The Microbiome-Gut-Brain Axis in Health and Disease

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KEYWORDS

• Microbiota • Psychobiotics • Short-chain fatty acids • Vagus nerve • GABA • Serotonin

KEY POINTS

- Gut microbes can communicate with the brain through a variety of routes, including the vagus nerve, short-chain fatty acids (SCFAs), cytokines, and tryptophan.
- Psychobiotics are bacteria that when ingested in adequate amounts produce a positive mental health benefit.
- The brain-gut-microbiota axis represents a paradigm shift in neuroscience and provides a novel target for treating not only irritable bowel syndrome (IBS) but also conditions, such as depression, autism, and Parkinson disease.

INTRODUCTION

The human adult gut contains more than 1 kg of bacteria, essentially the same weight as the human brain.[1] It is generally estimated that the gut is inhabited by 10^{13} to 10^{14} microorganisms, which is significantly more than the number of human cells in the body, and contains more than 100 times as many genes as in the genome.[2] Amazingly, the genomic and biochemical complexity of the microbiota exceeds that of the brain. Studies of the brain-gut-microbiota axis have been described as a paradigm shift in neuroscience.[3] Increasing evidence points to appropriate diversity in the gut microbiota that is essential not only for gut health but also for normal physiologic functioning in other organs, especially the brain. An altered gut microbiota in the form of dysbiosis at the extremes of life, both in the neonate and in the elderly, can have a profound impact on brain function. Such a dysbiosis might emerge for a variety of reasons, including the mode of birth delivery, diet, and antibiotic and other drug exposure. Given that the brain is dependent on gut microbes for essential metabolic products,

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it is not surprising that a dysbiosis can have serious negative consequences for brain function both from neurologic and mental health perspectives. Although much of the early data emerged from animal studies, mainly rodent based, there are now an increasing number of human studies translating the animal findings.

This review focuses on the routes of communication between the gut and brain, examines a prototypic disorder of the brain gut axis, explores the ways in which gut dysbiosis may evolve, and provides an up-to-date account of behavioral and neurologic pathologies associated with dysbiosis.

BRAIN-GUT-MICROBIOTA COMMUNICATION

The brain-gut-microbiota axis is a bidirectional communication system enabling gut microbes to communicate with the brain and the brain with the gut.[4] Although brain-gut communication has been a subject of investigation for decades, an exploration of gut microbes within this context has only featured in recent years. The mechanisms of signal transmission are complex and not fully elucidated but include neural, endocrine, immune, and metabolic pathways.[5,6] Preclinical studies have implicated the vagus nerve as a key route of neural communication between microbes of the gut and centrally mediated behavioral effects, as demonstrated by the elimination of central *Lactobacillus rhamnosus* effects after vagotomy[7] and that humans who have underwent vagotomy at an early age have a decreased risk of certain neurologic disorders.[8] The gut microbiota also regulates key central neurotransmitters, such as serotonin, by altering levels of precursors; for example, *Bifidobacterium infantis* has been shown to elevate plasma tryptophan levels and thus influence central serotonin (5HT) transmission.[9] Intriguingly, synthesis and release of neurotransmitters from bacteria has been reported: *Lactobacillus* and *Bifidobacterium* spp can produce γ-aminobutyric acid (GABA); *Escherichia*, *Bacillus*, and *Saccharomyces* spp can produce noradrenaline; *Candida, Streptococcus, Escherichia*, and *Enterococcus* spp can produce serotonin; *Bacillus* can produce dopamine; and *Lactobacillus* can produce acetylcholine.[10,11] These microbially synthesized neurotransmitters can cross the mucosal layer of the intestines, although it is highly unlikely that they directly influence brain function. Even if they enter the blood stream, which is by no means certain, they are incapable of crossing the blood-brain barrier (BBB). Their impact on brain function is likely to be indirect, acting on the enteric nervous system. SCFAs, which include butyrate, propionate, and acetate, are essential metabolic products of gut microbial activity and may exert central effects either through G-protein–coupled receptors, although such receptors are sparsely concentrated in the brain. It is more likely that they act as epigenetic modulators through histone deacetylases.[2] SCFAs are also involved in energy balance and metabolism and can modulate adipose tissue, liver tissue, and skeletal muscle and function.[12] Immune signaling from gut to brain mediated by cytokine molecules is another documented route of communication.[13] Cytokines produced at the level of the gut can travel via the bloodstream to the brain. Under normal physiologic circumstances, it is unlikely that they cross the BBB, but increasing evidence indicates a capacity to signal across the BBB and to influence brain areas, such as the hypothalamus, where the BBB is deficient. It is through the latter mechanism the cytokines interleukin (IL)-1 and IL-6 activate the hypothalamic-pituitary-adrenal (HPA) axis, bringing about the release of cortisol. This is the most potent activator of the stress system.

The HPA axis, which provides the core regulation of the stress response, can have a significant impact on the brain-gut-microbiota axis.[14–20] It is increasingly clear and probably of relevance in several pathologic conditions that psychological or physical

stress can significantly dysregulate the HPA axis and subsequently the brain-gut-microbiota axis, for example, in IBS[21] (**Fig. 1**).

Multiple lines of approach have been used to interrogate the brain-gut-microbiota axis, especially in animal model systems; these include the use of germ-free animals, potential probiotic agents, antibiotics, animals exposed to pathogens and the use of stress to determine the effects of dysregulating the axis. The largest naturalistic study of a gut pathogen and the impact on the brain-gut axis was as a result of the Walkerton catastrophe. The contamination of the Walkerton (Walkerton, Ontario, Canada) water supply occurred in 2000 claimed 7 lives and left more than 2000 people ill. The *E coli* outbreak was caused by farm runoff contaminating the town's water supply. Those infected had significant risk of developing postinfective IBS and many had comorbid depression/anxiety.[22] To a greater extent than any prior study, this natural disaster provided clear cut support for the notion of postinfective IBS.

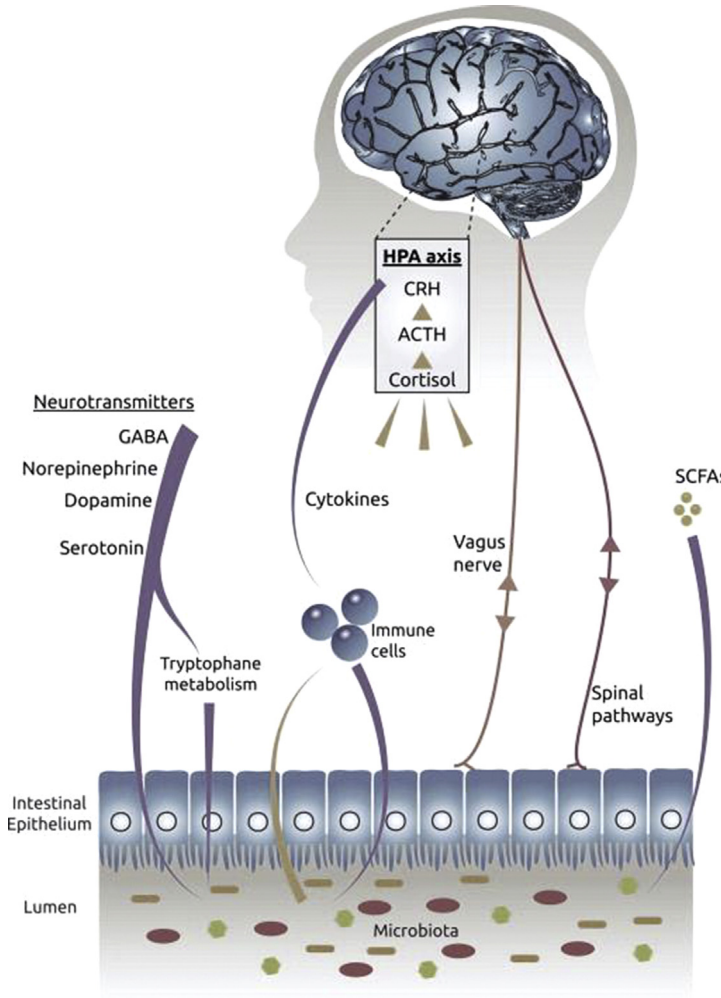

Fig. 1. Routes of communication between gut microbes and brain. These include the vagus nerve, SCFAs (butyrate, propionate, and acetate), cytokines, and tryptophan. ACTH, adrenocorticotropic hormone; CRH, corticotropin releasing hormone.

BRAIN-GUT-MICROBIOTA AXIS AND EXTREMES OF LIFE

The intestinal microbiota of newborn infants is characterized by low diversity and a relative dominance of the phyla Proteobacteria and Actinobacteria in the early postnatal period, a time at which there is enormous brain development. With the passage of time, the microbiota becomes more diverse, with the emergence and dominance of Firmicutes and Bacteroidetes.[23–25] Full-term, vaginally delivered babies born to healthy mothers who are breastfed and nonantibiotic treated have an optimal development of the neonatal microbiota.[26] The characteristic intestinal microbiota observed in healthy full-term infants is disturbed in preterm infants,[27] who are frequently delivered by caesarean section, receive antibiotics, and may have problems feeding.[28] Furthermore, preterm infants possess a functionally immature gut with low levels of acidity in the stomach, due to insufficient gastric acid secretion and their requirement for more frequent feeding.[28–30] These events lead to an increase in the prevalence of potentially pathogenic bacteria in the gastrointestinal (GI) tract and less microbial diversity than full term infants.[31–33] The extent to which these features play a role in the development of cerebral palsy and subsequent autism are the subject of research and ongoing debate.[34] What is clear is that complex brain maturation and the increasing sophistication of the gut microbiota are highly correlated. To date many assumptions are based on correlational data from which a causative impact cannot be conclusively concluded.

When the microbiota composition of elderly people in nursing homes are compared with those in the community, large-scale differences are detected. Those in nursing homes have a far less diverse microbiota and this has been attributed to a less varied diet.[35] It is possible, however, that pathologic factors that lead to admission into nursing homes, such as deteriorating cognitive function and less physical activity, might play an important role in the decreased microbial richness and not the less diverse diet. Ongoing studies should clarify this issue, and there is a challenge for the food industry to produce diets for the elderly that help to sustain microbial diversity.

What is abundantly clear is that a dysregulated gut microbiota either in early childhood or in an aging population significantly increases the likelihood of brain dysfunction. The precise relationship between these observations is far from understood. Determining the mechanisms and pathways underlying microbiota-brain interactions may yield novel insights into individual variations and perhaps enable the development of new treatments for a range of neurodevelopmental and neurodegenerative disorders, ranging from autism to Parkinson disease.

IRRITABLE BOWEL SYNDROME AS PROTOTYPE

IBS is the prototypic disorder of the brain-gut-microbiota axis, generally perceived as a having a biopsychosocial etiology[36] and frequently comorbid with depression or anxiety. The most important single risk factors are female gender, younger age, and preceding GI infections. Recent studies suggest that trauma in childhood, especially sexual abuse, may be an important risk factor.[37] The aspect of dysbiosis in IBS is important and is discussed elsewhere, but aspects of gut-to-brain communication are clearly altered. For example, elevated levels of plasma proinflammatory cytokines are found and there is an exaggerated pituitary-adrenal response to corticotropin-releasing hormone, together with augmented visceral pain responses. A recent study found that fasting serum levels of SCFAs did not differ between patients with IBS and controls.[38] The postprandial levels of total SCFAs, acetic acid, propionic acid, and butyric acid were found, however, significantly lower in patients with IBS compared

with healthy controls. An epigenetic model of IBS has been proposed,[36] which is consistent with the potential epigenetic modulating effects of butyrate, the levels of which are altered substantially in the postprandial state.

Treatments of IBS that do not take into account this complex pathophysiology[37] are likely to be of limited benefit (Fig. 2).

DEPRESSION

IBS and depression are frequently comorbid and the latter is associated with the presence of biomarkers of inflammation, such as elevated IL-6, tumor necrosis factor (TNF)-α, and the acute-phase protein, C-reactive protein.[39] Similar elevated biomarkers of inflammation have been seen in anxiety states and are known to occur as a result of stress. The site at which these proinflammatory molecules is produced in depression is not known and it has yet to be determined whether the elevation is core to the pathophysiology or merely epiphenomenal. There is evidence from rodent studies to indicate that stress alters the gut barrier function, allowing lipopolysaccharide (LPS) and other molecules to gain access to the bloodstream, stimulating Toll-like receptor 4 and other Toll-like receptors, resulting in the production of inflammatory cytokines.[39] If this does occur in depression, which has yet to be definitively demonstrated, it would explain the proinflammatory phenotype observed.

Bercik and colleagues,[40] using germ-free and specific pathogen-free mice, demonstrated that the early life stress of maternal separation alters the HPA axis and colonic

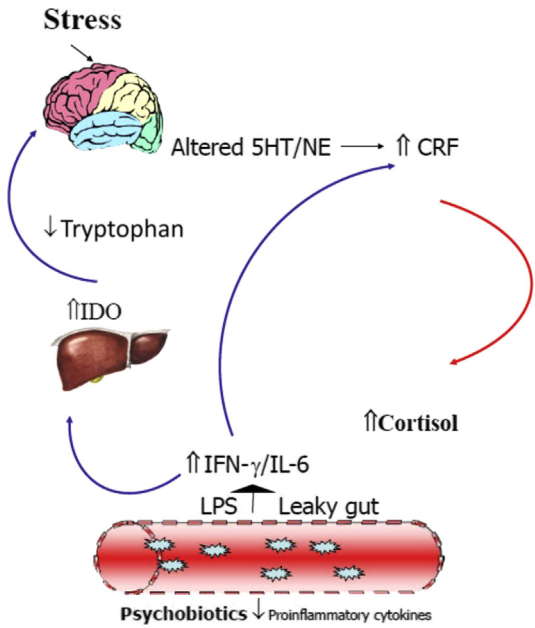

Fig. 2. Model of IBS. Psychological stress or infection leads to activation of the HPA axis, with elevation in cortisol and also changes in gut permeability. LPS enters the bloodstream, increasing proinflammatory cytokines and altering tryptophan metabolism. In turn this leads to alterations in serotonin (5HT) and glutamate neurotransmission. Psychobiotics may have an impact by decreasing gut permeability and signaling the brain via the vagus nerve and other routes. CRH, corticotropin releasing hormone; IDO, indoleamine 2,3-dioxygenase; IFN, interferon; NE, norepinephrine.

cholinergic neural regulation in a microbiota-independent fashion.[41] They showed, however, that the microbiota is required for the induction of anxiety-like behavior and behavioral despair. Colonization of adult germ-free maternally separated and control mice with the same microbiota produces distinct microbial profiles, which are associated with altered behavior in maternally separated mice but not in control mice. The results suggest that maternal separation–induced changes in host physiology lead to intestinal dysbiosis, which is a critical determinant of the abnormal behavior that characterizes this model of early-life stress. Prior studies in maternally separated rats demonstrated an altered behavioral phenotype when these animals reached maturity and also decreased diversity in the microbiota.[20] Does this decreased diversity translate to patients with major depression?

In a recent study the fecal microbiota was sequenced[41]; 46 patients with depression and 30 healthy controls were recruited. High-throughput pyrosequencing showed that, according to the Shannon index, increased fecal bacterial α-diversity was found in those currently depressed but not in a group who had responded to treatment. Bacteroidetes, Proteobacteria, and Actinobacteria were increased, whereas Firmicutes was significantly reduced. Despite the profound interindividual variability, levels of several predominant genera were significantly different between the depressives and controls. Notably, the depressives had increased levels of Enterobacteriaceae and Alistipes but reduced levels of Faecalibacterium. The investigators conclude that further studies are necessary to elucidate the temporal and causal relationships between gut microbiota and depression and to evaluate the suitability of the microbiome as a biomarker. When rats are given a humanized microbiota from depressed patients as opposed to healthy controls, they develop a depressive phenotype from a behavioral and immune perspective.[42]

AUTISM

Autism is a neurodevelopmental disorder whose prevalence is apparently on the increase. It is characterized by a failure of language acquisition and a lack of sociability. It is frequently associated with GI symptoms,[43] the relevance of which has been a longstanding source of controversy. Up to 70% of patients with the syndrome report abdominal symptoms and hence the view that it is a disorder of the brain-gut axis. The authors' group at the APC Microbiome Institute examined the behavior of mice raised in a germ-free environment.[44,45] The mice were tested in a 3-chamber apparatus, where a germ-free mouse was placed in the middle chamber with a familiar mouse in 1 chamber and a novel mouse in the third. The germ-free mouse spent as much time with the familiar as with the novel mouse; this is in contrast to the behavior of conventionally colonized mice who spend more time with the novel than the familiar mouse. Germ-free mice are also more likely to spend time with an empty chamber or an object than with another mouse, a decidedly abnormal behavior for a sociable animal. Colonization of the germ-free mice does partially normalize their behavior patterns. These behavioral changes are associated with significant alterations in underlying neurochemistry.

Work from the Patterson and Mazmanian[46] group in an animal model demonstrated that the microbiota modulates behavioral and physiologic abnormalities associated with neurodevelopmental disorders such as autism.[46] They used the maternal immune activation model induced by poly(I:C) injection during pregnancy and found altered GI barrier defects and microbiota alterations. Oral treatment with the human commensal *Bacteroides fragilis* was shown to correct gut permeability and interestingly stereotyped and other abnormal behaviors. Furthermore, a metabolite found in the abnormal

animals was observed to transfer the phenotype to naïve animals and to be reduced by *B fragilis*.

Increasing attention is being paid to oxytocin the hypothalamic peptide, which has been shown to increase sociability. The oxytocin receptor knockout mouse shows considerable deficits in social behavior and some small-scale preliminary studies in humans indicate that intranasally administered oxytocin may positively alter social behavior patterns. A few large clinical trials are under way to test oxytocin and related therapies for autism spectrum disorder. There is still considerable debate as to whether or not the preclinical findings translate to the clinical setting and if they do which patients and which aspects of the syndrome are likely to benefit most. Intriguingly, a recent study indicates that probiotic bacteria can influence hypothalamic posterior pituitary activity and increase oxytocin levels, raising the possibility of influencing social behavior by targeting the gut microbiota.[47]

The fecal microbiota in patients with autism spectrum disorder has been sequenced.[48] In the most recently published study, Tomova and colleagues[48] examined the microbiota in Slovakian children. The fecal microbiota of autistic children showed a significant decrease of the Bacteroidetes/Firmicutes ratio and elevation of the amount of *Lactobacillus* spp. There was a modest elevation in *Desulfovibrio* spp and a correlation with the severity of autism. A probiotic diet normalized the Bacteroidetes/Firmicutes ratio and *Desulfovibrio* spp levels. As recently summarized by Mayer and colleagues,[3] there is a paucity of large comprehensive studies of the microbiome in autism. Again the 'chicken or egg' issue emerges: Are these changes induced by stereotyped diets seen in many individuals as a product of obsessional behavior patterns? Also the heterogeneous nature of the disease needs to be taken into account and much more effort is needed to tease out the exact role of the microbiome in both the etiology and treatment of the disorder.

PARKINSON DISEASE

In marked contrast to autism, Parkinson disease tends to be diagnosed generally in old age; it is the second most common neurodegenerative disorder and affects 1% to 2% of the population over 65 years of age. It is a movement disorder characterized by degeneration of the zona compacta neurons of the substantia nigra. The most common GI symptoms are constipation, appetite loss, weight loss, dysphagia, sialorrhea, and gastroesophageal reflux.[49] α-Synuclein aggregates, the major neuropathologic marker in Parkinson disease, are present in the submucosal and myenteric plexuses of the enteric nervous system, prior to their detection in the brain, which may indicate a gut to brain prion-like spread.[50]

The gut microbiota has been sequenced in patients with Parkinson disease.[51] On average, the abundance of Prevotellaceae in the feces of Parkinson disease patients was reduced by almost 80% compared with controls. A logistic regression analysis based on the abundance of 4 bacterial families and the severity of constipation identified Parkinson disease patients, with 66.7% sensitivity and 90.3% specificity. The relative abundance of Enterobacteriaceae was highly correlated with the severity of postural instability and gait difficulty. The findings suggest that the intestinal microbiome is altered in Parkinson disease and is related to motor phenotype. Large prospective studies beginning in the early stages of the disorder are required.

It has been suggested that microbiota transplantation might benefit patients with Parkinson disease but there is as yet no conclusive evidence.[52] Neither are there any reports of controlled trials of probiotics/psychotiotics.

PSYCHOBIOTICS

Psychobiotics were first defined as the family of probiotics that, ingested in appropriate quantities, had a positive mental health benefit.[53] Recently, the definition has been expanded to include prebiotics, which are dietary, soluble fibers for example, galactooligosaccharides (GOS) or fructooligosaccharides (FOS) that stimulate the growth of intrinsic commensal microbiota. There is now an enormous volume of preclinical data to support the concept of psychobiotics. Understandably, clinical data are less abundant but nonetheless emerging. Given the demonstrated efficacy of probiotics in IBS[54] and the high comorbidity between IBS and stress-related mental health issues, such as anxiety and depression, it is not surprising that certain probiotics might have a positive impact on mental health.

Tillisch and colleagues[55] administered healthy female participants either a placebo or a fermented dairy drink made from the probiotics (*Bifidobacterium animalis lactis*, *Streptococcus thermophiles*, *Lactobacillus bulgaricus*, and *Lactococcus lactis*), which were consumed over 4 weeks. Participants underwent functional MRI to determine how probiotic ingestion affected neuropsychological activity. During image acquisition, participants were shown emotional faces that are known to capture attention and cause brain activation. Relative to placebo, probiotic-treated participants showed decreased activity in a functional network associated with emotional, somatosensory, and interceptive processing, including the somatosensory cortex, the insula, and the periaqueductal gray. In marked contrast, placebo participants showed increased activity in these regions in response to emotional faces. This is interpreted as evidence of a probiotic-induced reduction in network-level neural reactivity to negative emotional information.

A recent prebiotic study carried out in Oxford University found a significant impact on stress responses.[56] Healthy male and female participants consumed either Bimuno-GOS (BGOS), FOS, or a placebo. In comparison to the other 2 groups, participants who consumed BGOS showed significantly reduced waking-cortisol responses, which are a robust marker of anxiety, stress, and depression risk.[57] Furthermore, participants completed an emotional dot-probe task measuring vigilance, or attention to negative stimuli, which is also a marker of anxiety and depression. Participants taking BGOS showed substantially attenuated vigilance on this task, suggesting reduced attention and reactivity to negative emotions. Overall, the data support the view that the specific prebiotic has anxiolytic activity.

Takada and colleagues[58] examined the effects of *Lactobacillus casei* strain Shirota (LcS) on gut-brain interactions under stressful conditions. Double-blind, placebo-controlled trials were conducted to examine the effects of LcS on psychological and physiologic stress responses in healthy medical students while undergoing examination stress. Subjects received LcS-fermented milk or placebo daily for 8 weeks prior to taking an examination. Subjective anxiety scores, salivary cortisol, and the presence of physical symptoms were analyzed. In a parallel animal study, rats were fed a diet with or without LcS for 2 weeks, then submitted to water avoidance stress (WAS). Plasma corticosterone concentration and the expression of cFos and corticotropin-releasing factor in the paraventricular nucleus were measured immediately after WAS. Academic stress resulted in increases in salivary cortisol and an increase in physical symptoms, both of which were significantly suppressed in the LcS group. In rats pretreated with LcS, WAS-induced increases in plasma corticosterone were significantly suppressed, and the number of corticotropin-releasing factor–expressing cells in the paraventricular nucleus was reduced. Intriguingly, intragastric administration of LcS was found to stimulate gastric vagal afferent activity in a

dose-dependent manner. The results suggest that LcS may have a positive impact on stress responses by acting through the vagus nerve. In a study of university students, the authors have found that a *Bifidobacterium longum* decreased morning waking cortisol levels, reduced subjective levels of anxiety, and modestly improved aspects of cognitive functioning, an effect that was associated with altered encephalographic activity.

A large-scale cross-sectional study has examined the impact of probiotics on measures of social anxiety[59]; 710 young adults completed self-report measures of fermented food consumption, neuroticism, and social anxiety. An interaction model, controlling for demographics, general consumption of healthful foods, and exercise frequency, showed that exercise, neuroticism, and fermented food consumption significantly and independently predicted social anxiety. Furthermore, fermented food consumption also interacted with neuroticism in predicting social anxiety. For those with high neuroticism scores, a high frequency of fermented food consumption resulted in fewer symptoms of social anxiety. The data suggest that fermented foods containing probiotics may have a protective effect against social anxiety symptoms for those at higher genetic risk, as assayed by trait neuroticism.

Steenbergen and colleagues[60] tested a multispecies probiotic containing *Bifidobacterium bifidum*, *Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Lactobacillus casei, Lactobacillus salivarius*, and *Lactococcus lactis* in nondepressed individuals using a triple-blind, placebo-controlled, randomized, design; 20 healthy participants received a 4-week probiotic food-supplement intervention with the multispecies probiotics, whereas 20 control participants received an inert placebo for the same period. Subjects who received the 4-week multispecies probiotics intervention showed a significantly reduced overall cognitive reactivity to sad. The results provide evidence that probiotics may help reduce negative thoughts associated with sad mood.

Romijn and Rucklidge in their systematic review[61] add a note of caution to these optimistic findings, concluding that more trials are necessary before any definitive inferences can be made about the efficacy of probiotics in mental health applications. Further studies of a translational nature are required.

SUMMARY

The role of the microbiota-gut-brain access in the genesis of IBS symptoms is now largely accepted, although several questions remain unanswered. How does stress, especially early life stress, dysregulate the axis? Can IBS subtypes be delineated on the basis of the microbiota? If patients with IBS have comorbid psychiatric illness, does the latter resolve if the former is treated with probiotics?

There are an enormous number of preclinical studies implicating the gut microbiota in other stress-related conditions and in disorders at the extremes of life. Far more translational studies are required. The human studies to date support the view that the gut microbiota is altered in major depression and that psychobiotics, either in the form of prebiotics or probiotics, can have an impact on anxiety and depressive symptoms in healthy subjects. There is no clear indication of efficacy in diseased populations. In the neurodevelopmental disorder autism, which is usually diagnosed in early childhood, GI symptoms are common and an altered microbiota has been reported, whereas at the other end of the developmental spectrum, old age–related frailty correlates with decreased gut microbial diversity. Whether fecal microbiota transplantation is an appropriate therapeutic option in at least some brain-gut axis disorders remains to be determined.

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