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Neuromicrobiology: How Microbes Influence the Brain

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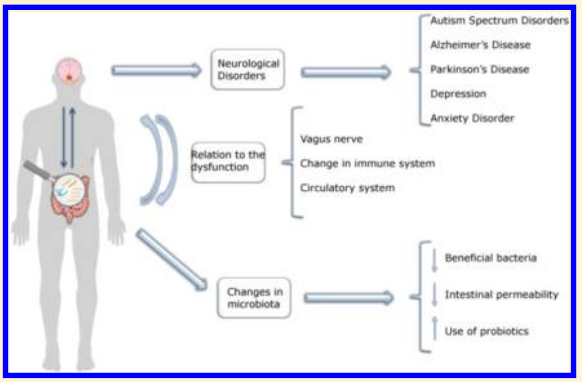
ABSTRACT: We review here recent discoveries in the exciting new field of neuromicrobiology. This field encompasses the interactions between the microbiome and the central nervous system. The microbiome has a tremendous impact on human health. In particular, the gut microbiota may play a key role in many essential processes in health and disease via the activity of the gut-brain axis, possibly contributing to autism spectrum disorders, Alzheimer's disease, Parkinson's disease, depression, and anxiety disorder. Gut microbes may also be involved in nociception, complex host behaviors, and brain development. Future efforts will be needed to determine whether the observed associations correspond to causative mechanisms, as well as to engineer effective interventions to modulate the effects of the microbiome on the central nervous system.



KEYWORDS: *Neuromicrobiology, gut microbiota, neurological disorders*

INTRODUCTION

Over 100 years ago, Nobel laureate Elie Metchnikoff proposed that lactic acid bacteria are beneficial to human health.[1,2] Since then, and most recently through technological advances in DNA sequencing, RNA sequencing, and metabolomics, we have come to realize that these microbes are part of complex multicellular communities that collectively constitute the gut microbiota. The microbiota comprises a multitude of symbiotic microorganisms that communicate with each other and with their host. Depending on their composition, these microbial communities have been directly linked to host health, as disturbances in the profiles of these communities correlate to a wide range of disease processes. Recent studies indicate that gut microbes may modulate development, metabolism, digestion, nutrition, and immunity, as well as the central nervous system (CNS). Their interactions with the CNS provide evidence of direct communication pathways between the gut and the brain,[3−8] and indeed signs of neurological disease have been observed after a partial gastrectomy.[9]

The composition of the microbiota evolves over time, which may have implications in brain development. From the first year of life, feeding modulates the composition of the microbiota.[10] With the introduction of novel foods, the microbiota begins to develop the microbial composition associated with adults.[11] During adult life, despite changes in lifestyle and food, the microbiota remains stable, consisting primarily (90%) of members of the phyla *Firmicutes* and *Bacteroidetes*.[12] These microbes perform important biological tasks for the host. For instance, bacteria from the genus *Bacteriodes* digest carbohydrates, leading to the generation of short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, which supply energy to the host.[13]

All in all, the relationship between the gut microbiota and the host provides beneficial effects by helping with digestion of food, energy storage, and modulation and protection of the intestinal barrier.[14] The activity of microorganisms present in the intestinal microbiota also influences the intestinal immune system, which is linked to the nervous system (Figure 1).

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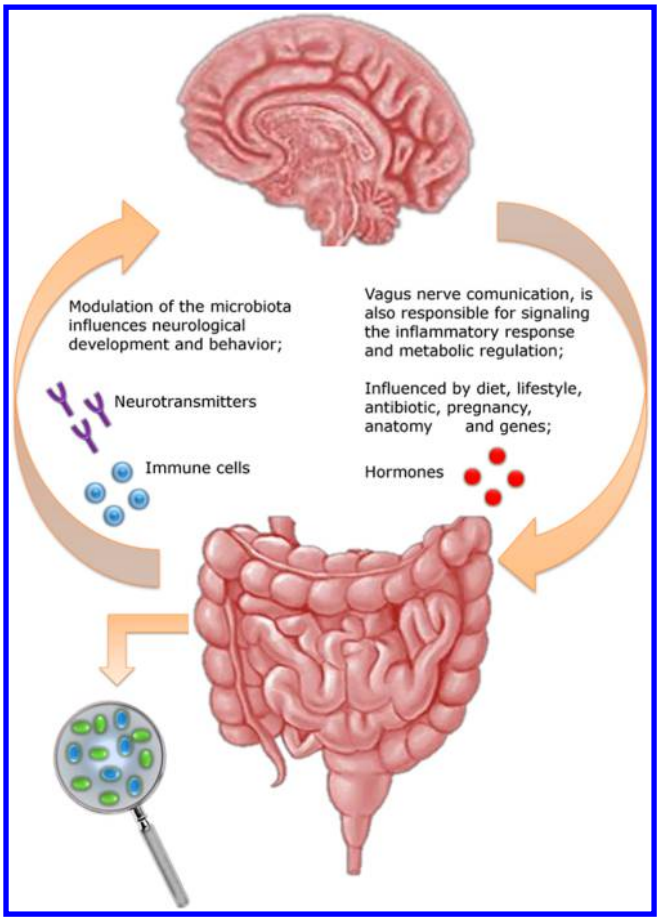

Figure 1. Schematic illustration showing bidirectional communication between the gut microbiota and the brain (i.e., gut−brain axis).

According to recent studies in rodents and humans, most of which were association studies, modifications in microbial diversity are linked to negative health outcomes and may cause alterations in the CNS (Table 1); these alterations are associated with autism spectrum disorders (ASD), depression, and anxiety.[14] In addition, conventional treatments for neurological diseases (e.g., antipsychotics) may alter the gut microbial composition, thereby influencing the response to treatment, as well as causing side effects.[15] Other studies have reported additional links between the microbiota composition and depression, anxiety, and ASD.[16−20] Thus, the composition of the microbiota, which evolves over time, may have implications in brain function.

In this Perspective, we review recent developments in the field of neuromicrobiology, particularly the links between the gut microbiota and neurological disease. In exploring the role that gut microbes play in neurological disorders, we specifically focus on ASD, Alzheimer's disease (AD), Parkinson's disease (PD), depression, and anxiety disorder.

OVERVIEW OF THE GUT−BRAIN AXIS

The enteric nervous system (ENS) is composed of millions of neurons, which are present in the mucosa of the gastrointestinal tract. These neurons are responsible for balancing intestinal functions. The most direct communication between gut and brain is mediated by the vagus nerve (Figure 1). This nerve serves as a major pathway for the transmission of signals originating from the foregut and colon and is responsible for primary parasympathetic control, such as basic intestinal activities.[21] The vagus nerve is known to be activated by gut microbes, which may lead to effects on the brain and behavior. In fact, the gut-brain axis is bidirectional, as blocking symbiosis between the microbiota and the host has been shown to be deleterious, causing neurological disorders among other diseases.[22−24] Thus, the gut may be considered a "second brain".[25] Changes in the gut−brain axis can be influenced by diet, lifestyle, genes, and anatomy (e.g., surgery).[26,27]

In light of this, the intestinal microbiota is increasingly being characterized as an important regulator of the CNS. Communication between the gut and the brain is influenced by the composition of the intestinal microbiota.[28,29] The gut−brain axis integrates the enteric and endocrine systems with the CNS. As a consequence, changes that occur in the microbiota may also modify the relative concentrations of important compounds such as growth factors and signaling proteins, which may lead to substantial physiological dysfunction.[30]

The microbiota can beneficially influence neurological disorders through endocrine and neural communication.[31] An example of such gut-brain interaction and modulation of cellular processes is the ability of bacteria to synthesize and release neurotransmitters, which may interact with enteric and endocrine cells. Beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* can produce gamma-aminobutyric acid (GABA), which is an inhibitory neurotransmitter in the mammalian CNS, and other bacteria have been shown to generate norepinephrine and serotonin.[31] The gut microbiota also appears to play other roles, such as modulating the immune response of the intestinal mucosa during infection, inflammation, or autoimmunity.[32]

AUTISM SPECTRUM DISORDERS

Several studies have reported links between the microbiota composition and ASD. ASD are a group of developmental disorders associated with a range of behavioral deficits. Individuals with ASD show alterations in brain development[33] and exhibit characteristic symptoms such as difficulty communicating, social development alterations, repetitive behaviors, and gastrointestinal disturbances (e.g., constipation and diarrhea).[34] Apart from the role of genetic alterations, the causes of ASD are not well established.

Recent studies have focused on elucidating novel contributors to the development of ASD.[35,36] About 70% of individuals with ASD have been found to have gastrointestinal distress, indicating potential involvement of the gut-brain axis in ASD.[37] A study performed by Gorrindo et al.[38] pointed out that the most common symptom among children with ASD was constipation, with an index of 85%. These insights hinted at a potential role of the intestinal environment in ASD. Indeed, most autistic children present gastrointestinal alterations similar to those displayed by patients with irritable bowel syndrome (IBS).[39] Children with ASD have a greater diversity in their intestinal microbiota than children without ASD,[40] further suggesting a link between the microbiota and ASD.[41,42] Association studies in humans report that obesity during pregnancy and gestational diabetes are directly linked to an increased risk of ASD.[43]

Recent work by Hsiao et al.[44] with a maternal immune activation (MIA) animal model of ASD showed increased intestinal permeability and microbiota dysbiosis compared to wild-type mice, along with the behavioral phenotypes associated with ASD. Oral administration of the gut bacterium *Bacteroides fragilis* ameliorated microbiota dysbiosis in MIA mice and also reduced several behavioral abnormalities characteristic of ASD, thus highlighting the potential of microbial interventions for the treatment of gut microbiota-mediated neurological disorders.

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Table 1. Studies That Discuss the Relationship between the Microbiota and Neurological Disorders

neurological disorders	human or animal	causation vs association (general observations)	behavioral changes	changes in microbiota	ref
depression	germ-free (GF) mice	GF animals colonized with a "depression microbiota" had more symptoms compared to control GF animals	GF mice present reduced behavior of depression, less anxiety behavior, and better memory performance	bacterial increase: Actinomycineae, Coriobacterineae, Lactobacillaceae, Streptococcaceae, Clostridiales, Eubacteriaceae, Lachnospiraceae, Ruminococcaceae, and Erysipelotrichaceae; bacterial decrease: Bacteroidaceae, Rikenellaceae, Lachnospiraceae, Acidaminococcaceae, Veillonellaceae, and Sutterellaceae	83
depression	humans	first indication of the association of supplementation of probiotics with reduction of symptoms associated with depression, humor alterations, and cognitive effect	ingestion of probiotics may reduce the symptoms of depression, such as aggressive and sad mood	probiotic supplementation containing: *Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W37, *Lactobacillus brevis* W63, *Lactobacillus casei* W56, *Lactobacillus salivarius* W24, and *Lactococcus lactis* (W19 and W58)	109
depression	humans	analysis of fecal samples from individuals with and without depression led to a better understanding of the relationship of the microbiota and incidence of specific microorganisms with symptoms associated with depression	relationship between *Faecalibacterium* and the prevalence of depressive symptoms	bacterial increase: Acidaminococcaceae, Enterobacteriaceae, Alistipes, Fusobacteriaceae, Porphyromonadaceae, and Rikenellaceae; bacterial decrease: Bacteroidaceae, Erysipelotrichaceae, Lachnospiraceae. Prevotellaceae, Veillonellaceae and Ruminococcaceae; most abundant genera in major depressive disorder group: *Alistipes, Blautia, Clostridium XIX, Lachnospiracea incertae sedis, Megamonas, Parabacteroides, Parasutterella, Phascolarctobacterium, Oscillibacter*, and *Roseburia*	110
depression	Sprague–Dawley rats	probiotic reduced symptoms associated with depression, besides regularizing the immune response, improving behavioral defects, and balancing noradrenaline concentrations	reduction in the symptoms associated with depression and regulation of CNS-mediated psychobiological systems	probiotic supplementation containing *Bifidobacterium infantis* 35624	111
depression	humans	patients with major depressive disorder display reduced abundance of *Bifidobacterium* and *Lactobacillus*; fecal samples were analyzed and allowed visualization of the association between bacterial population and irritable bowel syndrome (IBS)	prevalence of IBS in individuals with depression	bacterial decrease: *Bifidobacterium* and *Lactobacillus*	112
Alzheimer's disease	humans	first indication that bacterial population and viral and infectious load may be associated with the development of AD symptoms. Each pathogen influences cognitive decline	aged individuals with high infectious load had inferior cognition	associated with infectious load, being viral (HSV-1 and CMV) or bacterial *Borrelia burgdorferi, Chlamydia pneumoniae*, and *Helicobacter pylori*	113
Alzheimer's disease	humans	supplementation with probiotics did not significantly affect other parameters such as oxidative stress and inflammation, but probiotics may positively influence the cognitive function of individuals with AD	individuals with AD presented improvement in mental state and cognitive function with probiotic supplementation for 12 weeks	probiotic supplementation containing: *Lactobacillus acidophilus, Lactobacillus casei, Bifidobacterium bifidum,* and *Lactobacillus fermentum*	114
anxiety	Wistar rats and humans	the probiotic complex was associated with reduced anxiety in rats and improved psychological effects in healthy humans	influence on the psychological state of healthy individuals in daily activities; individuals treated with probiotics had reduced symptoms associated with anxiety	probiotic supplementation containing *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175	85
anxiety	BALB/c mice	these studies show that probiotic supplementation is associated with reduced symptoms related to stress and anxiety, correlating effectively *Bifidobacteria* with the treatment of neurological disorders	probiotic led to improvement in locomotor activity, better memory skills and fewer anxiety symptoms	probiotic supplementation containing *Bifidobacterium longum* 1714 and *Bifidobacterium breve* 1205	107, 115
anxiety	germ-free swiss Webster mice	this study that shows the association of the intestinal microbiota with neurological interferences and behavior	GF mice presented fewer symptoms associated with anxiety in relation to specific pathogen-free (SPF) animals		93
anxiety	humans	administration of probiotics is associated with improved mental health; this probiotic complex did not affect the Hypothalamic Pituitary Adrenal (HPA) axis	the probiotic-treated group presented fewer symptoms associated with anxiety, as well as beneficial mental health effects	probiotic supplementation *Bifidobacterium logum, Lactobacillus acidophilus* LA5 and *Bifidobacterium lactis* BB12, *Lactobacillus casei, Lactobacillus rhamnosus, Lactobacillus bulgaricus, Bifidobacterium breve, Streptococcus thermophilus*	116
Parkinson's disease	humans	mucosal and fecal samples were analyzed, and some genes were downregulated in the fecal microbiota of individuals with PD; the microbiota composition of the mucosal and fecal samples was associated with significant changes in patients with PD		bacterial increase: Proteobacteria (class Betaproteobacteria), *Blautia, Roseburia, Coprococcus, Akkermansia, Oscillospira Bacteroides*; bacterial decrease: *Faecalibacterium* (*Firmicutes*, class *Clostridia*)	117

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Table 1. continued

neurological disorders	human or animal	causation vs association (general observations)	behavioral changes	changes in microbiota	ref
Parkinson's disease	humans	the alteration of the fecal microbiome may be associated with the development of PD; *Prevotellaceae* was reduced in PD patients, and high abundance of this genera was unlikely to have PD; high abundance of *Prevotellaceae* may constitute biomarker to exclude PD	*Enterobacteriaceae* was directly related to motor impairments	bacterial decrease: *Provotellaceae*; the abundance of *Ruminococcaceae* could be directly linked to levels of *Provotellaceae*	62
autism	children	changes in the microbiota may cause the development of symptoms associated with autism; bacteria associated with healthy status had a low abundance in children with AD, which suggests probiotic supplementation may be a promising strategy for modulating the microbiota for the treatment of autism spectrum disorders		bacterial increase: Bacteroidetes, Proteobacteria, Actinobacteria, Lachnospiraceae, Porphyromonadaceae, Rikenellaceae, Prevotellacea, Enterobacteriaceae, Alistirpes, *Akkermansia muciniphila* and Clostridiaceae; bacterial decrease: Firmicutes (e.g., *Enterococcus* species), and *Bifidobacterium* species from the phylum Actinobacteria	118
autism	children	there is an association between gastrointestinal (GI) changes and autism symptoms; the imbalance of bacteria associated with healthy status may be associated with the development of autism		bacterial increase: *Lactobacillus;* bacterial decrease: *Bifidobacterium* and *Enterococcus;* autism group more likely to have increased levels of *Bacillus* spp. and decreased *Klebsiella oxytoca*	119
autism	children	identified a relationship between genes expressed in the intestine of children with autism and bacterial populations; the cause for these intestinal changes remains to be investigated	GI symptoms are linked to dysbiosis in patients with autism, and correlated with enzymatic deficiency within the intestine	bacterial increase: Firmicutes to Bacteroidetes ratio (e.g., *Lachnospiraceae* and *Ruminococcaceae*), *Betaproteobacteria* in ilea; bacterial decrease: Bacteroidetes	120
autism	children	increased abundance of *Suterella* spp. in feces of children with autism; *Ruminococcus torques* is also increased and may be associated with GI disturbances in children with autism		bacterial increase: *Suterella* and *Ruminococcus torques*	121
autism	children	the intestinal microbiota was associated with GI disorders, and children with autism had a less diverse microbiota		bacterial decrease: *Prevotella*, *Coprococcus* and *Veillonellaceae;* dominant phyla in microbiota of patients with autism: *Firmicutes* and *Bacteroidetes*; most abundant genera: *Bacteroides, Faecalibacterium, Bifidobacterium, Akkermansia,* and *Subdoligranulum*	122

ALZHEIMER'S DISEASE

AD is a neurodegenerative disorder with complex etiologies that are still poorly understood. The severe cognitive impairments associated with AD correlate with the accumulation of protein aggregates composed of amyloid plaques (amyloidosis) and tau protein tangles in tissues of the CNS. Growing evidence suggests that microbial infections targeting the CNS constitute additional factors associated with increased incidence of AD.[45−50] These infections likely promote chronic inflammation in the CNS, which could lead to amyloidosis and synaptic degeneration. Indeed, bacterial infections have been shown to induce the formation of amyloid peptide oligomers. These oligomers (amyloid beta, or Aβ) exhibit antimicrobial activity and, in fact, may eliminate infections in the brain.[51] On the other hand, Aβ, which forms pathogenic plaques, promotes the pathology of AD. Taken together, the evidence suggests that bacterial-induced amyloid formation may lead to AD.

APP/PS1 mice, which overexpress amyloid precursor protein and presenilin 1 [important for the production of amyloid beta (Aβ) from amyloid precursor protein (APP)], were found to have fewer bacteria in their gut belonging to the genera *Allobaculum* and *Akkermansia* and more bacteria belonging to the family *Rikenellaceae* compared to wild-type (WT) controls.[52] Treatment of APP/PS1 mice with an antibiotic cocktail reduced the accumulation of microglia and astrocytes surrounding amyloid plaques in the hippocampus.[53] Germ-free (GF) APP/PS1 mice exhibited reduced cerebral and serum Aβ levels, reduced microglial activation, and increased levels of Aβ-degrading enzymes compared to conventional APP/PS1 mice, further supporting the notion that host-associated microbes are involved in AD pathology.

Another association study identified more inflammation-associated bacteria, such as *Escherichia* and *Shigella*, in fecal samples from AD patients compared to control groups, which correlated with increased levels of pro-inflammatory cytokines (e.g., IL-1β) in the blood (Figure 2).[54] Further supporting the link between AD and bacteria, intestinal permeability increases with age,[55] thereby allowing bacteria to translocate from the lumen of the gut and mediate neuro-inflammation. The hypothesis of bacterial translocation has been supported by the observation that there are higher levels of lipopolysaccharide (LPS) in the brain of AD patients than in healthy individuals.[56] Transgenic 5xFAD mice rapidly develop amyloid plaques and exhibit significant neuron loss, and therefore represent an excellent model for AD. These mice had an altered microbiota compared to WT mice, with an increase in the ratio of *Firmicutes* to *Bacteroidetes*. These recent developments indicate that more work is needed to elucidate which bacterial species of the gut microbiota are functionally involved in AD and to develop interventions that may be useful for treating AD.

PARKINSON'S DISEASE

PD is a neurodegenerative motor disorder that affects an estimated 1 million people and 1% of the United States population > 60 years of age.[57] PD is commonly associated with impaired gastric motility[58] and elevated levels of alpha-synuclein in the intestine.[59] The diagnosis of PD relies on a basic symptom, motor difficulty. This debilitation is caused by a decrease in dopamine and the subsequent death of the dopaminergic neurons in the substantia nigra.[60] Treatment of PD often includes dopamine supplementation, but currently there are no drugs that delay the

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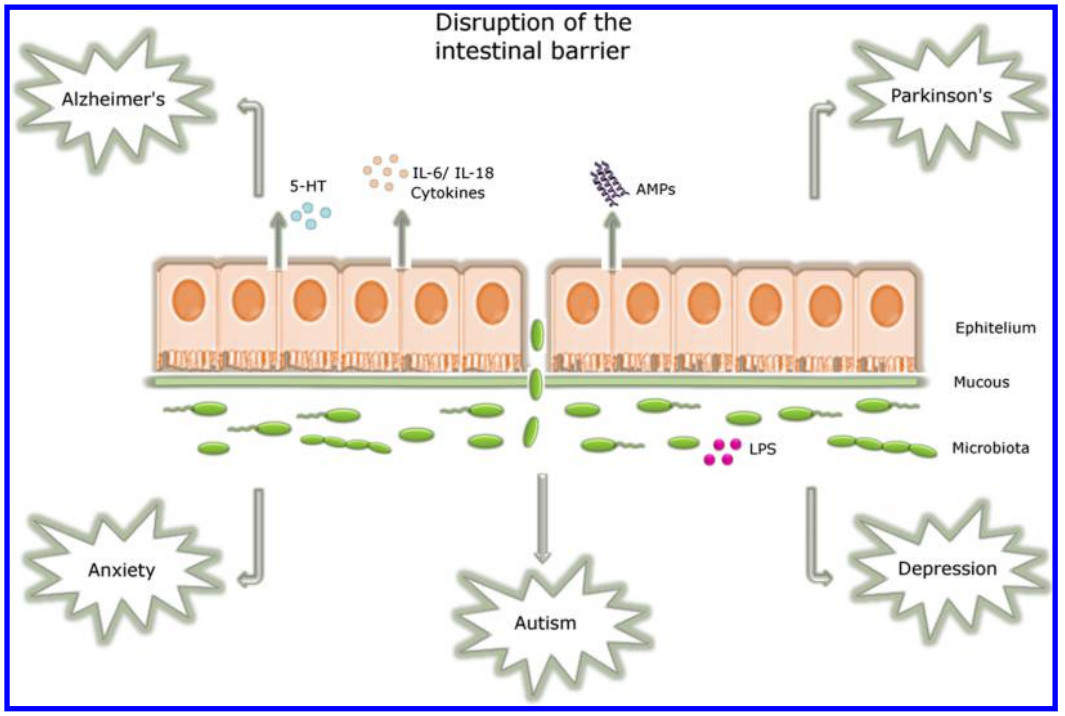

Figure 2. Influence of the gut microbiota on the development of neurological disorders. Abbreviated terms in the figure correspond to 5-HT: 5-hydroxytryptamine; IL: interleukin; LPS: lipopolysaccharide; AMPs: antimicrobial peptides.

neurodegeneration associated with PD, and the originating cause of this disease remains poorly defined.[61]

Several associations between the gut microbiota and PD have been established. For example, the fecal microbiota of PD patients displayed a reduced abundance of *Prevotellaceae* and elevated levels of *Enterobacteriaceae* compared to healthy control groups.[62] Patients with PD also present hallmark pathophysiological signs such as increased intestinal permeability and inflammation.[63,64] Recently, Sampson et al.,[65] using a mouse model of PD, demonstrated that the gut microbiota is required for symptoms associated with PD. In this model, mice overexpress alpha-synuclein, a protein that aggregates in PD neurons, causing synucleinopathies that result in the motor dysfunction characteristic of PD. Importantly, the study revealed a causative effect of the gut microbiota in PD, as colonization of mice with microbiota from patients with PD exacerbated movement impairments compared to mice colonized with microbiota from healthy donors.

DEPRESSION

Depression, the most common mental disorder, is a multifactorial disease that can result from biological, psychological, and social factors.[66] Although its etiology is not well understood, depression has been correlated with the individual's stress levels and lifestyle,[67] but can also be induced by anxiety (see below) and chronic diseases.[68] Symptoms include depressed mood, lack of inclination for daily activities, decreased appetite, and altered sleep and libido; severe depression is directly linked to self-harm and suicide.[69] Depression and anxiety are reported by about 70−90% of individuals with intestinal inflammation, thereby indicating a potential link between the human gut microbiota and these disorders.[70−72] Several factors have been shown to correlate with changes in the intestinal microbiota, including maternal separation, isolation, social phobia conditions, and other types of stress.[73−75]

A link between depression and the microbiota has been established by research with mouse models. GF mice, lacking a normal gut microbiota, display less of the behavioral impairment associated with depression and anxiety than mice having a microbiota.[76] Microorganisms may influence the development of depression via the gut-brain axis by modulating inflammation, the hypothalamic−pituitary−adrenal (HPA) axis, or signaling neurotransmitters.[77] The HPA axis balances the stress response, acting directly on the microbiota−gut−brain axis.[75,78−82]

Zheng et al.[83] demonstrated that the gut microbiota may play a role in the development of depression in mice, altering the metabolism of the host. In the same study, the authors, using clinical samples, found that patients with major depressive disorder displayed differences in the relative abundance of *Firmicutes, Bacteroidetes*, and *Actinobacteria* when compared with healthy individuals. Transplantation of GF mice with fecal microbiota from patients with depression resulted in depression-like behaviors compared with colonization with microbiota from healthy individuals, demonstrating that dysbiosis may have a causal role in the development of depression.

Studies with healthy human volunteers have reinforced the link between the microbiota and diseases such as anxiety and depression. According to Dinan et al.,[84] the stress caused by physical or psychological factors may be directly related to the imbalance of the microbiota−gut−brain axis. Messaoudi et al.[85] showed that probiotics consisting of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 greatly reduced the symptoms of depression (and anxiety, see below) in healthy human volunteers. Given the relationship between host metabolism, the microbiota, and the manifestation of depression, new therapies could be developed that modulate the intestinal microbiota for the treatment of depression. A deeper understanding of the causative microbes and mechanisms involved in the microbiota−depression relationship is needed in order to target these with appropriate microbiome engineering strategies.

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ANXIETY DISORDER

According to the American Psychiatric Association,[86] anxiety disorder is characterized by behavioral disturbances, phobia, and panic syndrome, as well as fear. Anxiety can also be understood as social phobia, which consists of fear in social situations that interferes directly with social conviviality.[87,88]

Because microorganisms can act within the autonomic nervous system, as well as in the HPA axis, changes in alimentary behavior may have consequences for anxiety disorder.[89] Several studies have analyzed the microbiota−brain−behavior relationship by inducing dysbiosis with antimicrobials.[88] Some of these studies have reported that infection by pathogenic bacteria may cause the increase of symptoms associated with anxiety.[90−92]

Neufeld et al.[93] also observed that GF animals exhibited less anxiety behavior than specific-pathogen-free (SPF) animals, which are free of particular pathogens. These data were corroborated by Arentsen and colleagues,[94] who showed that GF animals displayed greater social conviviality. The GF group of mice presented an anxiogenic (i.e., anxiety causing) mechanism, which indicates a role for the gut microbiota in modulating conditions associated with anxiety.[95]

Lactobacilli and *Bifidobacteria* have shown therapeutic potential in the context of neurological disorders, including anxiety.[85,96,97] A study by Rao et al.[98] showed that strains of *Lactobacillus* can improve symptoms associated with anxiety in patients with chronic fatigue syndrome. Other studies with BALB/c mice demonstrated the efficacy of *Lactobacillus rhamnosus* in reducing symptoms associated with anxiety as well as stress.[99]

A study by Messaoudi et al.[85] emphasized the importance of *Lactobacillus* and *Bifidobacteria* for the reduction of anxiety symptoms. *L. helveticus* R0052 and *Bifidobacterium longum* R0175 conferred beneficial therapeutic activity in this study. Sudo et al.[100] demonstrated that the hypothalamic−hypophyseal−adrenal axis response to stress was potentiated in GF animals, and this relationship was reversed by colonization with *Bifidobacterium* species. Both increased motor activity and reduced anxiety were observed in GF animals compared with mice with a normal gut microbiota in a number of other studies.[31,76,93] Other studies linking the gut microbiota to anxiety include one by Clarke and colleagues,[31] who showed that GF mice have a higher serotonin and 5-hydroxyindoleacetic acid (5HIAA) index and exhibit less anxiety than animals colonized with a normal microbiota.[31] Desbonnet et al.[101] found that adolescent mice treated with antibiotics to remove the resident microbiota presented cognitive alterations, decreased anxiety, memory deficits, and difficulty identifying new and habitual objects.[101]

CONCLUSIONS

Over the years, many studies have found correlations between the gut microbiota and the CNS (The Human Microbiome Project Consortium, 2012).[102] The points at which these studies intersect constitute the new field of neuromicrobiology. Although it is clear that the gut microbiome plays a significant role in the health and disease states of the host, much of the research done to date on this topic has only demonstrated associations between bacterial profiles and certain clinical conditions. It remains to be elucidated whether these links are causative, promoting disease, or are, rather, consequence of unrelated pathophysiology. Several recent causative studies of mice convincingly show the role of the gut microbiota in PD[65] and depression.[83] Similar studies are needed to determine whether causation, as opposed to association, is involved in other neurological diseases.

Although extensive research has already been done on the gut−brain axis (Figure 1), we still do not fully understand the mechanisms by which the intestinal microbiota is modified or the relationship between those modifications and the associated neurological effects. These questions will need to be addressed in future studies. A great deal of the work so far has involved comparisons between GF mice and mice having a microbiota. Such comparisons have been useful to establish behavioral and neurological distinctions between these populations but, nevertheless, represent an extreme "all or nothing" scenario. The next steps to be taken clearly should include comparisons of microbiota having or lacking particular bacterial species or perhaps particular strains.

Thus, existing approaches for microbiome modulation, e.g., broad-spectrum antibiotics and fecal transplants, may broadly disturb the microbiome but do not precisely target individual types of bacteria. Current methods for microbiome characterization, such as metagenomic sequencing, only provide a relative census of the bacteria present in a community, rather than readouts on their specific function. Novel engineering strategies are needed to restructure these microbial communities in order to study the relationships between them and the functioning of the CNS. Potential solutions include the precise reconfiguring of complex microbial consortia through additive, subtractive or modulatory technologies.[103−106]

In addition, all the gradations of neurological manifestations in humans are only weakly recapitulated in animal models, making it difficult to interpret results. Therefore, the development of animal models that more closely mimic human neurological disease will enable the design of more realistic experiments for the field of neuromicrobiology. Ultimately, it will be important to translate observations made with animal models to humans, to further verify the connection between the gut microbiota and the human brain.

There is substantial evidence to suggest that probiotics may offer approaches to improving the health of individuals with neurological disorders. In addition to improving cognitive ability, probiotics modulate the response to stress,[85,99,107] a condition that has been increasingly associated with disease. Indeed, Dinan et al.[108] have referred to probiotics as "psychobiotics". However, the molecular mechanisms regulating the relationships between microbiota and the brain need to be understood in much greater depth before probiotics can begin to be developed as targeted therapeutics.

Future work should extend the foundational research done so far to functional studies aimed at identifying specific bacteria or consortia of bacteria (or their secreted products) responsible for health and disease and to causal studies using existing and improved animal models. Elucidation of the relevant bacterial determinants will certainly impact the diagnosis, treatment, and prevention of neurological diseases.

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