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Chapter 3

# Consequences of Gut Dysbiosis on the Human Brain

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**Abstract**

The central nervous system (CNS) and the gastrointestinal (GI) tract develop in parallel and communicate with each other throughout life using neural, endocrine, and immune pathways, giving rise to the concept of a 'gut-brain axis' in which both organ systems intimately interact. Fundamental to the axis is the GI microbiome, which is the collective genomic aggregate of bacteria and other microorganisms that dwell within the lumen of the GI tract. Increasing evidence gathered from animal models and human studies demonstrates that perturbation of the microbiome, otherwise known as dysbiosis, can lead to specific neurological and psychiatric disorders. This chapter will provide a brief review of the literature that reveals the influence of the microbiome in CNS disease and provide perspectives in treatment through modification of the microbiome.

**Keywords:** microbiome, dysbiosis, brain, multiple sclerosis, Parkinson's disease

## 1. Introduction to the brain-gut-microbiome axis

The human microbiome has emerged as an entity with a tremendous degree of influence in health and disease.[1] Bacteria within the GI tract perform a wide range of symbiotic functions for their host, which range from digestion and the production of bioactive metabolites to influencing the healthy development and function of the immune system [1, 2]. All of these local effects on the GI tract have the ability to impact the brain through neural connections,

[1] For the purposes of this chapter, the microbiome mentioned herein refers to the combined aggregate of bacteria, viruses, fungi, archaea, and protista.

such as the vagus nerve, and by endocrine means [3, 4]. The awareness of this two-way interaction between the gut and the brain has now provided more explanations for some conditions otherwise labeled as 'functional disorders' and has also laid fertile ground for the discovery of new treatment modalities in modulating the microbiome in treating CNS disease [5–7].

Significant development of the microbiome begins at the time of delivery. Vaginally, born infants are colonized by maternal fecal and vaginal microorganisms, whilst those born by Cesarean section are colonized by skin flora [8, 9]. Although the dogma has been that the antenatal intrauterine environment is sterile, this notion has been challenged by several findings, most notably that meconium contains bacterial colonies. Therefore, this implies that the influence of the microbiome may extend into the prenatal period [10, 11]. Following birth, the microbiome adapts according to factors such as dietary intake, antibiotic use, and living conditions. As the CNS and microbiome develop in parallel, significant changes in the microbiota occur at critical neurodevelopment time periods [12, 13]. Disruptions in the evolutionary progression of the microbiome may therefore have a lasting impact on the healthy development of the brain and *vice versa* because of this close interaction between the two systems.

The term 'dysbiosis' refers to an imbalance of microorganisms within the mucosal flora. Hepatic encephalopathy is the archetypal example of how a GI dysbiosis can result in CNS damage. Liver cirrhosis results in a distinct microbiota signature that differs significantly from healthy, control subjects [14]. Accumulation of toxic mediators, such as ammonium produced by urease-producing bacteria, enters the portal circulation. Blood ammonium concentrations rise, cross the blood-brain barrier, and accumulate within the brain leading to astrocytic damage and cerebral edema [15–17]. Curiously, however, treatment with the oral antibiotic rifaximin does not cause clinical improvement through changing the proportions of bacteria in the microbiome, but rather, the improvement in endotoxemia and cognition appears to be through modulation of bacterial metabolism [18–21]. Therefore, derangements of the microbiome that result in CNS dysfunction are not limited to constitutional changes but may also be influenced by its metabolic activity.

## 2. The influence of the microbiome in multiple sclerosis

### 2.1. Epidemiology and pathogenesis

Multiple sclerosis (MS) is the most common CNS demyelinating disease and is classically depicted by the acquisition of discrete demyelinating plaques within the grey and white matter of the CNS [22–24]. The acute MS plaque is characterized by infiltration of inflammatory cells with concomitant demyelination and edema [25]. Perivascular lymphocytic cuffing comprised predominantly of T cells is seen. There is a reactive astrogliosis with variable amounts of oligodendrocyte apoptosis within the plaque [26]. Over time, the plaques become sclerotic, representing the final pathological event at that location after a period of marked inflammation, astrogliosis, demyelination, remyelination, and axonal loss.

Despite this common pathological hallmark of the disease, MS is remarkably heterogeneous in terms of clinical presentation and prognosis [27]. Furthermore, the exact pathogenesis still remains poorly understood, although it is clear that both genetics and the environment have significant influences in the onset of MS and a complex interplay exists between these elements [28, 29]. Certainly, inflammation plays a key role in the pathophysiology of the disease. Most researchers favor an autoimmune hypothesis whereby autoreactive immune cells targeting myelin antigens are activated, likely incited by an environmental trigger [30].

Migrational studies have provided insight into how environmental changes may influence the risk of development of MS. Generally, populations further away from the equator have an increased risk of developing MS than those closer to the equator [31, 32]. Many studies have demonstrated that people migrating from high-risk areas to low-risk areas can be at sustained risk if the migration occurred after a certain critical age point [33]. Conversely, if the age of migration is younger than the critical age point, the individual is conferred the risk of the new region. The human microbiome is recognized to exhibit great geographical variation between populations and the local environment has a marked influence on the development of the microbiota [34, 35]. Given that the microbiota influences neurodevelopment and immunity early in life, one can speculate that this may explain why the conferred migrational risk of MS is age-dependent.

The first suggestion that MS may be related to hygienic living conditions was reported by Liebowitz et al. in 1966 [36]. By examining the degree of crowded living conditions, they found that the incidence of MS was higher in those that are more sanitary. The hygiene hypothesis, formulated later by Strachan in 1989, proposed that allergy and autoimmune diseases are, at least in part, the consequence of inadequate immune stimulation against pathogens during the early years of life that causes aberrant responses to self in later years [37, 38].

One MS epidemic occurred during the British occupation of the Faroe Islands during World War II. Prior to the arrival of British troops in 1940, there were no documented cases of MS in the native born Faroese on the islands. After 1943, there were four MS epidemics and the patients were located in proximity to the British encampments [39, 40]. The conclusion was that somehow the British troops had introduced an unknown pathogenic organism into the islands. Interestingly, the incidence of several infections increased during the occupation that coincided with MS epidemics, notably gastroenteritis and mumps infections, suggesting an association between MS and dysbiosis [41].

Aside from geographical predispositions for MS, other risk factors such as obesity, cigarette smoking, female sex, and low vitamin D levels are all associated with differences in the composition and/ or metabolic activity of the microbiota [42–49]. These epidemiological findings insinuate a potential role for the human microbiome in predisposing MS.

### 2.2. Bacterial dysbiosis and MS

Some of the initial indications that the GI microbiota may play a role in the pathogenesis of MS arose from work on experimental allergic encephalomyelitis (EAE) in germ-free (GF) mice. For decades, EAE has been used extensively as an animal model of demyelinating

disease in which exposure to CNS myelin components, such as spinal cord homogenate or specific myelin proteins, triggers a T-cell-mediated autoimmune response that leads to CNS demyelination [50, 51]. Although there are similarities to relapsing remitting MS, there are notable differences that have been reviewed elsewhere (refer to Sriram and Steiner for a detailed review [50]).

Evidence that the gut microbiota can influence autoimmunity has been gathered from experiments that contrast conventionally housed animals with a normal composition of microbiota [also known as specific pathogen free (SPF) or conventionally colonized (CC)], and those maintained in a sterile environment [germ-free (GF) animals], thus removing the possibility of postnatal colonization of their GI tract. The absence of gut microbiota at birth affects the gut-associated lymphoid tissue (GALT), such that GF mice have hypoplastic Peyer's patches and mesenteric lymph nodes. Furthermore, the lymph nodes have fewer germinal centers and IgA-producing plasma cells than normally present in controls [49, 52]. Beyond the GI tract, the spleen and lymph nodes are also poorly developed [53]. This maldevelopment of the lymphoreticular system provides an explanation as to why GF mice are more prone to infection and why the risk of developing autoimmune disease is modified [54]. The gut microbiome has been shown to influence the probability of developing EAE in GF and SPF mice. Berer et al. showed that in SJL/J mice that have autoreactive CD4 T cells to myelin oligodendrocyte protein, the presence of the GI microbiota promoted the development of EAE [55]. Furthermore, the absence of GI microorganisms in GF mice and the consequent limited production of $T_H17$ cells within the GI tract and spleen appear to be protective against EAE unlike in controls [56, 57]. When segmented filamentous bacteria, which are known to induce the production of $T_H17$ cells, are inoculated into the GF mice, these animals developed EAE with antigenic stimulation, demonstrating that specific bacterial species within the gut microflora can predispose autoimmune demyelinating disease [56].

Several studies have demonstrated changes in the abundances of various bacterial taxa in MS compared with controls. Miyake et al. investigated fecal samples collected during the remission phase from patients with relapsing remitting MS and demonstrated 21 species that were significantly different in relative abundance [58]. Fourteen of these species belonged to the *Clostridia* clusters XIVa and IV, which were reduced in MS patients and are recognized to have an anti-inflammatory role [59]. Furthermore, *Bacteriodes* and *Prevotella* species were less prevalent in MS, although the exact pathogenic significance of this is yet defined. Of note, however, they did not discuss the possible confounding influence of medical therapy that may have been administered to these patients.

Rumah et al. identified *Clostridium perfringens* type B in the stool of a patient 3 months after the onset of MS symptoms [60]. *C. perfringens* B has the capability of producing Epsilon toxin (ETX), which can cross the blood-brain barrier and have toxicity to oligodendrocytes, thus providing a possible mechanism for demyelination in MS [61, 62]. Their analysis also revealed a reduced frequency of *C. perfringens* A in the GI tract of MS patients and that ETX reactivity was ten times more common than in controls. Another group identified a significantly increased Archaea (*Methanobrevibacteriaceae*) in MS contrasted with controls [63]. *Methanobrevibacter smithii* is considered to be strongly immunogenic and may be pro-inflammatory in the

host. The same researchers also identified several organisms that were anti-inflammatory and were seen in a lower abundance in MS. Significant differences in microbiota in *Proteobacteria*, such as enrichment of *Shigella* and *Escherichia*, were also observed in pediatric MS when compared with controls [64].

### 2.3. Viral etiology

Many viruses have been implicated as risk factors for the development of MS [65]. Perhaps the most discussed has been the Epstein-Barr virus (EBV), which can be present in the oral microbiome and can be transmitted by saliva [66]. Humans are the obligate host for EBV and while many healthy controls are infected, nearly all patients with MS have seropositivity for EBV [67]. Furthermore, infectious mononucleosis (IM) resulting from EBV infection doubles the risk of developing MS. Similarly, a recent meta-analysis revealed significant associations between anti-EBNA (EBV nuclear antigen) IgG positivity, infectious mononucleosis, and smoking in conferring an increased risk of developing MS [68–70].

Several research findings have identified the presence of EBV within B cells from MS patients. One study identified the presence of EBV latent proteins being expressed in B cell follicles within the cerebral meninges and that the infiltrating B cells had EBV infection [71]. Interestingly, the cerebrospinal fluid (CSF) in MS is usually associated with oligoclonal bands, which is the product of IgG secretion from clonally expanded B cells [72]. Screening of these oligoclonal antibodies has identified BRRF2 and EBNA-1, which are EBV-related proteins, as possible targets of the CSF IgG immune response [73]. Exactly, how EBV fits into the pathogenesis of MS remains to be determined; however, its association with the oral microbiome in MS is evident.

### 2.4. Altering the microbiome-protection against MS by helminth infection

Certain helminthic infections appear to reduce the risk of developing MS [74]. Infection with *Trypanosoma cruzi* and *Paracoccidiodes brasiliensis* in MS patients causes lymphocytes to produce higher amounts of interleukin-10 (IL-10) and neurotrophic factors, such as brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF), in comparison with controls [75]. In MS, there is usually a low amount of IL-10 secretion favoring a $T_H1$ response, rather than a $T_H2$ response as is present in helminthic infections [76]. *Trichuris suis* is a helminth that has efficacy when administered orally in inflammatory bowel disease (IBD). Treatment with this helminth in MS is associated with elevated IL-4 and IL-10 as well as radiological improvements on MRI [77]. Another group demonstrated reduction in IFN-$\gamma$ and IL-2 as well as an increase in IL-10 and IL-4 in secondary progressive MS following *Trichuris suis* administration, suggestive of a shift toward a $T_H2$ response [78]. In summary, therapeutic manipulation of the gut microbiome that favors an overall anti-inflammatory phenotype appears to have great promise in the treatment of MS. Further trial data are needed in this field to evaluate its efficacy and safety.

## 3. Parkinson's disease (PD)

PD typically manifests with bradykinesia, postural instability, and resting tremor that results from progressive neurodegeneration within the basal ganglia that is associated with abnormal $\alpha$-synuclein accumulation and Lewy body formation [79, 80]. Until recently, it was thought that PD originated within the CNS, in which the pathological protein $\alpha$-synuclein spread from the dorsal motor nucleus of the vagus to involve the basal ganglia and thenceforth the cerebral hemispheres [81]. However, the characteristic pathology has since been found in tissues outside of the CNS. Slow transit constipation is now well recognized to be a common finding that predates the motor symptoms in PD and can be present many years prior to diagnosis [82–84].

Findings from autopsy and surgical pathology studies have demonstrated the presence of phosphorylated $\alpha$-synuclein in the sublingual glands and within Auerbach's and Meissner's plexuses of the enteric nervous system [85–87]. Consistent with these findings, GI pathology and concomitant symptoms predate motor features by at least 2 years [88, 89]. Transmission of $\alpha$-synuclein from neuron to neuron has been elegantly shown in a rotenone animal model of PD wherein ingestion of rotenone causes release of $\alpha$-synuclein and retrograde spread toward the brainstem; hemitransection of the vagal nerve, however, protects against ipsilateral synuclein pathology [90]. Similarly, neuronal transmission of $\alpha$-synuclein occurs in human mesencephalic fetal transplants of PD patients in which $\alpha$-synuclein is detected within the grafted cells at *postmortem* examination [91, 92]. The vagus nerve can therefore act as a conduit for the proteopathic spread of $\alpha$-synuclein from the periphery to the brainstem. In a novel retrospective study, Svensson et al. showed a reduction in PD risk in patients who had truncal vagotomy compared with super-selective vagotomy and between truncal vagotomy and the general population [93]. While the study did not reach statistical significance, the complete severance of neural bidirectional communication between the GI tract and the brain may be beneficial in preventing the proteopathic spread of $\alpha$-synuclein and hence avert PD neurodegeneration that might otherwise be inevitable.

Several pieces of data suggest that the microbiome has an involvement in PD. Scheperjans and colleagues demonstrated in a case control study of PD that *Prevotellaceae* species were significantly reduced whilst *Enterobacteriaceae* species were increased in patients with motor-predominant rather than tremor-predominant PD [94]. Furthermore, the quantity of *Enterobacteriaceae* correlated with the degree of postural instability. However, the question remains as to whether the dysbiosis is a consequence or cause of gastrointestinal dysmotility and also how this could influence the production, aggregation, or release of $\alpha$-synuclein within the GI tract. Regardless of the microbiome's significance in the pathogenesis of PD, bacterial overgrowth that occurs in a proportion of patients with GI dysmotility can influence the symptomatic response to drug treatment. Malabsorption of drugs, in particular levodopa, is the probable reason for the increased motor fluctuations seen in this dysbiosis. Administration of rifaximin has shown improvement in these cases [95, 96].

The oral and nasal microbiota may also be relevant in PD and require investigation. The nucleus of the glossopharyngeal nerve exhibits $\alpha$-synuclein deposits, and similarly to the

vagus, the glossopharyngeal nerve may act as a route for peripheral entry of PD pathology into the brain. Furthermore, the olfactory bulb is frequently affected by PD pathology prior to the onset of motor symptoms and this is an explanation for the often prodromal anosmia [97]. Whether there is a dysbiosis in the oral or nasal cavity has yet to be ascertained, but may offer clues as to why PD pathology occurs in these other peripheral anatomic sites.

## 4. The role of the microbiome in psychiatric disease

The impact of the GI microbiome on human behavior and psychiatric disease is complex, but there are several observations that demonstrate strong associations between the two entities. First, anxiety and depression frequently co-exist with chronic gut disorders [98–102]. Second, mouse models of GI infection demonstrate elevated levels of anxiety-like behavior and alterations in CNS biochemistry [103]. Third, it was realized decades ago that stress occurring early in life or later in adulthood can alter the microbial composition of the gut [104]. More recent investigations conducted in animal models, and human patients have delved deeper into these associations and have attempted to elucidate how the commensal microbiome influences behavior.

The hypothalamic-pituitary-adrenal (HPA) axis is fundamental to the stress response, and endocrine disturbances of this axis have been attributed to depression and anxiety. In patients with severe depression, overactivation of the HPA axis causes hyper-secretion of catecholamines, corticotropin-releasing factor (CRF), and vasopressin [105]. Patients with depression often show elevated plasma cortisol levels, elevated CRF concentrations within the CSF and increased limbic concentrations of CRF [106, 107]. Sudo et al. conducted the first study to demonstrate the involvement of the gut microbiome in the normal development of the HPA axis [108]. GF mice were shown to have an exaggerated elevation of plasma adrenocorticotrophic hormone (ACTH) and corticosterone (the dominant glucocorticoid in rodents) in response to stress compared to SPF mice [108, 109]. However, when *Bifidobacterium infantis*, a bacterial species found in the infant gut, was inoculated in these mice, the exaggerated stress response was normalized. Importantly, this reversal took place only when the bacterial inoculation occurred by 6 weeks *postnatum*, suggesting a neurodevelopmental window of susceptibility to the effects of this bacteria-host interaction [108].

Other studies have focused on the effect of the microbiome on behavior and brain biochemistry. One of the challenges in interpreting the results of these reports is that they differ in animal strain, sex, and sourcing as well as overall experimental design. Despite these differences, it is clear that the microbiome influences both behavior and brain neurobiology. With regard to behavior, the majority of studies that compare GF with SPF mice report a decreased anxiety-like behavior in GF mice, in spite of an exaggerated HPA axis response to acute stress [109–111]. The one notable exception in mice, however, was a study by Nishino et al. [112]. This group compared GF mice with gnotobiotic mice, which are mice born in germ-free conditions to parents fed stools of SPF mice, and found that the ex-GF mice are less anxious. In this model, the transfer of the bacterial species *Brautia coccoides* reduces anxiety-like behavior [112].

Further evidence of the microbial influence on behavior derives from transferring microbiota between mouse strains of inherently different behavioral phenotypes. In a study by Bercik et al. colonization of GF mice with gut bacteria from donor mice with differing anxiety phenotypes transferred the behavioral phenotype of the donor to the recipient [113]. Again, it appears that there is a critical neurodevelopmental time point before which behavioral profiles are modifiable as adult GF mice colonized with SPF feces retain the anxiolytic behavioral phenotype of GF mice [110].

Animal studies that have investigated brain neurochemistry and examined monoamine concentrations and turnover rates have provided some insight into explaining these behavioral phenomena. Alterations in central monoamine neurotransmission, specifically serotonin (5-HT), norepinephrine (NE), and dopamine (DA), are known to play a role in anxiety and depression [114–119]. As would be predicted based on their behavioral patterns, ex-GF mice exhibit higher NE and DA turnover rates and have higher 5-HT concentrations in the striatum than their GF counterparts [112]. Furthermore, stress-sensitive rats that exhibit anxiety-like behavior when GF had a reduced DA turnover in the frontal cortex, striatum, and hippocampus than SPF rats [120]. Many of the studies that investigate monoamine transmission examine tryptophan levels as well, as tryptophan is required for the synthesis of 5-HT and may be low in depression [121]. Kynurenine, a metabolite of tryptophan, is increased in depression and the kynurenine:tryptophan ratio in blood correlates with anxiety [103, 122, 123]. Accordingly, the less anxious GF mice exhibit a decreased kynurenine:tryptophan ratio and increased plasma tryptophan concentrations compared to the more-anxious SPF mice [109]. In male GF mice, there is a significant sex-dependent increase in 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) concentrations. Notably, the reduced anxiety seen in GF animals is normalized following microbial colonization as well as normalization of both the tryptophan concentrations and the kynurenine:tryptophan ratio. Interestingly, however, the increased 5-HT and 5-HIAA concentration in GF animals remains resistant to colonization [109]. Using mass spectrometry, Matsumoto et al. analyzed the cerebral metabolome of GF mice and ex-GF mice and identified 38 metabolites that differed significantly [124]. Notably, concentrations of DA were twofold higher in GF than in ex-GF mice; consistent with the findings that GF mice display increased motor activity and reduced anxiety-like behavior compared with their ex-GF counterparts. In the cerebrum of GF mice, the concentration of tryptophan was decreased but the study failed to find differences in 5-HT levels.

Various receptors, with known roles in depression and anxiety, are influenced by the microbiome. For instance, the 5HT1A receptor, which is associated with anxiety, has decreased expression in the dentate granule layer of the hippocampus in GF female mice [111]. The N-methyl-D-aspartate (NMDA) receptors are important for learning and memory. Sudo et al. reported downregulation in the NMDA receptor subunit 2A (NR2A) mRNA in the cortex and the hippocampus of GF mice compared to SPF mice [108]. Neufeld et al., however, did not detect differences in the hippocampal subregions by *in situ* hybridization but rather demonstrated a decrease in NMDA receptor subunit 2B (NR2B) mRNA expression in the central amygdala of GF mice [111]. Additionally, DA D1 receptor mRNA was significantly higher in the hippocampus of GF mice than in SPF mice [110].

In patients with depression, hippocampal neurogenesis is reduced along with levels of BDNF, and recent evidence indicates that increased hippocampal BDNF is associated with anxiolytic and antidepressant behavior [125]. In the studies of GF mice, the exact influence of the microbiome on BDNF is uncertain; some studies show increased hippocampal BDNF expression, while others show the opposite at both the mRNA and protein level. The differences in BDNF appear to be sex-related, with reductions in BDNF being observed mainly in male GF animals, and not in female animals [108, 109, 111]. Heijtz et al. showed that in GF mice, mRNA expression of nerve growth factor-inducible clone A (NGFI-A), implicated in the development of anxiety-like behavior, was significantly lower in the orbital frontal cortex, striatum, hippocampus, dentate gyrus, and amygdala compared with SPF mice [110]. These studies highlight a role for neurotrophic factors in the microbiome-gut-brain axis and its influence on anxiety and depression and further indicate that regulation of this axis may be sex dependent. The microbiome also appears to have a broader impact on neurobiology, as evidenced by a study looking at the amygdala that showed an altered transcriptome in GF compared to SPF mice. Specifically, in GF mice, there is upregulation of immediate early response genes with differential expression of genes involved in neurotransmission, plasticity, and metabolism [126].

### 4.1. Treatments that alter the microbial flora

#### *4.1.1. Probiotics*

Associations between the microbiome and behavior are reinforced by studies using probiotic therapy, which alter the gut microbial environment through the ingestion of live bacterial cultures. There is increasing evidence that certain strains are able to attenuate various behavioral and biochemical effects of stress. Adult rats given *Lactobacillus helveticus* NS8 display reduced stress-induced anxiety and depression that is comparable to citalopram therapy [127]. Biochemically, these rats had lower corticosterone and ACTH concentrations in the plasma, increased hippocampal monoamine concentrations and BDNF transcription as well as higher levels of plasma IL-10, which is an anti-inflammatory cytokine that is reduced in depressed patients [127]. Similarly, the probiotic formulation of *L. helveticus* R0052 and *B. longum* R0175 attenuated the response to chronic stress, with decreased levels of corticosterone, epinephrine, and NE within the plasma [128]. Furthermore, the probiotics *B. longum 1714* and *B. breve 1205* are anxiolytic in mice [129]. Probiotics with *B. infantis*, previously discussed as being capable of reversing the exaggerated stress response in GF mice, have shown various results in rats; one showed attenuation of behavioral and biochemical abnormalities associated with maternal separation, whilst another showed minimal behavioral effects, despite changes in the levels of various cytokines and metabolites [130, 131].

Chronic treatment with *L. rhamnosus* (JB-1) in mice lowered stress-induced corticosterone as well as anxiety- and depression-related behavior and caused alterations in Gamma-Aminobutyric acid [GABA(B1b) and GABA(A$\alpha$2)] receptor mRNA in specific regions within the brain [132]. Interestingly, these findings were not found in vagotomized mice [132]. The vagus is known to mediate communication between the gut microbiota and the HPA axis, with

increased CRF mRNA, plasma ACTH, and corticosterone concentrations in rodents following vagal stimulation [133]. Furthermore, in humans, vagal nerve stimulation has antidepressant effects, including normalization of the HPA axis [134, 135]. In mice, JB-1 increased the firing rate of the mesenteric nerve bundle, which was prevented by subdiaphragmatic vagotomy [133]. Similarly, in a mouse model in which chronic mild DSS colitis induces anxiety-like behavior, *B. longum* NCC3001 normalized the anxiety-like behavior and CNS changes induced by chronic gut inflammation but not in mice that had undergone vagotomy [136]. Similar results were obtained in a *T. muris* parasite model of chronic colonic infection, further supporting a neurally mediated mechanism of the probiotic effect [103].

From an immunological perspective, Smith et al. demonstrated that *Recombination activating gene-1* (*Rag1*) knockout mice, which are B and T cell deficient, had a dysbiosis, altered behavior, and heightened HPA axis activity [137]. When pretreatment with *L. rhamnosus* (R0011) and L. *helveticus* (R0052) was administered, the microbiota and behavioral changes were normalized [137]. In rats, myocardial infarction (MI) is accompanied by increased cellular apoptosis in the limbic system and a depression-like behavior [138]. In this model, administration of probiotics that combined *L. helveticus*and *B. longum* ameliorated post-MI depression through reduction in pro-inflammatory cytokines and restoration of barrier integrity in the GI tract [139]. Interestingly, using the IL-10 knockout mouse, which is a model of colonic inflammation similar to IBD, Ohland et al. showed that the ingested diet and the presence or absence of inflammation within the GI tract can influence probiotic efficacy [140]. This suggests a role for immune cells in the intestinal and behavioral health in rodents. Collectively, these studies overwhelmingly support a role for probiotic strains in modulating various aspects of brain function and behavior, some of which appear to be at least partly vagal dependent.

#### *4.1.2. Prebiotics*

Prebiotics are food components that modulate the microbiota by enhancing the growth of probiotic microbes and have been used in several studies to further define a role for the microbiome in behavior. Human milk oligosaccharides (HMO) promote the growth of specific bacteria including probiotic members of the genus *Bifidobacterium* and *Lactobacillus*. Mice fed the prebiotic containing the human milk oligosaccharides 3′Sialyllactose (3′SL) or 6′Sialyllactose (6′SL) showed less anxiety-like behavior, and less microbiota alteration in response to stress [141]. The prebiotics, fructo-oligosaccharide (FOS), and galacto-oligosaccharides (GOS) promoted the growth of the *Lactobacilli* and *Bifidobacteria* in the gut and raised hippocampal BDNF and NR1 subunit expression compared with controls. GOS also increases hippocampal NR2A subunits and NR1 expression within the frontal cortex and increases plasma D-alanine, which acts as an agonist at the NMDA receptor [142]. These studies show that prebiotic-mediated proliferation of gut microbiota, like probiotics, can affect brain neurochemistry and animal behavior.

#### *4.1.3. Antimicrobials*

Administration of oral, but not intraperitoneal, antimicrobials (neomycin, bacitracin, and pimaricin) to SPF mice increased the proportion of *Lactobacilli* and *Actinobacteria* populations,

while decreasing the proportion of *γ-proteobacteria* and *Bacteroidetes* populations [113]. These microbiota changes were associated with improvements in standardized tests of less apprehensive behavior, effects that were reversible after discontinuation of antimicrobial treatment and return to pretreatment microbiota profiles. These changes were independent of inflammation, levels of gastrointestinal neurotransmitters, and nervous system integrity.

### 4.2. Evidence from human studies

#### *4.2.1. Microbial diversity among different populations*

Cross-sectional studies in humans have begun to investigate the gut microbial composition and its association with mood. Naseribafrouei et al. analyzed fecal samples from patients with depression and controls and found no overall significant difference in species diversity between depressed and non-depressed samples but rather a general overrepresentation of the order *Bacteroidales* in depression and a decrease in family *Lachnospiraceae* [143]. At a genus level, *Alistipes* and *Oscillibacter* were associated with depression. Jiang et al. analyzed fecal samples from patients with active major depressive disorder (active-MDD), responded major depressive disorder (responded-MDD) and healthy controls. In contrast to the first study, Jiang et al. found increased fecal bacterial $\alpha$-diversity in the active-MDD group when compared with controls; however, this was not found between the responded-MDD when compared with controls [144]. The three dominant phyla *Bacteroidetes, Proteobacteria*, and *Actinobacteria* were increased, while *Firmicutes* were significantly reduced in both active-MDD and responded-MDD groups than in controls. *Faecalibacterium* was associated with a negative correlation with the severity of depressive symptoms. Concordant with Naseribafrouei et al., an increase in *Oscillibacter* and *Alistipes* was found in depression compared to controls [144]. Of note, possible confounding elements to explain the differences between these two studies may be related to the recruitment of controls from an outpatient neurology clinic by Naseribafrouei et al., unlike Jiang who recruited healthy subjects as controls [143, 144]. Additionally, differences between ages of subjects as well as geographic locations between the studies could contribute to differences in bacterial diversity, as diversity of gut bacteria is known to be influenced by several factors including health status, age, diet, and antibiotic use [47]. In a cross-sectional observational study examining associations between the gut microbiome and maternally rated temperament in toddlers, it was found that certain dimensions of temperament could be associated with differences in phylogenetic diversity [145]. In addition, they found certain sex-specific associations between temperament and the gut microbiome.

These studies begin to identify bacterial groups potentially harmful in the pathogenesis of mood disorders, though further studies will be needed to elucidate temporal and causal relationships between gut microbiota and depression as well as to evaluate their utility as biomarkers of disease. Again, as in the rodent studies, these results point to sex-related differences in how the microbiome may be regulated and how it affects the CNS and behavior.

*4.2.2. Human probiotic studies*

To date, few probiotic studies have been conducted in humans to evaluate their effects on mood. The consumption of a 3-week course of a yogurt containing *Lactobacillus casei* Shirota (LcS) improved mood in patients with low mood (as evaluated by a questionnaire-based assessment) [146]. In healthy volunteers who received a 30-day course of *L. helveticus* R0052 and *B. longum* R0175 compared to placebo, probiotic-treated subjects displayed lower somatization, depression, and anger hostility [147]. A study by Steenbergen et al. aimed to complement these findings and showed that participants who received a multispecies probiotic had reduced cognitive reactivity to sad mood [148]. In a randomized double-blind placebo trial with chronic fatigue syndrome (CFS) patients, two months of daily LcS induced a significant rise in both *Lactobacillus* and *Bifidobacterium* and a concomitant significant decrease in anxiety symptoms compared to controls [149]. These results provide evidence that the intake of probiotics may help reduce negative thoughts associated with sad mood. Probiotic supplementation warrants further research as a potential treatment or preventative strategy for depression.

## 5. Perspectives

The topics discussed in this review emphasize the broad influence that the microbiome has in a wide range of psychiatric and neurologic diseases. Changes in the microbiome are relevant to many brain diseases and understanding what the abnormal changes in the GI microflora are in these conditions is necessary to identify novel targets for therapies. Recognition of pathological changes in the constitution of the microbiome offers a possible means of anticipating or prognosticating future disease. It also provides an opportunity to intervene and correct a dysbiosis with beneficial effects on the disease.

The role of the microbiome in neurodevelopment cannot be underestimated. As previously discussed, exposure to particular microorganisms at specific time points in animal models can have lasting impacts on neurological disease risk and behavior. Treatments that alter the microbial flora may influence healthy brain development and further work in this area is needed to appreciate how significant this may be in humans.

Future work that expands on our current understanding of the dysbioses that occur in CNS diseases should hopefully provide further insight into microbiota-related disease mechanisms and provide additional therapeutic options for patients.

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