bacterial species (VSL#3) was shown to dampen sickness behavior by a mechanism involving reduced activation of microglial cells and reduced infiltration of monocytes into the brain.⁷² The authors convincingly showed that the amelioration of behavioral symptoms was related to changes in systemic immune activation, such as lowered

TNF- α levels. These data are in agreement with an older report showing that VSL#3 treatment reduced circulating TNF- α levels, which were associated with improved neuropsychiatric outcomes in patients with chronic liver disease.⁹⁰ Thus, TNF- α causes sickness behaviors in the murine model by cerebral microglial activation and the recruitment of monocytes into the brain vasculature and brain parenchyma.⁷² The mechanisms of organ inflammation in the peripheral organs, leading to alteration of brain functions, are of great importance for the design of clinically acceptable therapeutic agents to prevent peripheral inflammation.

The influence of gut microbiota on neuroinflammation and motor deficits was demonstrated recently in an animal model of Parkinson's disease.⁸⁴ Sampson et al.⁸⁴ demonstrated that the gut microbiome plays a role in nervous and intestinal dysfunctions specific to Parkinson's disease in a mouse model. Briefly, it was shown that the presence of the normal gut microbiome is required for Parkinson's disease-related motor and brain pathology and that the production of short-chain fatty acids promoted microglial activation and enhanced Parkinson symptoms. When the microbiome was depleted in these mice, reduced activation of microglia and a reduced level of pathology were observed, providing the direct evidence of the contribution of the gut microbiome to Parkinson's disease pathophysiology in this model.⁸⁴ In addition, mice that received fecal transplantation from patients with Parkinson's disease, but not mice that received fecal samples from healthy controls, exhibited significant impairment of motor functions, again providing strong evidence of the involvement of the gut microbiome in the pathophysiology of Parkinson's disease. Taken together, these data add to the understanding of how probiotics may influence brain function by modifying immune system signaling to the brain.

MODULATION OF MAMMALIAN BEHAVIOR BY GUT MICROBIOTA

Depression and anxiety

Major depressive disorder, specifically recurrent unipolar depression (normally referred to as depression), is a common, serious, stress-related, debilitating, and, if untreated, life-threatening psychiatric disorder, affecting over 100 million individuals worldwide.⁹¹ The HPA axis is dysregulated in depressive patients, which leads to abnormally high circulating levels of corticotropin-releasing factor and cortisol. Often, elevated concentrations of proinflammatory cytokines are also found in the plasma of patients with depression. In recent years, the prospect of using compounds that modulate the gut microbiome, such as probiotics, for treating psychiatric

Table 1 Animal studies providing evidence of the modulatory role of the microbiota in brain-related pathological conditions

Reference(s)	Treatment	Animal model	Pathological condition	Outcome	Proposed mechanism of action
Sudo et al. (2004) ⁶⁹ , Desbonnet et al. (2010) ⁷⁰	<i>Bifidobacterium infantis</i>	Germ-free mice	Anxiety	Antidepressant-like activity	Modulation of HPA response
Bravo et al. (2011) ⁵¹	<i>Lactobacillus rhamnosus</i> JB-1	BALB/c mice	Anxiety	Antidepressant-like activity	Modulation of neurotransmission, expression of GABA _A and GABA _B receptors
Bravo et al. (2011) ⁵¹ , Stilling et al. (2015) ⁷¹	<i>Lactobacillus rhamnosus</i>	Germ-free mice	Anxiety	Reduced stress-induced corticosterone levels and anxiety- and depression-related behavior	Higher expression of GABA _A and GABA _B receptors; higher expression of serotonin receptor 1A; involvement of vagus nerve
D'Mello et al. (2015) ⁷²	Model of liver inflammation and probiotic mixture VSL#3	C57BL/6 mice	Sickness behavior	Reduced symptoms	Monocyte recruitment to the brain in response to systemic TNF- α signaling, leading to microglial activation
Messaoudi et al. (2011) ⁷³	<i>Lactobacillus helveticus</i> R0052 and <i>Bifidobacterium longum</i> R0175	Rats	Anxiety	Reduced anxiety-like behavior	Crosstalk between the microbiome and enteric nervous system as well as CNS
Desbonnet et al. (2008) ⁷⁴	<i>Bifidobacterium infantis</i>	Rats	Anxiety	No effect in naive rats but significantly attenuated IFN- γ , TNF- α , and IL-6 cytokines following mitogen stimulation	Increased plasma concentrations of tryptophan and kynurenic acid; reduced 5-HIAA concentration in the frontal cortex in DOPAC in the amygdaloid cortex
Gareau et al. (2007) ⁷⁵	<i>Lactobacillus rhamnosus</i> R0011 and <i>Lactobacillus helveticus</i> R0052 (5%)	Rat pups	Stress due to maternal separation leading to dysbiosis	Change in gut flora composition observed	Normalization of HPA axis activity
Desbonnet et al. (2015) ³⁴ , Frohlich et al. (2016) ⁷⁶	Antibiotic treatment	Mice	Depletion of the gut microbiota	Altered gut microbiota, decreased spleen weights in adulthood. Reduced anxiety, induced cognitive deficits, altered dynamics of the tryptophan metabolic pathway	Change in microbial metabolites and in expression of BDNF, NMDA receptor subunit 2B, serotonin transporter, neuro-peptide Y, and vasopressin
Li et al. (2009) ⁷⁷	Ground beef diet	CF1 mice	None	Increased bacterial diversity in the beef-supplemented diet; improved working and reference memory	TBD

(continued)

Table 1 Continued

Reference(s)	Treatment	Animal model	Pathological condition	Outcome	Proposed mechanism of action
Ohland et al. (2013) ⁷⁸	Western-style diet and <i>Lactobacillus helveticus</i> R0052	WT and IL-10-deficient 129/SvEv mice	Western diet increased weight gain, changed gut microbiota and cytokine expression, and altered anxiety-like behavior	Probiotics alone decreased anxiety-like behavior in WT mice on a chow diet	Inflammatory pathways
Davari et al. (2013) ⁷⁹	<i>Lactobacillus acidophilus</i> , <i>Bifidobacterium lactis</i> , <i>Lactobacillus fermentum</i>	Diabetic rats	Memory impairment related to diabetes mellitus	Improved the impaired spatial memory in diabetic animals	Stimulation of Schaffer collaterals in hippocampus, restoration of long-term potentiation, activation of superoxide dismutase, and increased serum insulin level
Gareau et al. (2011) ⁸⁰	<i>Citrobacter rodentium</i> in addition to water avoidance stress	C57BL/6 mice and germ-free Swiss-Webster mice	None	Memory impairment observed in C57BL/6 after stress Memory impairment observed in germ-free mice	Modulation of HPA axis and hippocampal plasticity
Lyte et al. (1998) ⁸¹	<i>Campylobacter jejuni</i>	CF-1 male mice	Anxiety	Decreased exploratory behaviors and increased nonexploratory behaviors	Activation of immune–neural mechanisms
Lyte et al. (2006) ⁵²	<i>Citrobacter rodentium</i>	CF-1 male mice (model of IBD)	IBD	Increased anxiety-like behavior	Mediated via vagal sensory neurons
Smith et al. (2014) ⁶⁸	<i>Lactobacillus rhamnosus</i> R0011 and <i>Lactobacillus helveticus</i> R0052	B- and T-cell-deficient Rag1 ^{-/-} mice	Memory deficit, anxiety, dysbiosis	Improved baseline impairments	Modulation of intestinal microbiota, HPA axis, and cFos expression
Bercik et al. (2011) ²⁷	Microbiota of SPF NIH Swiss and BALB/c mice	Germ-free mice	Baseline behavior of germ-free mice	Altered the composition of the microbiota and increased exploratory behavior	Changes in brain chemistry and in hippocampal expression of BDNF
Gacias et al. (2016) ⁸²	Microbiota of nonobese diabetic mice	C57BL/6 mice	Social avoidance	Improvement in social avoidance observed	Changes in gene expression and in myelination in frontal cortex
Zheng et al. (2016) ⁸³	Microbiota of humans with depression	Germ-free mice	Depression	Mice developed depressive symptoms	Neurotransmission, others
Sampson et al. (2016) ⁸⁴	Microbiota of patients with PD	α -Synuclein over-expressing germ-free mice	PD pathology	PD-like motor deficits observed in transplanted germ-free mice	Changes in SCFAs modulated neuroinflammation and microglia activation

Abbreviations: BDNF, brain-derived neurotrophic factor; DOPAC, 3,4-dihydroxyphenylacetic acid; HPA, hypothalamic–pituitary–adrenal; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; NIH, National Institutes of Health; NMDA, *N*-methyl-D-aspartate; PD, Parkinson’s disease; Rag1, recombination activating gene 1; SCFAs, short-chain fatty acids; SPF, specific pathogen-free; TBD, to be determined; TNF, tumor necrosis factor; WT, wild type; 5-HIAA, 5-hydroxyindoleacetic acid.

disorders has gained great interest among neuroscientists, even though the mechanisms of action of the microbiota on mood in humans remain elusive.

Several lines of evidence in preclinical models that include bacterial infections, probiotic treatment, fecal transplantation, and analysis of germ-free animals suggest that the gut microbiota can influence brain function and, consequently, alter behavior.¹⁶ Anxiety and depression are among the brain-related behavioral changes that are modified by changes in the gut microbiome.^{34,51,70,73,87,92–95} Sudo et al.⁶⁹ were among the first groups to study the effect of the microbiome on the HPA axis. Their seminal study showed that stressed germ-free mice have an overly responsive HPA axis. The overreaction of the HPA response was reduced by supplementing mice with a single bacterial strain, *Bifidobacterium infantis*.⁶⁹ In 2011, Bravo et al.⁵¹ showed that chronic treatment of BALB/c mice with *Lactobacillus rhamnosus* JB-1 moderated anxiety and antidepressant-related behavior, probably by inducing neurochemical changes. The lower anxiety level of *L. rhamnosus*-treated animals was concomitant with alterations in the expression of γ -aminobutyric acid (GABA) receptors, both GABA_A and GABA_B receptors, across a variety of brain regions. Importantly, the neurochemical and behavioral effects were not found in vagotomized mice, thus identifying the vagus as a major modulatory pathway between the gut and the brain.⁵¹ This study showed that *L. rhamnosus* had antidepressant/anxiolytic activity and demonstrated that, in this animal model, dietary intake of a bacterial strain may alter brain function and behavior. Moreover, the authors identified the vagus nerve as the route of communication between the gut microbiome and the brain. Bercik et al.^{27,50} showed that fecal transplantation may result in the transfer of behavioral traits from the donor mouse to the recipient mouse. A recent study confirmed the above findings by showing that the gut microbiome determines behavioral changes in another model, ie, the nonobese diabetic mouse.⁸² The transfer of intestinal microbiota from nonobese diabetic mice to C57BL/6 mice was sufficient to induce social avoidance and changes in gene expression and myelination in the prefrontal cortex in the C57BL/6 mice, a phenotype of the nonobese diabetic mouse. In conclusion, these animal data provide evidence that microbes of the GI tract are implicated in the pathophysiology of depression and anxiety and that some strains confer a certain degree of resilience against these conditions.

Several studies in humans (Table 2)^{73,96–104} support the data from animal studies and show that the gut microbiota may play a role in modulating depression and anxiety.^{73,97,105} Some researchers have reported that the composition of the microbiome was different

in patients with major depressive disorder than in their healthy counterparts,^{83,106} but others failed to confirm this.¹⁰⁷ Mechanistically fascinating is the result of 1 study (Table 1) in which germ-free mice were inoculated with fecal samples of depressive patients. The transplanted mice developed depressive-like behaviors.⁸³ This strongly hints for the involvement of the gut microbiome in regulating depressive symptoms in humans. In a randomized, double-blind, placebo-controlled trial, petrochemical workers who consumed a probiotic yogurt or a multispecies probiotic capsule for 6 weeks showed improved mental health as measured by a general health questionnaire and a depression anxiety and stress scale.⁹⁶ These data are in line with those from an older study in which supplementation with probiotic yogurt improved the mood status of healthy elderly individuals, especially those with decreased mood scores at baseline.⁹⁷ Lastly, probiotic treatment (containing *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum*) for 8 weeks in patients with major depressive disorder was reported to improve clinical signs of depression as assessed by the Beck Depression Inventory in a recent randomized, double-blind, placebo-controlled trial performed in central Iran (Table 2).¹⁰³

Analysis of fecal samples reveals that the microbiome of depressive patients differs from that of healthy controls.^{51,106} Indeed, changes in the microbiome of depressive patients can be linked to the severity of depression. These reports revealed a negative correlation between *Faecalibacterium* organisms and the severity of depressive symptoms and an altered composition of the gut microbiota in acutely depressed patients.¹⁰⁶

Patients with depression show changes in counts of both gram-positive and gram-negative bacteria.^{106,107} Increases are reported for *Roseburia*, *Phascolarctobacterium*, *Megamonas*, *Clostridium*, *Lachnospiraceae incertae sedis*, *Blautia*, *Oscillibacter*, *Parasutterella*, *Parabacteroides*, and *Alistipes*, whereas *Ruminococcus*, *Dialister*, *Prevotella*, *Faecalibacterium*, and *Bacteroides* are reduced in people with depression.¹⁰⁸ The genus of *Bifidobacterium* has been studied in detail in relation to depression. *B. infantis* was found to normalize the exaggerated HPA axis response and ameliorate depressive symptoms in animal models.^{70,109} Another gram-positive bacterium, *Lactobacillus farciminius*, is also able to reverse stress-induced elevation of HPA axis activity and neuroinflammation in vivo.¹¹⁰ *Lactobacillus rhamnosus* was shown to alter emotional behavior and central GABA receptor expression in vivo via the vagus nerve, thereby decreasing both anxiety and depression-like symptoms in mice.⁵¹ Messaoudi et al.⁷³ studied probiotic treatment with a combination of *L. helveticus* and *Bifidobacterium longum* in rats and

Table 2 Nonexhaustive list of human studies supplementing probiotics to normal and diseased human populations

Supplementation	Study population	Behaviors tested	Outcome	Reference(s)
Probiotic yogurt or a multi-species probiotic capsule (<i>Lactobacillus acidophilus</i> LA5 and <i>Bifidobacterium lactis</i> BB12)	Normal population	Depression, anxiety, stress	Improvement in participants supplemented with probiotic yogurt or probiotic capsule	Mohammadi et al. (2016) ⁹⁶
Probiotic yogurt (<i>Lactobacillus casei</i> Shirota)	Normal elderly individuals with decreased mood	Mood status	Improvement in participants in bottom third of the depressed/elated dimension at baseline	Benton et al. (2007) ⁹⁷
<i>Lactobacillus helveticus</i> & <i>Bifidobacterium longum</i> <i>Bifidobacterium longum</i> 1714	Healthy individuals	Anxiety, stress	Alleviation of psychological distress	Messaoudi et al. (2011) ⁷³
	Healthy individuals	Stress response, cognition, brain activity	Change in electroencephalographic activity, dampened stress response, and enhanced cognitive performance	Allen et al. (2016) ⁹⁸
Fermented milk product with probiotic containing <i>Bifidobacterium animalis</i> subsp <i>lactis</i> , <i>Streptococcus thermophilus</i> , <i>Lactobacillus bulgaricus</i> , <i>Lactobacillus lactis</i> subsp <i>lactis</i>	Healthy women	Emotion, attention	Altered activity of brain regions that control central processing of emotion and sensation by functional MRI	Tillisch et al. (2013) ⁹⁹
<i>Lactobacillus helveticus</i> IDCC3801	Healthy elderly individuals	Cognition	Improvement in cognitive functioning during cognitive fatigue tests	Chung et al. (2014) ¹⁰⁰
<i>Bifidobacterium infantis</i> 35624	Patients with IBS	IBS symptoms	Improvement in symptoms	Brenner et al. (2009) ¹⁰¹
Probiotic milk containing <i>Lactobacillus acidophilus</i> , <i>Lactobacillus casei</i> , <i>Bifidobacterium bifidum</i> , and <i>Lactobacillus fermentum</i>	Patients with Alzheimer's disease	Cognitive functions, antioxidative status	Significant improvement in MMSE score (in plasma malondialdehyde, serum hs-CRP, and serum TG	Akbari et al. (2016) ¹⁰²
<i>Lactobacillus acidophilus</i> , <i>Lactobacillus casei</i> , <i>Bifidobacterium bifidum</i>	Patients with major depressive disorder	Depression	Improvement in clinical signs	Akkasheh et al. (2016) ¹⁰³
FOS or Bimuno GOS	Healthy individuals	Stress	Significantly lower cortisol awakening response (assessed in saliva) after Bimuno GOS intake	Schmidt et al. (2015) ¹⁰⁴

Abbreviations: FOS, fructooligosaccharides; GOS, galactooligosaccharide; hs-CRP, high-sensitivity C-reactive protein; IBS, irritable bowel syndrome; MMSE, Mini-Mental State Examination; MRI, magnetic resonance imaging; TG, triglycerides.

humans. The treatment was effective in decreasing stress levels, anxiety, and depressive scores in both the animal experiment and the clinical trial, providing the evidence that, in this case, animal models are a reliable model for conditions in humans. Resident gut bacteria can also have negative effects on the host. For example, *Campylobacter jejuni* was shown to induce anxiety-like behavior without inducing immune activation in mice.⁸¹ As noted, in vivo models provide evidence that a heightened HPA axis response and depressive-like symptoms can be reversed by the administration of probiotic bacteria such as *B infantis*.⁷⁰ Moreover, probiotics may elevate blood tryptophan concentrations, modulate serotonin levels in the frontal cortex, and

modulate cortical dopamine metabolites, thereby ameliorating depressive symptoms.⁷⁴ In addition, rat studies showed that the consumption of *L rhamnosus* is associated with improved depressive scores.⁵¹ Together, these studies suggest that probiotics may improve mood status in humans and that (unhealthy) nutrition may be a risk factor for depression. Therefore, a healthy diet could have a preventive effect against depression.¹¹¹

Stress

The gut microbiome and the stress response are interrelated in mammals. Sudo et al.⁶⁹ showed that germ-free

mice under stress conditions exhibited a strong HPA response when compared with control animals. Their study showed that fecal transfer from specific pathogen-free mice was able to partially normalize the exaggerated stress response in germ-free animals. Most interestingly, the abnormal stress response was age-dependently reversed when animals were treated with the probiotic *B infantis*.⁶⁹ Supporting data were provided by a study in which probiotic treatment of rat pups normalized corticosterone release and ameliorated colonic dysfunction induced by stress due to maternal separation.⁷⁵ These data are in line with a report in healthy volunteers following prebiotic supplementation. Prebiotic supplementation with fructooligosaccharides or a commercially available powder containing galactooligosaccharides (Bimuno, DSM Nutritional Products, Basel, Switzerland) for 3 weeks revealed that the cortisol awakening response, as assessed by salivary samples, was significantly lower after intake of Bimuno powder than after placebo intake. In addition, participants showed decreased attentional vigilance to negative vs positive information in a dot-probe task after Bimuno powder intake than after placebo intake. No effects were found after the administration of fructooligosaccharides, indicating the specificity of the observed effects.¹⁰⁴ Thus, the above-mentioned studies indicate that the neuroendocrine function of the brain can be affected by the gut microbiome. As outlined in the Introduction, the interaction between the gut and the brain is bidirectional in both rodents and humans. Evidence for the effect of the brain on the gut microbiome can be found in studies documenting that parental stress,^{112,113} early-life stress,^{114,115} and psychological stress^{116–119} change the composition of the gut microbiota.

To further validate that preclinical results could be translated to healthy humans, Allen et al.⁹⁸ tested whether the consumption of *B longum* strain 1714 affects brain-related functions. They showed that this probiotic modulated electroencephalographic activity, dampened the stress response, and enhanced cognitive performance in healthy volunteers.⁹⁸ This study confirmed older data showing that supplementing healthy women with a fermented milk product containing *Bifidobacterium animalis* subsp *lactis*, *Streptococcus thermophilus*, *Lactobacillus bulgaricus*, and *L lactis* subsp *lactis* altered activity of brain regions that control central processing of emotion and sensation in a functional magnetic resonance imaging study.⁹⁹

Cognition

The chance of providing cognitive support to humans may be greatest during gestation, infancy, and older

age,¹²⁰ as these are periods of life with the highest vulnerability and the greatest demand for nutrients. To date, the majority of mechanistic evidence for the involvement of the gut microbiota in cognition is provided by animal experiments of induced infections,^{80,121} antibiotic and dietary manipulations,^{34,76,77,78} and probiotic interventions.^{78,79}

Animal studies suggest that the microbiome may influence neurodevelopment.^{87,88,71} Short-chain fatty acids, the major metabolites produced by the microbiome, are implicated in the functionality of the blood–brain barrier and thus have a direct role in determining the accessibility of circulating factors to the brain.¹²² Short-chain fatty acids may control gene transcription in the brain via epigenetic mechanisms. Among these, butyrate is shown to be brain active and capable of facilitating long-term potentiation and the formation of long-term memory in rats via an extracellular signal-regulated kinase (ERK)-dependent signaling mechanism.¹²³ These early reports were confirmed by subsequent studies showing that sodium butyrate facilitates neuronal plasticity and memory formation¹²⁴ via a pathway that mimics the beneficial effects of environmental enrichment.¹²⁵ These studies have also pointed to butyrate as the most important short-chain fatty acid involved in epigenetic modulation of brain function. The positive effect of butyrate on cognition after systemic and local injections prompted scientists to test it in models of neurodegenerative diseases to counteract cognitive impairment. In animal models of Alzheimer's disease, butyrate showed positive effects on pathology and memory performance.^{125,126} In models of other neurodegenerative disorders, including Parkinson's disease,¹²⁷ amyotrophic lateral sclerosis,¹²⁸ Huntington's disease,¹²⁹ and ataxia,¹³⁰ butyrate exhibited neuroprotective effects and helped restore, at least partially, neuronal function.

At the cellular level, butyrate's effects are mediated by various receptors, including G protein–coupled receptors, free fatty acid receptors, and transporters,⁷¹ and by the utilization of butyrate as an energy source via the β -oxidation pathway.⁷¹ Butyrate inhibits histone deacetylase, thereby promoting histone acetylation and the epigenetic regulation of gene expression in human cells. Therefore, some have proposed it be tested experimentally to treat cognitive impairment and neurological disorders ranging from depression to neurodegenerative diseases in humans (reviewed by Stilling et al.⁷¹).

Probiotics were also employed in human studies examining cognitive performance of both healthy and diseased study participants. Probiotic treatment (*L helveticus* IDCC3801) of healthy elderly individuals was shown to improve scores on cognitive fatigue tests.¹⁰⁰ Another study suggests that consumption of a

fermented probiotic milk product modulates brain activity during an emotional attention test in healthy women.⁹⁹ In addition, prebiotic intake reduced the waking cortisol response and altered emotional bias in healthy volunteers, resulting in improved performance of healthy individuals in an emotional attention task.¹⁰⁴

Promising results were reported recently by Akbari et al.,¹⁰² who showed that supplementing Alzheimer's disease patients with a probiotic milk containing *L acidophilus*, *L casei*, *B bifidum*, and *Lactobacillus fermentum* (each organism: 2×10^9 CFU/g of milk) for 12 weeks positively affected cognitive function. If these results can be replicated by independent research groups, they would be groundbreaking because they would indicate the potential usefulness of probiotics as a viable and affordable strategy for improving cognitive capacity in both healthy individuals and patients with Alzheimer's disease.

Mechanistic evidence of microbial influence on neuronal signaling

The gut and the brain communicate with each other via central and systemic routes. The major route of central communication between the gut and the brain is the vagus nerve.^{16,131} The incoming information from the gut via the vagus nerve to the brain is processed in the nucleus tractus solitarius, which has large projections that include the parabrachial nucleus, which further projects to the prefrontal cortex as well as the amygdala, a region susceptible to microbial transcriptional regulation.^{71,132} Moreover, recent findings indicate that gut microbes induce excitability of the intrinsic primary afferent neurons in the intestine after hyperpolarization. This elevated excitability was not observed in germ-free animals, suggesting that colonization restores normal neuronal excitability.⁵⁷ Furthermore, neuroactive metabolites of microbiota, such as short-chain fatty acids, constitute a route of information flow between the gut and the brain.¹³³ Despite the information provided by the above-mentioned research, the role of microorganisms in the regulation of neuronal activity is far from being fully understood. The mechanisms of involvement of the gut microbiota in brain function and disorders, including anxiety and depression, may be related to the ability of the microbiota to synthesize soluble factors (eg, neuromodulators) and modulate their absorption and function.¹³⁴ A study by Neufeld et al.⁸⁶ showed that the expression of an *N*-methyl-D-aspartate (NMDA) receptor subunit (NMDArec2B) is reduced in the amygdala (a region implicated in the emotional processing of external cues) of germ-free animals. Savignac et al.⁹³ confirmed the involvement of the microbiome in gene expression by showing that feeding

prebiotics elevates levels of brain-derived neurotrophic factor, NMDA receptor subunits, and D-serine in the rat brain. They also showed that prebiotic supplementation normalizes lipopolysaccharide-induced anxiety and cortical levels of serotonin 2A receptor and interleukin 1 β in male mice. Moreover, supplementing germ-free animals with *L rhamnosus* led to higher expression of both the GABA receptor in the amygdala and the serotonin 1A receptor in the hippocampal formation.^{51,71} These data suggest the possibility of treating neuropsychiatric disorders by manipulating the microbiome with specific prebiotics and probiotics.

Gaseous metabolites of bacteria, including carbon monoxide, hydrogen sulfide, nitric oxide, and others, are also implicated in the neuronal control of gut functions by muscarinic cholinergic, vasoactive intestinal peptide.^{135,136} The interaction with the nerve cells is indirect and involves enteric glia, a collection of glial cells residing within the walls of the intestinal tract^{137,138} and within epithelial and smooth muscle cells,⁴⁶ interstitial cells of Cajal,¹³⁵ and immune cells.¹³⁹ Enteric glia are crucial for the above-mentioned interactions, as they also communicate with various types of non-neuronal cells in the gut wall, such as enterocytes, enteroendocrine cells, and immune cells and are therefore important local regulators of diverse gut functions. Several studies have emphasized the importance of enteric glia as modulators of ENS function, owing to the responsiveness of enteric glia to microbial, luminal, and inflammatory signals.^{46,138,140–143} Thus, enteric glial cells regulate intestinal barrier function, immune responses, intestinal secretion, and gut motility and are hypothesized to be moderators of neurotransmission and neuroplasticity in the intestine.¹⁴⁴

Bile acids are also able to modulate neuronal activity, thus affecting both the host and microbiota, for example, by activating G protein-coupled bile acid receptors on intrinsic primary afferent neurons.¹⁴⁵ This effect, however, depends on the specific bile acid, as some promote bacterial growth, whereas others inhibit it.^{146,147}

Quorum sensing, used by bacteria to coordinate gene expression according to the density of their local population, represents a communication route within the gut by which bacteria may react to external (ie, host) factors. The centrally produced neurotransmitter noradrenalin, a major catecholamine neurotransmitter in the sympathetic nervous system, is known to serve as a potent quorum sensing signal in bacteria such as *Escherichia coli*.^{148–152} Hence, the host nervous system may regulate bacterial growth, biofilm formation, and virulence mechanisms, including toxin production in the intestine, via noradrenalin-dependent neurotransmission. Catecholamines, on the other hand, have been

linked to the virulence of 2 pathogenic bacteria, namely enterohemorrhagic *E coli* and *C jejuni* (reviewed by Savidge¹⁵³). These varying effects of the same neurotransmitter on different microbes demonstrate that much about the optimal utilization of microbes for specific health benefits is still unknown. Nevertheless, it is evident from the above data that microbes in the GI tract interact with the ENS, thereby influencing both host and microbial functions.

There is considerable evidence that the microbiome plays a role in modulating mood disorders, stress, and anxiety, all conditions that are influenced by serotonergic neurotransmission.^{33,154} Bravo et al.⁵¹ demonstrated that *L rhamnosus* regulates emotional behavior in mice by modulating central GABA receptor expression via a mechanism that involves the vagus nerve. They also showed that mice are less anxious and exhibit less depressive-like behavior following *L rhamnosus* supplementation. Undoubtedly, nutrition has a major influence on the composition of the gut microbiome. For example, it is known that a Western diet changes the gut microbiome and induces anxiolytic effects in mice.¹⁵⁵ The synthesis of serotonin as well as the availability of its precursor tryptophan is highly regulated during the life span. Metabolism of tryptophan, however, is altered after consumption of a Western diet.¹⁵⁵ Tryptophan is the precursor of serotonin, which therefore links diet, the microbiome, neurotransmission, and effects on behavioral change to each other.¹⁵⁶ This is supported by the findings of Desbonnet et al.,³⁴ who depleted the gut microbiome in mice by antibiotic treatment, resulting in a dramatic reduction in tryptophan levels in blood as well as a reduction in BDNF levels in the hippocampus. It is worth noting that several bacterial strains, such as *L lactis* subsp *cremoris*, *L lactis* subsp *lactis*, *Lactobacillus plantarum*, and *S thermophilus*, have been shown to produce monogenic amines, including serotonin.¹⁵⁷ Moreover, levodopa, serotonin, dopamine, and noradrenaline were detected during the late growth phase of *E coli* K-12 cultures,¹⁵⁸ and *L plantarum* has been reported to produce acetylcholine.⁹⁴ Lastly, several strains of *Lactobacillus brevis*, *Bifidobacterium adolescentis*, *Bifidobacterium dentium*, and *B infantis* have been reported to be GABA producers.¹⁵⁹ In addition, Romano et al.¹⁶⁰ reported that the intestinal microbiota composition modulates the bioavailability of choline. Most efficient in this regard were strains of *Anaerococcus hydrogenalis*, *Clostridium asparagiforme*, *Clostridium hathewayi*, *Clostridium sporogenes*, *Edwardsiella tarda*, and *Escherichia fergusonii* isolated from human samples.¹⁶⁰ It is therefore possible that microbial-derived neurotransmitters can alter the activity of the ENS and, perhaps, the CNS. Lastly, changes in the microbiome composition have a

profound effect on the function and responsiveness of the HPA axis.⁶⁹ This effect is age dependent, as the abnormal HPA response could be partially normalized after recolonization at an early stage, but not at a late stage, clearly showing that the microbiome modulates the HPA response to stress, an effect that is most prominent during the postnatal period.⁶⁹

DISCUSSION

Elucidating the mechanisms by which microbes affect brain function constitutes an exciting field of research. In vivo data have been instrumental in showing that the excitability of enteric and vagal afferent neurons may be modulated by the microbiota¹³¹ and that the brain modulates intestinal motility, intestinal secretion, and immune function.¹⁶¹ Research in preclinical models suggests that the effect of the microbiome on behavior may be related to changes in the amygdala and hippocampus.^{67,162} A significant difference in the volume and dendritic morphology of the amygdala and hippocampus was observed between conventionally colonized mice and germ-free mice, including shorter neurites, a smaller degree of branching, and thinner spines in germ-free mice,¹⁶² suggesting that the microbiota is required for the normal morphology and ultrastructure of brain neurons. The authors argue that dysbiosis and the consequent neural remodeling may contribute to the maladaptive stress responsivity and behavioral profile observed in germ-free mice.¹⁶² On the other hand, the nervous system controls the intestinal physiology. The involvement of neural circuits, neurotransmitters, and receptors in the sympathetic regulation of intestinal function is well established. Dysregulated neurotransmission, altered HPA response, and damage of enteric neurons result in an abnormal microbiome. For example, stress conditions can cause abdominal pain and constipation.^{148,152,163} In addition, psychological stress has been shown to shift the microbial colonization on the mucosal surface and alter the susceptibility of the host to infection.¹⁵² Moreover, the ENS and the immune system both play important roles in the development of irritable bowel disease. Both the ENS and the CNS can modulate intestinal inflammation through secretion of neuropeptides or other soluble molecules.¹⁶⁴ In addition, the innervation of the GI tract by the sympathetic nervous system controls the motility, fluid exchange, and blood flow in the gut of healthy individuals.¹⁶⁵ Lastly, human studies show that the HPA axis is dysregulated in depression; however, this can be reversed after the resolution of depression.²⁶ The stress response is immature at birth. Its maturation is governed by genetic factors of the host, as different mouse strains have been shown to exhibit different

stress and behavior responses to environmental stimuli.²⁶

CONCLUSION

Many of the state-of-the art therapies for brain disorders aim to restore dysregulated neurotransmission in affected brain areas. As noted in this review, data are increasingly showing that bacteria can produce important neurotransmitters such as GABA, acetylcholine, and serotonin. Research aiming to understand the communication between the intestinal microbiota and the brain peaked in recent years but revealed multiple mechanisms by which the human host responds to commensal and pathogenic bacteria.¹⁶ Communication between the brain and the microbiota involves epithelial receptor-mediated signaling, immune modulation, and stimulation of enteric neurons by bacterial metabolites. Important for this crosstalk is the ability of the microbiota to regulate the availability of circulating tryptophan, which affects serotonin synthesis, and to alter the expression of some CNS receptors, thereby enabling them to directly influence brain excitability and function as well as to exert epigenetic control of gene expression.

Industry has undertaken enormous effort to tackle diseases of old age, such as Parkinson's disease and Alzheimer's disease, as well as diseases that affect younger persons, such as attention-deficit/hyperactivity disorder and autism, but results have been disappointing at times. Future research will show whether microbes can be used to produce therapeutic neurotransmitters for treating psychiatric disorders. For therapy to be successful, any potential adverse effects must be studied, such as those caused by the presence of receptors or epigenetic processes in tissues other than the brain. In conclusion, regulation or modification of the GI microbiome through diet may provide critical benefits for preventing and treating brain-related disorders, which has prompted several experts to propose specific developments of the microbiota for use as potential psychotropic therapies.¹⁰⁹ Since there are seemingly endless possibilities to combine pre- and probiotics with other nutritional compounds, future mechanistic studies are needed to determine the true potential of such psychotropic therapies to produce the envisaged benefits in the targeted populations.

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