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REVIEW

Recent developments in understanding the role of the gut microbiota in brain health and disease

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There is a growing appreciation of the role of the gut microbiota in all aspects of health and disease, including brain health. Indeed, roles for the bacterial commensals in various psychiatric and neurological conditions, such as depression, autism, stroke, Parkinson's disease, and Alzheimer's disease, are emerging. Microbiota dysregulation has been documented in all of these conditions or in animal models thereof. Moreover, depletion or modulation of the gut microbiota can affect the severity of the central pathology or behavioral deficits observed in a variety of brain disorders. However, the mechanisms underlying such effects are only slowly being unraveled. Additionally, recent preclinical and clinical evidence suggest that targeting the microbiota through prebiotic, probiotic, or dietary interventions may be an effective "psychobiotic" strategy for treating symptoms in mood, neurodevelopmental disorders, and neurodegenerative diseases.

Keywords: microbiota; brain; aging; neurodegeneration; behavior; diet

Introduction

Advances in sequencing and metabolomics technologies have enabled scientists to explore the full extent of how microbes affect every aspect of the world we live in. Indeed, almost all areas of medicine have been shown to be affected by the trillions of bacteria that reside within the gut, including neurology and psychiatry. However, it is easily forgotten that the concept of microorganisms conferring host health benefits has existed for some time. It is now over 100 years since the Nobel laureate Elie Metchnikoff of the Pasteur Institute in Paris proposed that lactic acid bacteria may play a key role in the programming of the aging process.[1] Hubert J. Norman, working at the Camberwell House asylum, and George Porter Phillips, working in the Bethlem Royal Hospital, both in London, tested the concept that lactic acid bacteria may ameliorate the symptoms of depression.[2,3] Since then, considerable advances have been made in understanding how the bacterial commensals within our gastrointestinal system can influence host health.

Over the past decade, it has become clear that the bidirectional communication pathway between gut bacteria and the central nervous system (CNS), the microbiota–gut–brain axis, exerts a profound influence on key brain processes, such as neuroinflammation, activation of the stress axes, neurotransmission, and neurogenesis, in addition to modulating complex behaviors, such as sociability and anxiety.[4–13] Gut bacteria influence these central processes through their ability to synthesize neurotransmitters (i.e., γ-amino butyric acid (GABA), noradrenaline, and dopamine) and modulate activation of the immune system, along with their ability to produce metabolites, such as short-chain fatty acids (SCFAs), that possess neuroactive properties.[4] Moreover, the gut microbiota and the brain are linked through additional pathways, such as the vagus pathway, and through the modulation of key dietary amino acids, such as tryptophan[14–17] (Fig. 1). Given the close association between the gut microbiota and the brain, it is not all that surprising that gut bacteria have roles to play in neurological and psychiatric diseases. Evidence now suggests that

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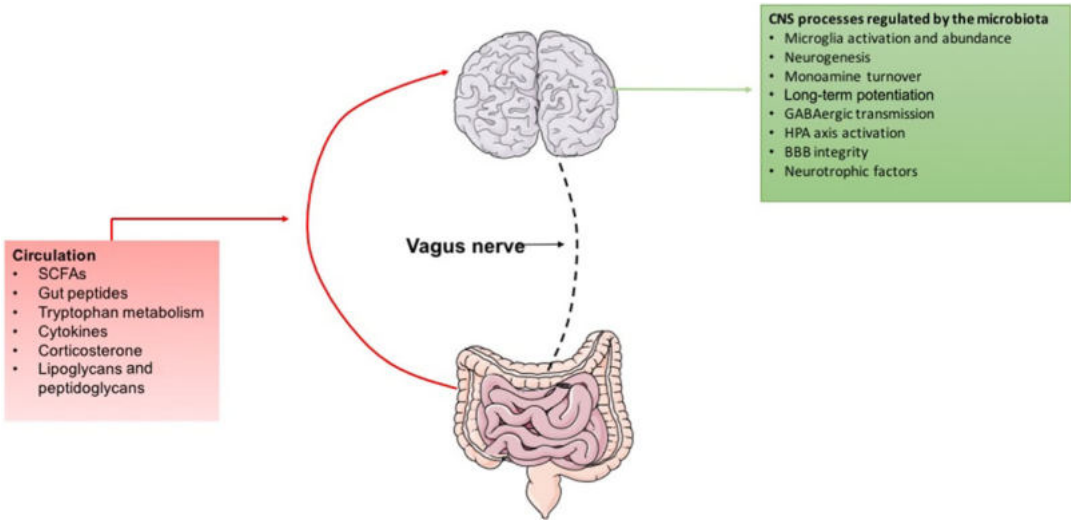

Figure 1. Key mechanisms underlying communication between the gut microbiota and the brain. Gut bacteria can signal to the brain through a variety of mechanisms. These include the production of SCFA metabolites, modulation of immune signaling to the brain, and transmission via the vagus nerve. These mechanisms serve to influence central homeostatic processes, such as neurotransmission, neurogenesis, and neuroinflammation, all of which are involved in several neurological and psychiatric conditions. SCFAs, short-chain fatty acids; BBB, blood–brain barrier; HPA, hypothalamic–pituitary–adrenal axis; CNS, central nervous system; GABA, γ-amino butyric acid.

the bacterial commensals of the gut can influence conditions such as depression, schizophrenia, and autism.[5,15,18–20] While great strides have been taken in elucidating how bacterial commensals may be involved in these conditions, more insight is required before microbiota-based therapies can be rationally employed as viable treatment options. Perhaps what is surprising, however, is the extent to which gut bacteria appear to be involved in other facets of brain health and behavior. Emerging research now suggests that the microbiota–gut–brain axis is also involved in neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease, and psychiatric conditions, such as addiction.

Microbiota and neurology: aging and neurodegeneration

With an increase in the age that humans are living there is a need to better understand the biological mechanisms underpinning the aging process. In light of this, it is important to elucidate how aging affects the microbiota–gut–brain axis, as it may provide us with a greater insight into age-related diseases, such as stroke and neurodegenerative diseases (i.e., Parkinson's disease and Alzheimer's disease).

Aging

Advances in modern medicine have led to an increase in human longevity. While humans are living longer, the onus is now on medical and scientific research to mitigate the severity of age-related diseases (i.e., cognitive decline, stroke, gastrointestinal disturbances, cancer, etc.). Although there is a growing body of evidence as to how bacterial commensals are affected with age,[21–23] there is limited information to date about how such changes may affect the microbiota–gut–brain axis. However, this deficit is beginning to be addressed.[24] For instance, we have recently characterized the microbiota composition of aged (20–21 months old) versus young (2–3 months old) mice.[25] Aged animals displayed an altered microbiota, with changes observed in phyla that have previously been associated with inflammation, which is consistent with previous reports.[25,26] Aged animals display increased basal intestinal permeability, which is exacerbated in response to stress. Such increases in intestinal permeability are likely to increase the risk for the translocation of bacteria or bacterial components (i.e., lipopolysaccharide (LPS)) from the gut lumen into the circulation, where they can elicit an inflammatory response. Indeed, aged mice display an increase

in several circulating inflammatory cytokines (i.e., interleukin (IL)-1β, IL-4, IL-2, and tumor necrosis factor (TNF)-α) compared with young mice.[25]

Other studies assessing the effect of age on the murine microbiota have reported conflicting results. For instance, Conley *et al.* reported no differences in the relative ratio of Firmicutes to Bacteroidetes in aged mice (26 months old), with an increase in the *Muscipirillum* genus their most significant outcome.[27] However, a separate study by Kim *et al.* identified an increase in the level of Firmicutes and Actinobacteria with a corresponding decrease in Bacteroidetes in aged animals (18 months).[28] However, these differences may be due to the fact that aged female C57 mice were used in the study by Conley *et al.*, while Kim *et al.* used aged male C57 mice.[27,28] Although these studies report different alterations to the microbiota with respect to age, both argue that the altered microbiota may be driving the observed elevated inflammatory response in aged animals.[27,28] The elevated circulating inflammatory profile of aged animals may help to explain the cognitive behavioral deficits reported in these animals.[25]

While the microbiota appears to be affected by aging, evidence suggests that targeting the gut commensals provides an opportunity to mitigate age-associated inflammation and other deleterious conditions associated with age. For instance, treatment of aged mice (18 months old) with the *Lactobacillus brevis* OW38 strain was found to improve the expression of intestinal tight junction proteins, along with reducing the Firmicutes to Bacteroidetes ratio.[29] Moreover, this *Lactobacillus* strain attenuated circulating levels of inflammatory markers (IL-1β and TNF-α) while also increasing the expression of the neurotrophin brain-derived neurotrophic factor in the hippocampus.[29] Polyphenols have also demonstrated efficacy in attenuating age-associated inflammation and dysregulation to the gut microbiota of mice. Treatment of middle-aged mice (40 weeks old) with the polyphenolic lignan syringaresinol reduced circulating levels of the proinflammatory marker LPS binding protein (LBP), while also increasing the humoral response to vaccination with the influenza virus.[30] Moreover, syringaresinol modulated the gut microbiota by increasing the relative abundance of *Lactobacillus* and *Bifidobacterium* species, while decreasing levels of the potentially harmful *Akkermansia* genus.[30] While these are promising preclinical studies, such modulators of the microbiota will need to be assessed in humans in order to determine whether they display efficacy in mitigating the deleterious effects of aging in humans. Nonetheless, these are promising findings that suggest that targeting the microbiota may help to prolong human longevity and attenuate age-related inflammation.

Parkinson's disease

Parkinson's disease is characterized pathologically by degeneration of the pars compacta of the substantia nigra of the mid brain and widespread neuroinflammation. Neurologically, parkinsonian patients exhibit a range of motor-related impairments, such as bradykinesia, resting tremors, muscular rigidity, and impairments in posture. Before the development of the motor symptoms, parkinsonian patients often report prodromal nonmotor-related symptoms, such as depression, sleep disturbances, and constipation, suggestive of gastrointestinal dysfunction.[31,32] Moreover, gastrointestinal function is further exacerbated following progression of the disease with constipation, impaired gastric emptying, and difficulties with defecation all reported in patients.[32] Furthermore, α-synuclein, the protein aggregate hallmark of Parkinson's disease pathology in the brain, has also been identified in the mucosal and submucosal nerve fibers and ganglia of the enteric nervous systems (ENSs)[33,34] of parkinsonian patients, with some preclinical evidence even suggesting that α-synuclein in the gut can transport to the brain via the vagus nerve.[35]

Given the gastrointestinal disturbances reported in Parkinson's disease, it stands to reason that the microbiota–gut–brain axis is affected in this neurodegenerative disease. Indeed, Scheperjans *et al.* identified a reduced abundance of the *Prevotella* species in fecal samples from parkinsonian patients.[36] A more recent clinical study also identified a reduction in the abundance of *Prevotella* species (*P. copri)* in the microbiota of parkinsonian patients, along with increases in *Akkermansia muciniphilia.*[37] Given that *Prevotella* species produce mucin, which serves to enhance the integrity of the intestinal barrier, a reduced abundance of this bacteria strain may lead to increases in intestinal permeability in parkinsonian patients and subsequent bacterial translocation.[36] In a separate study, the fecal microbiota of

parkinsonian patients were found to contain lower levels of anti-inflammatory–associated bacteria from the genera *Blautia* and *Roseburia* and a higher level of proinflammatory-associated bacteria from the genus *Faecalibacterium* when compared with healthy controls.[38] Recent work by Hill-Burns *et al.* also identified alterations to the gut microbiota in parkinsonian patients; however, the changes reported were different from those in previous reports.[39] Although these studies report different alterations to the gut microbiota of parkinsonian patients, they collectively suggest that there is dysregulation to the gut commensals that may facilitate inflammation and bacterial translocation. Further corroborating a dysregulated microbiota–gut–brain axis in Parkinson's disease was a recent observation by Unger *et al.*, who demonstrated that the levels of SCFAs—the microbial metabolites acetate, butyrate, and propionate—were all significantly lower in parkinsonian patients relative to age-matched healthy controls.[40] Given that SCFAs are able to cross into the brain and possess neuroactive properties, such as regulating microglial activation,[41] a reduction in their production in Parkinson's disease may affect disease pathology. Other reports of microbiota dysregulation in Parkinson's disease include small intestine bacterial overgrowth or high prevalence rates of colonization with the Gram-negative bacterium *Helicobacter pylori*.[42,43] Parkinsonian patients are found to have increased urinary indoxyl sulfate levels, which is indicative of overgrowth of bacteria in the small intestine or constipation.[44] *H. pylori* infection may contribute to the development of parkinsonian symptoms through degenerating dopaminergic neurons in the brain.[45] Interestingly, eradication of *H. pylori* has been shown to enhance the onset time of levodopa (first-line pharmacological treatment for Parkinson's disease symptoms) while also improving tremor, rigidity, and walking ability.[43,46] Metagenomic analysis has revealed that genes involved in the biosynthesis of LPS are upregulated in parkinsonian patients, suggesting that a dysregulated microbiota is potentially driving aberrant immune activation in the neurodegenerative disease.[38]

Animal models of Parkinson's disease have provided considerable insight into the pathology of the neurodegenerative disease. Recent intriguing work has identified a potential mechanism for how the microbiota–gut–brain axis may be involved in the neurodegenerative disease. In the absence of a microbiota, α-synuclein–overexpressing mice do not show as great an impairment in motor and gastrointestinal symptoms when compared with conventionally colonized mice of the same genotype.[47] Microglia in the striatum of germ-free (mice completely devoid of a microbiota; see Fig. 2 for a summary of the neurobiological changes observed in germ-free mice), α-synuclein–overexpressing mice displayed a less activated phenotype relative to conventional colonized mice of the same genotype, suggesting a less reactive phenotype in the absence of a microbiota, which is consistent with reports from previous studies in germ-free mice.[47,48] Moreover, administration of SCFAs (acetate, butyrate, and propionate) to germ-free, α-synuclein–overexpressing mice exacerbated motor deficits to a level comparable to conventionally colonized mice of the same genotype, suggesting that SCFAs are the facilitators of the adverse effect that the microbiota has on motor symptoms.[47] It is worth noting, however, that the concentration of SCFAs administered to mice in this study were considerably higher than what would expected to normally be found *in vivo*. While the data are compelling in suggesting that SCFAs are the mediators of motor dysfunction in these experiments, more work is required to determine whether elevated levels of endogenous SCFAs are driving these behavioral changes. Moreover, the authors demonstrate that microbiota derived from parkinsonian patients exacerbated motor symptoms of α-synuclein–overexpressing mice compared with those mice who received a microbiota from a healthy human, suggesting that dysregulation of the microbiota can worsen motor symptoms when combined with a genetic predisposition.[47] Given these observations, targeting the microbiota–gut–brain axis may help alleviate some of the neurological and gastrointestinal symptoms of Parkinson's disease.

Alzheimer's disease

Alzheimer's disease is the most common form of age-related dementia worldwide. The neurodegenerative disease is characterized by the accumulation of amyloid plaques, tau fibrils, and widespread neuroinflammation that culminates in severe cognitive impairments, such as long-term memory loss, and other symptoms, such as physical disability and exhaustion. Given the neurological

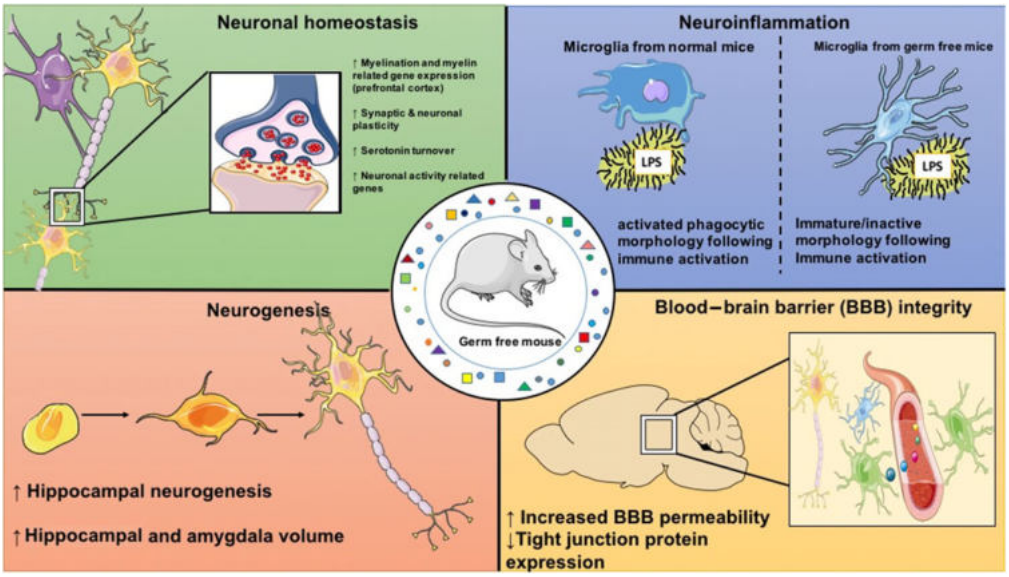

Figure 2. Germ-free mice display alterations to central physiological processes. In the absence of a microbiota, germ-free mice display several unique alterations to brain physiology and chemistry. Microglia from germ-free mice display an immature phenotype and fail to respond to bacterial-associated molecular patterns (i.e., LPS) as efficiently as specific pathogen-free or conventional mice. These mice also display increases in the permeability of the blood–brain barrier (BBB), which corresponds to a reduction in the expression of tight junction proteins. Reduction in the integrity of the BBB may allow for the translocation of immune cells and bacterial components into the brain of germ-free mice. Germ-free mice also exhibit increased neurogenesis, which corresponds to an increase in the volume of the hippocampus of these animals in comparison with specific pathogen-free or conventional animals. Finally, germ-free mice display several unique alterations to neuronal homeostasis. For instance, these animals exhibit hypermyelination of the frontal cortex along with increases in serotonin turnover in the hippocampus. Moreover, there is an increase in markers of synaptic and neuronal plasticity in these animals, which may correspond to the increases in neurogenesis and brain volume observed in these animals. For a comprehensive review on germ-free mice, see Ref. 122. LPS, lipopolysaccharide.

nature of this disease, perhaps it is surprising that emerging evidence suggests that the microbiota–gut–brain axis may have a role to play in this condition. Recently, Cattaneo *et al.* identified that the *Escherichia/Shigella* bacterial genera, which are associated with mediating inflammation, were increased in fecal samples from Alzheimer's patients relative to control subjects. Moreover, the increase in these bacterial taxa positively correlated with an increase in the expression of the proinflammatory cytokines IL-1β and CXCL2 in whole blood.[49] Such results suggest a causal link between dysregulation of the microbiota and systemic inflammation, which may initiate or exacerbate the neurodegeneration that is occurring in the brain in Alzheimer's disease. Given that intestinal permeability increases with age,[25] bacteria or bacterial components (i.e., LPS) may translocate from the lumen of the gut and mediate systemic and neuroinflammation. In support of this, levels of LPS and the *Escherichia coli* K99 pilli protein have been found to be higher in the brain parenchyma and blood vessels of Alzheimer's patients.[50] Moreover, LPS was found to colocalize with $A\beta_{1-40}$ in amyloid plaques, suggesting that bacterial components do indeed translocate from the gut into the systemic circulation and reach the brain in Alzheimer's disease.[50] The microbiota may influence neurodegeneration in conditions like Alzheimer's disease through molecular mimicry. Dendritic cells in the intestine may sense and transport bacterial amyloid antigens to immune cells in Peyer's patches. For instance, the bacterial extracellular amyloid protein curli is sensed by the A11 Aβ oligomer antibody, which suggests that there are configurations of amyloid proteins that have the same structure as microbial proteins.[51]

Preclinical data have provided a greater insight into the role that the microbiota–gut–brain axis may play in Alzheimer's disease. In a recent study, both young and old germ-free mice overexpressing amyloid precursor protein and presenilin 1 (APP/PS1) were found to display reduced levels of $A\beta_{42}$

compared with conventional APP/PS1 mice, along with a reduction in microglial activation.[52] Additionally, these germ-free APP/PS1 mice displayed increased levels of Aβ-degrading enzymes, insulin-degrading enzyme, and neprilysin-degrading enzyme, suggesting that, in the absence of a microbiota, the murine brain is better equipped to target the amyloid pathology.[52] Moreover, in a separate study, chronic treatment of APP/PS1 transgenic mice with an antibiotic cocktail reduced microglial and astrocyte accumulation surrounding amyloid plaques in the hippocampus.[53] This observation is not all that surprising, given that the microbiota can influence glial cell activation and neuroinflammation in the brain.[48] Interestingly, however, antibiotic treatment was found to decrease insoluble Aβ plaques while leading to a corresponding increase in the concentration of soluble Aβ levels, which suggests that the gut microbiota has some bearing on amyloid load in the brain.[53] Other animal models of Alzheimer's disease have also documented alterations to the microbiota–gut–brain axis. In the 5xFAD transgenic model of Alzheimer's disease (which rapidly develops amyloid plaques), levels of human Aβ precursor protein are elevated not only in the brains of these mice compared with wild type but also in the gastrointestinal system (cecum, colon, jejunum, etc.).[54] Moreover, these 5xFAD mice displayed an altered microbiota relative to wild-type controls; with an increase observed in the Firmicutes/Bacteroidetes ratio. Within the Firmicutes phylum, levels of the *Clostridium leptum* species, which are associated with inflammation, were found to be transiently increased in 5xFAD mice.[54] While these preclinical studies suggest that the microbiota–gut–brain axis may be involved in Alzheimer's disease, more work is required to determine whether alterations to the bacterial commensals are facilitating the disease or whether a dysregulated microbiota is a consequence of the central neurodegeneration.

While little is currently known regarding the role of the microbiota–gut–brain axis in Alzheimer's disease, preliminary preclinical evidence indicates that diet or modulators of the microbiota (i.e., probiotics) may provide a means to ameliorate the neurodegenerative disease. For instance, grape seed polyphenol extracts 3-hydroxybenzoic acid and 3-(3-hydroxyphenyl)propionic acid were shown to prevent the assembly of $A\beta_{1-42}$ into toxic fibrils *in vitro*, suggesting that a polyphenol-rich diet may attenuate amyloid accumulation in the brain.[55] The probiotic mixture VSL#3 (combination of *Streptococcus thermophilus, Bifidobacterium breve, B. longum, B. infantis, Lactobacillus acidophilus, L. plantarum, L. paracasei*, and *L. delbrueckii* subspecies Bulgaricus) attenuated age-related deficits in long-term potentiation, suggesting an improvement in memory, in rats while also ameliorating age-related microglial activation.[56] Despite these interesting preliminary preclinical studies, more studies are required with both aged and transgenic animals to determine whether diet or probiotics may be employed into the clinical setting as a preventative measure for the onset of Alzheimer's disease.

Multiple sclerosis

Multiple sclerosis is an autoimmune neurodegenerative disease caused by the progressive loss of myelin sheaths surrounding the axons of neurons. The degenerative disease is characterized by a variety of neurological symptoms, such as vision impairment, ataxia, muscle spasms, paralysis in severe cases, and fatigue. Moreover, bladder- and gastrointestinal-related symptoms have also been reported in multiple sclerosis. For instance, Buscarinu *et al.* recently demonstrated that there is an increase in intestinal permeability along with a reduction in intestinal absorption in patients with the relapsing/remitting form of the condition.[57] A separate clinical study identified an increase in circulating LBP in patients with relapsing/remitting multiple sclerosis, potentially indicating an increase in the translocation of Gram-negative bacteria from the intestinal lumen to the circulation.[58] In addition to alterations in gastrointestinal physiology, there are reports of dysregulation to the composition of the gut microbiota in patients with the condition. Relapsing/remitting multiple sclerosis patients displayed high interindividual variability compared with healthy patients, while there were no significant differences reported in terms of species richness.[59] At the genus level, the gut microbiota of patients with multiple sclerosis displayed significant reductions in *Faecalibacterium*, *Prevotella*, and *Anaerostiples*.[59] More clinical data are required to determine whether these alterations to the gut microbiota and the gastrointestinal system in multiple sclerosis are driving the pathology or if these alterations are simply a comorbid consequence of the disease.

Preclinical studies have provided a greater insight into how the microbiota–gut–brain axis is affected in multiple sclerosis and whether it contributes to the observed pathology. For instance, recent work from our group has demonstrated that the gut microbiota influences myelination within the prefrontal cortex of mice.[60] Germ-free mice displayed increased expression of myelin-related genes (i.e., *Mag, Mbp, Mog*, and *Mobp*) in the prefrontal cortex relative to conventional mice, which was restored to control levels following recolonization with microbiota.[60] Antibiotic administration also increased the expression of myelin-related genes in the prefrontal cortex of nonobese diabetic mice, which further corroborates that the gut microbiota influences myelination within the CNS.[61] Interestingly, germ-free mice display significantly attenuated experimental autoimmune encephalomyelitis (EAE; animal model of multiple sclerosis) scores relative to conventional controls.[62] Kyung *et al.* demonstrated that the reduced EAE severity in germ-free mice was due to a reduced ability of dendritic cells in these animals to activate proinflammatory T cells.[62] Consequently, in the absence of a microbiota, activation of proinflammatory T_H17 cells, which drive neurological damage in multiple sclerosis, is blunted. Interestingly, when germ-free mice were colonized with segmented filament bacteria, EAE deficits were restored in these animals, further corroborating that the microbiota modulates the proinflammatory status of the brain during EAE. In a separate study, Miller *et al.* demonstrated that TNF receptor signaling plays a role in the cross talk between the host microbiota and the immune system during multiple sclerosis. Inhibition of TNFR2 signaling exacerbated multiple sclerosis pathology in myelin oligodendrocyte glycoprotein-specific 2D2 T cell receptor (TCR) mice.[63] However, antibiotic administration attenuated disease severity and mortality in these mice.[63] These findings are likely to have an important bearing on anti-TNF therapies for multiple sclerosis in patients. Interestingly, the composition of the gut microbiota may determine the severity of multiple sclerosis. Rats subjected to EAE were more resistant toward developing multiple sclerosis–like symptoms if they contained a higher diversity of *Lactobacillus spp.* relative to those rats that had a lower diversity of the lactic acid bacteria.[64] Such results suggest that targeting the microbiota using beneficial *Lactobacillus* strains could be used as a preventative strategy for multiple sclerosis.

Stroke and brain injury

To date, only a few studies have investigated how the gut microbiota may be involved in stroke and ischemic brain damage. Clinical data are limited at the moment, but some studies have identified dysregulation to the microbiota in patients following stroke.[65] Alterations to the human microbiota following stroke have been observed, with specific decreases observed in the *Bacteroides fragilis* group and increases in an *Atopobium* cluster noted.[66] Moreover, microbial metabolism was affected by stroke, with decreases in fecal concentration of acetic acid and increases in valeric acid and isovaleric acid.[66] Preclinical data have provided us with a greater insight into how the microbiota–gut–brain axis may be affected in stroke and brain injury. For instance, depletion of gut bacteria through antibiotic administration worsened the survival rate of mice following the induction of ischemia.[67] In the middle cerebral artery occlusion (MCAO) preclinical model of stroke, cerebral ischemia is associated with a dysregulation of the murine microbiota, with a reduction in bacterial α-diversity, along with reductions in intestinal motility and intestinal barrier dysfunction observed as an increase in intestinal permeability.[68,69] Microbial-derived metabolites may also influence stroke susceptibility through modulating platelet activation and thrombosis. For instance, elevated levels of the microbial metabolite trimethylamine *N*-oxide (TMAO; produced from the microbial metabolism of TMA-rich foods) were shown to correlate with an increased risk for thrombosis in cardiovascular patients.[70] TMAO promoted platelet aggregation in the presence of thrombin through enhancing intracellular calcium release.[70] Interestingly, while dietary choline or TMA increased TMAO production and thrombosis in conventional mice, this effect was not observed in germ-free mice, indicating that the microbiota facilitates the production of TMAO through the metabolism of choline-rich foods. Bacteria within the *Allobaculum* taxa (associated with choline metabolism) were positively correlated with thrombosis, indicating that these bacteria may drive TMAO production.[70] Wang *et al.* demonstrated that inhibiting microbial TMA lyases with the choline analogue 3,3-dimethyl-1-butanol reduced

in vivo levels of TMAO while also reducing atherosclerosis, which suggests that the microbiota may present a therapeutic target for the treatment of cardiovascular diseases, such as stroke.[71]

There is considerable evidence to suggest that the gut microbiota modulates immune signaling during stroke to influence the pathological outcome. Transplantation of a dysregulated poststroke microbiota into germ-free mice exacerbated the subsequent cerebral damage mediated by MCAO in these animals, which was associated with increased trafficking of proinflammatory T lymphocytes (T_H1 and T_H17 phenotype) of intestinal origin to the cerebral infarct site.[68] Further evidence for a role of intestinal IL-17 in mediating ischemic damage comes from comprehensive experiments performed by Benakis *et al.* The authors eloquently demonstrated that dysregulation of the murine microbiota following antibiotic treatment resulted in a reduction in the trafficking of the proinflammatory IL-17$^+$ $\gamma\delta$ T cells, which was associated with a reduction in IL-17–associated chemokine expression in brain parenchyma, along with reduced neutrophil accumulation and a reduction in infarct volume of the ischemic site.[72] Thus, the gut microbiota appears to influence the magnitude of poststroke neuroinflammation by modulating intestinal T cell trafficking to the meninges. A recent study by Sadler *et al.* highlighted that mice of the same strain (C57BL/6) but provided from different vendors harbor distinct microbiotas, which influences the neuroinflammatory response following experimental stroke.[73] Notably, the absence of segmented filamented bacteria in C57 mice from one vendor resulted in a reduced ability to produce anti-inflammatory T_{reg} cells following stroke.[73] Such results suggest that the use of mice from different vendors introduces a potential confound of differing microbiotas, even if they are the same strain of animal.

Increased activation of the sympathetic nervous system may also influence dysregulation to the microbiota following stroke or traumatic brain injury. MCAO or traumatic brain injury in mice resulted in a dysregulation to the microbiota, observed as a decrease in the relative abundance of Prevotellaceae and Peptococcaceae, which correlated with an increase in noradrenaline release and noradrenergic innervation of the cecum.[74] Noradrenaline has been shown to inhibit the expression of certain species of *Prevotella in vitro*.[75] Increased activation of the sympathetic nervous system following stroke or traumatic brain injury may lead to increased noradrenergic innervation of the gastrointestinal system that may help to account for the observed dysregulation to the microbiota in these conditions. In support of this, Houlden *et al.* administered 6-hydroxy-dopamine, which results in a potent increase in systemic noradrenaline release, to mice and observed a decrease in the abundance of Prevotellaceae.[74] Additionally, recent preclinical data suggest that the gut microbiota can influence the severity of neurological damage following spinal cord injury.[76]

An additional complication associated with stroke is poststroke infection, which has a high mortality rate. Evidence suggests that the gut microbiota can influence the severity of poststroke infection. For instance, poststroke infection was observed only in specific pathogen–free mice and not germ-free mice, indicating that the bacteria driving the systemic inflammation following stoke originated from within the host.[69] Moreover, it has been demonstrated that, following MCAO in mice, there is an increase in intestinal permeability, which suggests that intestinal bacteria are capable of translocating from the lumen of the gut into the systemic circulation whereby they can invade other organs (i.e., the lungs) and elicit an inflammatory response.[69] In support of this, 16S sequencing of the lung microbiota revealed an increase in bacterial species in the lungs that were predicted to originate from the small intestine.[69] Thus, it appears that the intestinal microbiota can not only influence the neuroinflammatory status through influencing T cell migration to the brain but also systemic inflammation following stroke through bacterial translocation from the gut into the circulation, whereby they can invade other organs. On the basis of these results, targeting the increased intestinal permeability in stroke patients may help to limit bacterial translocation leading to sepsis. Moreover, developing a probiotic strain that could modulate T lymphocyte signaling to the brain may help to ameliorate the neuroinflammatory status in patients following stroke. While data are currently limited, there is some preclinical evidence to suggest that probiotic strains can ameliorate the neurological damage caused by stroke. *Clostridium butyricum,* for instance, prevented neuronal apoptosis mediated by bilateral

common carotid artery occlusion (BCAO) in mice while also improving performance in the Morris water maze task, suggesting an improvement in cognition in these animals.[77] This bacterial strain also increased levels of beneficial gut bacteria, such as *Bifidobacteria*, *Lactobacilli*, and *Fecalibacterium prausnitzii*, in BCAO mice.[77]

Microbiota and stress-related disorders

Accumulating evidence suggests that there is dysregulation to the microbiota–gut–brain axis in stress-related disorders, such as depression and anxiety. Understanding how the gut microbiota is affected in such conditions will allow for the development of microbiota-based therapies, which may provide us with safer and more efficacious treatment options for such conditions.

Depression

Evidence for a role of the gut microbiota in depression and other stress-related disorders has predominantly arisen from preclinical studies (for reviews on this topic, see Refs. 4, 14, 18, and 78). For instance, it was recently shown that chronic stress results in dysregulation of the microbiota and metabolome of rats, with decreases observed in the Firmicutes/Bacteroidetes ratio, and, more specifically, decreases in the relative abundances of *Lactobacillus* and increases in *Oscillibacter*.[79] While the preclinical evidence is rather compelling, only a few clinical studies to date have performed microbiota analysis in depressed patients to assess for any potential dysregulation. We have recently combined both clinical and preclinical research to demonstrate how the microbiota–gut–brain axis is affected in depression. Depressed patients were found to have a dysregulated microbiota, observed as a reduction in species richness and microbial diversity.[80] Interestingly, when the fecal microbiotas of these depressed patients were transplanted into microbiota-depleted (antibiotic cocktail) rats, the depression behavioral phenotype was also transferred into the animals.[80] Rats that received the microbiota from depressed patients displayed a dysregulated microbiota themselves, along with an anhedonic and anxiety-related behavioral phenotype. Moreover, these animals displayed an elevated kynurenine/tryptophan ratio, indicating that perhaps the depressed microbiota is facilitating the conversion of tryptophan into the deleterious metabolite kynurenine, which is implicated in depression.[80] Zheng *et al.* corroborated these results when they demonstrated that transplantation of a dysregulated microbiota from depressed humans to germ-free mice conferred a depressive-related phenotype in these animals.[81] Recent work from De Palma *et al.* also demonstrated the successful transfer of behavior from humans to mice following microbiota transplantation. Transplantation of microbiota from patients with irritable bowel syndrome with anxiety to germ-free mice resulted in gastrointestinal symptoms and anxiety-related behaviors in these animals.[82] These findings indicate that dysregulation to the gut microbiota is capable of facilitating the behavioral and physiological symptoms of depression and anxiety.

Microbiota-based therapies for the treatment of depression

While a greater understanding is required as to how the gut microbiota can mediate the behavioral and physiological symptoms of depression, emerging preclinical and clinical data are highlighting how modulators of the gut commensals (i.e., prebiotics and probiotics) may be an appropriate therapy for depression and other stress-related disorders. For instance, recent work from our group has shown that a prebiotic combination of fructooligosaccharide and galactooligosaccharide (GOS) improved depression and anxiety-related behavior in mice subjected to chronic psychosocial stress.[83] Moreover, this prebiotic combination reduced stress-induced activation of the hypothalamic–pituitary–adrenal (HPA) axis while also ameliorating dysregulation of the microbiota brought about by chronic stress.[83] Other groups have also documented the beneficial effects of GOS and other prebiotics on anxiety-related behavior in preclinical models.[84,85] Additionally, GOS supplementation was found to lower the cortisol awakening response in healthy volunteers while shifting attention from negative toward positive stimuli, demonstrating that the prebiotic also displays efficacy in humans.[86]

The probiotic strain *Lactobacillus rhamnosus* JB-1 has been shown to improve anxiety and social-related behaviors in mice following chronic social defeat,[87] which is in line with previous work from our group, which also demonstrated the beneficial behavioral effects of this particular strain.[88] Such findings support the use of microbiota modulators

for the treatment of depression and stress-related disorders. However, we must err on the side of caution with our use of animal models for assessing the efficacy of prebiotics and probiotics and how this translates to the clinical setting. For instance, we have recently shown that the *L. rhamnosus* JB-1 strain, which has demonstrated efficacy preclinically, displayed no effect in healthy male volunteers in terms of cognition, subjective stress measures, activation of the HPA axis, or immune activation.[89] A lack of clinical efficacy of *L. rhamnosus* JB-1 may be due to the fact that mice possess a different gut microbiota than that of humans and, thus, may react differently to the probiotic strain. Moreover, the two studies that demonstrated beneficial behavioral effects in mice used either an inherently anxious mouse strain (BALB/c) or employed chronic stress to promote an anxious phenotype, which would have different reactions to stress than healthy male humans.[87,88] Despite the probiotic effects of *L. rhamnosus* JB-1 strain being somewhat lost in translation, other candidate bacterial strains have demonstrated efficacy as potential psychobiotics in humans. For instance, the probiotic strain *B. longum* 1714 demonstrated efficacy in reducing stress-induced cortisol release, along with attenuating daily self-reported levels of stress and improving visuospatial memory in healthy male volunteers.[90] A fermented milk product containing several different probiotic strains altered the activity of brain regions associated with emotionality in healthy female participants, suggesting potential psychobiotic potential.[91] Moreover, a recent pilot study in patients with irritable bowel syndrome demonstrated that the probiotic *B. longum* NCC3001 improved scores of depression, but not anxiety, while also improving gastrointestinal symptoms.[92] While these studies are certainly promising, future studies assessing the therapeutic potential of psychobiotics for the treatment of depression will require large-scale recruitment of depressed patients to determine the extent of their efficacy. In addition to prebiotics and probiotics, emerging evidence has demonstrated that microbiota transplantation is also capable of modifying behavior and may be an effective treatment strategy for depression and other neuropsychiatric disorders.[93,94] However, robust clinical data are required to support the use of microbiota transplantation as a potential treatment strategy for depression.

Microbiota, sociability, and neurodevelopmental disorders

There is growing evidence to support a role for the microbiota in regulating social behaviors in mammals.[4] How the gut microbiota modulates social behavior remains to be fully elucidated. Given the complexity and variability of the gut microbiota from one species to another, identifying specific microbial compositions or even specific bacterial species that promote social behavior is challenging. Moreover, the mechanisms through which the gut microbiota promote social behavior remain unknown. However, there is growing evidence to suggest a role for microbiota modulation of certain neurotransmitter/peptide systems in the brain.[95,96]

The gut microbiota influences social behaviors

The concept that the gut microbiota may influence social behavior arose from experiments with germ-free mice. Germ-free mice were found to have deficits in social behavior in the three-chamber interaction test.[97,98] Germ-free mice exhibit social avoidance behavior in this test, observed as an increase in the time spent in the empty chamber and a decrease in time spent in the chamber with a novel conspecific mouse.[98] This social deficit is reversed following recolonization with microbiota, further confirming that gut bacteria modulate social behaviors. However, microbiota reconstitution did not improve social cognitive deficits in the three-chamber test, as these mice failed to spend more time with a novel conspecific mouse over a familiar animal.[98] Moreover, the transfer of cued food information through social contact and communication in the social transmission of food preference test was similar between conventional controls and germ-free mice. Such results suggest that some facets of social behavior are amenable through manipulation of the microbiota, while others are not. Further reaffirming this association between microbiota and social behavior is that depletion of microbiota of conventionally colonized mice following antibiotic treatment also results in social deficits.[99] Interestingly, Arentsen *et al.* reported opposing behavioral results, demonstrating that germ-free mice display increases in social behaviors in the three-chamber test relative to specific pathogen–free mice.[100] This may be due to differences in performing the three-chamber social interaction test or to differences in animal breeding/husbandry techniques.

A growing body of evidence suggests that, in a variety of animal models of autism spectrum disorders, there is an altered microbiota composition. For instance, in the *in utero* valproate animal model of autism, the pups display an autistic-like phenotype along with intestinal inflammation and dysregulation to the microbiota.[101,102] Specifically, these animals display an increase in the Firmicutes/Bacteroidetes ratio along with increases in cecal butyrate concentrations, suggesting alterations to microbial metabolism.[101] Interestingly, there were specific increases reported in the operational taxonomic units assigned to the genera *Alistipes, Mollicutes, Lactobacillales,* and *Enterorhabdus* in valproate *in utero*–exposed males. Given that autism is more prevalent in males than in females, perhaps the microbiota has a bearing on the greater risk of autism in males than in females. Alterations in gut microbiota composition have also been reported in other environmental models of autism, such as the maternal immune activation model, suggesting that exposure to an *in utero* stress (i.e., teratogens or inflammagens) negatively affects gut commensals and behavior.[103] Genetic animal models of autism allow us to investigate the relationship between the host genome and the microbiota. The BTBR animal model of autism displays a robust autistic-like phenotype, with deficits in social behaviors, repetitive behaviors, and anxiety-related behaviors frequently reported.[104–106] Recently, it was demonstrated that BTBR mice display a reduction in the Firmicutes/Bacteroidetes ratio, along with increases in the abundance of species such as *A. mucinphilia* and reductions in *Bifidobacterium spp*, suggestive of microbiota dysregulation.[107] This strain may prove a useful model for dissecting the association of host genetics and microbiota in facilitating an autistic-like phenotype.

Microbiota-based therapies for the treatment of autism: hype or hope?

Although more insight is required into how gut bacteria influence social behaviors and other behavioral aspects associated with autism, recent evidence suggests that modulating the microbiota through diet, probiotics, and microbiota transfer is capable of modifying some aspects of behavior relevant to autism. While most studies that have demonstrated the beneficial effects of modulating the microbiota have been preclinical, a recent small open-label study by Kang *et al.* demonstrated that transfer of a standardized human gut microbiota mixture was capable of improving gastrointestinal and behavioral symptoms in autistic children.[108] Moreover, these improvements to the gastrointestinal and behavioral symptoms through microbiota transfer were maintained for up to 8 weeks following the cessation of the therapy, suggesting that the intervention had long-lasting effects on the microbiota.[108] While these results are preliminary and lack appropriate controls, it lends support to the use of microbiota-based therapies in the future to manage the gastrointestinal and behavioral symptoms of autism. However, as autism is a genetic disorder,[109] more research is needed to investigate the relationship between host genetics and the gut microbiota in shaping behaviors in autism.

Emerging preclinical research suggests that candidate bacterial strains are capable of improving the core behavioral symptoms of autism. In the maternal immune model of autism, Hsiao *et al.* demonstrated that *B. fragilis* was capable of improving stereotyped and anxiety-related behaviors in this animal model while also improving intestinal permeability.[103] This led Gilbert *et al.* to provocatively and, perhaps prematurely, propose the concept of probiotics for treating autism.[110] More recently, Buffington *et al.* identified that, in an animal model of autism (offspring of mothers fed a high-fat diet), fecal levels of *Lactobacillus reuteri* were lower compared with nonautistic controls.[97] These autistic-like mice also displayed reduced oxytocin immunoreactivity in the paraventricular nucleus (PVN) of the hypothalamus.[97] Interestingly, supplementation with *L. reuteri* ameliorated autistic-related behaviors in these mice through enhancing oxytocin immunoreactivity in the PVN of the hypothalamus.[97] These behavioral and physiological effects appeared to be specific to *L. reuteri,* as supplementation with *L. johnsonii*, which was also found to be reduced in these mice with autistic-like behaviors, had no effect on these parameters.[97] Given the association of oxytocin with social behaviors and autism, it is likely that the hormone plays a role in the psychobiotic effects of the bacterium. It may be a peptide released from the bacterium, as health-killed *L. reuteri* failed to increase oxytocin immunoreactivity within the PVN,[97] while lysed *L. reuteri* was still capable of mediating this effect.[111]

There remain several unanswered questions regarding the prosocial effects of *L. reuteri.* For instance, more information regarding *L. reuteri's* effect on other brain regions related to the processing of social behaviors (i.e., the amygdala) is required. In the study by Buffington *et al.*, the authors noted that there was no effect of the bacterial strain on repetitive or anxiety-related behaviors. Thus, a greater understanding of the neurocircuitry affected in autism is required. Moreover, is this behavioral effect a direct consequence of consuming *L. reuteri*? Or does this bacterial strain alter the host microbiota, which then improves social behavior? Aside from probiotics, the SCFA butyrate has recently been shown to improve autistic-related behaviors in BTBR mice.[106] Butyrate treatment improved deficits in social and repetitive behaviors in BTBR mice while also modulating the expression of genes related to excitatory and inhibitory neurotransmission, suggesting that such microbial metabolites may present a potential therapeutic opportunity for the treatment of autistic behavior.[106]

Microbiota and addiction

While considerable advances are being made with regard to the role of the microbiota–gut–brain axis in conditions, such as neurodegenerative diseases, and psychiatric conditions, such as depression, little is known with regard to the role that gut microbes play in substance abuse disorders. When we consider drug addiction and any potential association with a dysregulated microbiota, it is important to consider comorbidities, such as depression and anxiety. Moreover, adequate and nutritional dietary intake is likely to be lacking in drug addicts, which may also affect the composition of the microbiota. Finally, many pharmacological agents,[112] including psychotropics,[113] can have direct effects on the microbiota, which complicates any interpretation. Nonetheless, there is growing evidence that the microbiota can modulate behaviors and physiological changes relevant to substance abuse.

Alcohol

Chronic abuse of alcohol can lead to several serious health complications, such as addiction, nutrient deficiency, liver disease, and colorectal cancer. Moreover, chronic alcohol exposure can lead to psychological and cognitive deficits, such as major depression and Korsakoff's syndrome. Recent preclinical and clinical data suggest that chronic alcohol consumption can negatively affect the gut microbiota, which is perhaps not all that surprising given that ethanol is consumed via the gastrointestinal system.[114,115] Recently, it was shown that chronic ethanol consumption in alcoholics results in alterations to their microbiota; decreasing the relative abundance of the Bacteroidetes phylum and increasing the relative abundance of Proteobacteria.[116] Intestinal permeability is also found to be increased in a subset of alcoholics, increasing the risk for bacterial translocation in these individuals.[117] Moreover, alcoholics with increased intestinal permeability also displayed a dysregulated microbiota, with increases in the *Blautia* and *Megasphera* genera, and decreased levels of the anti-inflammatory *Faecalibacterium prausnitzii.*[117] *F. prausnitzii* has been shown to inhibit the production of the proinflammatory cytokine IL-8 *in vitro,* demonstrating its anti-inflammatory potential.[118] Interestingly, low levels of *F. prausnitzii* were correlated with elevated levels of circulating IL-8 in alcoholics with increased intestinal permeability, suggestive of proinflammatory immune activation.[117] Additionally, the psychological status of alcoholics with increased intestinal permeability was worse than that of controls and alcoholics with regular intestinal permeability, which suggests that a dysregulated microbiota–gut–brain axis is facilitating the psychological symptoms observed in alcoholics.[117]

Recent work from our group demonstrated that chronic intermittent ethanol exposure in mice, which models chronic alcohol exposure in humans, resulted in an increase in the *Allistipes* genera along with a reduction in *Clostridium* cluster IV.[115] Bacterial species found within *Clostridium* cluster IV, such as *F. prausnitzii*, possess anti-inflammatory properties.[114] Thus, a loss of this genus following chronic alcohol exposure may promote a proinflammatory immune state in the gastrointestinal system. In support of this, germ-free mice that were humanized with the microbiota of alcoholics with hepatitis displayed a higher level of inflammation and permeability of the intestine compared with controls.[114] Moreover, the microbiota of these humanized germ-free mice lacked known beneficial bacteria, such as *F. prausnitzii,* and contained bacterial species that have been previously associated with intestinal inflammation, such as *Bilophila wadsworthia.*[114,119] Thus, chronic alcohol exposure appears to lead to a

dysregulation in the composition of the microbiota; promoting a proinflammatory state within the gut, which could affect the microbiota–gut–brain axis and behavior in these individuals.

Cocaine and cannabis

Data regarding the effects of other drugs of abuse upon the microbiota are currently limited. However, there is some preliminary evidence to suggest that certain drugs, such as cannabis and cocaine, affect the microbiota–gut–brain axis. Depletion of the murine gut microbiota with an antibiotic cocktail served to enhance unbiased conditional place preference for cocaine, which was reversed following treatment of antibiotic-treated mice with SCFAs.[120] Such data indicate that the gut microbiota can influence drug-seeking behavior. Δ^9-Tetrahydrocannibinol (THC), the active psychotropic chemical in cannabis, was shown to alter the microbiota of mice, observed as an increase in *Clostridium leptum* and decreases in *Roseburia* species.[121] Moreover, in diet-induced obese mice, THC lowered the Firmicutes/Bacteroidetes ratio while also increasing *A. muciniphilia*, a bacterial strain that has been previously associated with weight loss, reducing insulin resistance, and improving intestinal barrier function.[122] Whether THC affects gut–brain signaling to improve weight loss (i.e., modulation of satiety peptides) is unknown. However, such preliminary data warrant a greater investigation into how THC can influence gut microbiota composition and weight loss. While more work is required into understanding how drugs of abuse, such as cannabis and cocaine, affect the microbiota and, subsequently, the gut–brain axis, these studies provide an insight into how this axis may be affected in addicts.

The diet–microbiota–gut–brain axis: toward nutritional psychiatry

Diet has been shown to have an important bearing on the composition of the microbiota.[123] For instance, the fecal microbiota of the rural Hadza community, who mostly forage in the wild for their diet, was found to be unique from that of a Westernized population.[124] Moreover, the diets of Westernized countries are found to be suboptimal with respect to the consumption of nutrients and high in saturated fats, which may help to explain the prevalence of diseases and psychiatric conditions in such countries.[125,126] It is important to consider the factors that have driven the evolution of the Westernized microbiota from that of more rural microbiotas, such as that of the Hadza community. Sonnenburg *et al.* demonstrated that a diet consisting of low microbiota–accessible carbohydrates resulted in a reduction in the diversity of gut bacteria of mice (that harbored a human microbiota), which was largely reversible following changing the diet to a higher level of carbohydrates that bacteria could consume. Interestingly, however, gut bacterial diversity worsened across generations in mice on a low microbiota–accessible diet, which became less amenable to improvement following restoration with a high-carbohydrate diet.[123] The results of this study highlight how the human gut microbiota may have potentially evolved to be less diverse and amenable to modulation following the movement of humans from more indigenous, rural communities to Westernized lifestyles. Moreover, these results may help to explain why diseases and psychiatric disorders are more prominent in Western countries. Despite this, modifying one's diet has been shown to improve many facets of behavior.

The consumption of a Mediterranean-style diet (i.e., fruits, vegetables, unsalted nuts, fish, lean red meat, etc.) is linked to a reduced likelihood for the development of depression in comparison to the Western-style diet, which is linked to a greater risk for developing the mood disorder.[126] Preclinical evidence has identified the beneficial effects of polyunsaturated fatty acids. For instance, perinatal omega-3 fatty acid supplementation lowered stress-induced activation of the HPA axis in adolescent mice while also enhancing cognition in adulthood, demonstrating the beneficial effects of dietary supplements on stress and behavior.[127] Moreover, the omega-3 fatty acids docosahexaenoic acid and eicosapentaenoic acid have been shown to improve anxiety and depression-related behaviors in female rats.[128] Given that stress-related psychiatric conditions are more prevalent in females, these preclinical data suggest that diet may be an effective strategy for treating stress-related disorders in women. Until recently, most evidence regarding the effect of diet on mood had been limited to animal studies and observational studies in humans.[126] However, in a recent randomized controlled trial conducted by Jacka *et al.*, the authors demonstrated that a modified version of the Mediterranean diet

(Mod*i*MedDiet) was a beneficial adjunctive therapy for the treatment of clinical depression.[129] Individuals with mild depression reported improvement in depression symptomology as assessed by the Montgomery-Åsberg Depression rating scale (MADS) following the 12-week dietary trial. While these are preliminary results, they are rather promising, and they suggest that modifying one's diet to incorporate a more Mediterranean-style of food intake is a beneficial strategy for the treatment of depression. Indeed, dietary changes may be a less-costly and safer strategy for the treatment of mild depression for patients rather than conventional antidepressant medication. However, much more research is needed to give an evidence base to such hypotheses.

Additionally, emerging preclinical data suggest that the diet is also capable of improving autistic-related behavior. For instance, a high-fat, low-carbohydrate ketogenic diet has also been shown to improve deficits in social behaviors and repetitive behaviors in the BTBR mice as well as in the environmental models of autism, maternal immune activation, and valproate *in utero* exposure.[130–132] Moreover, a modified amino acid diet (containing high concentrations of histidine, lysine, and threonine, with low concentrations of leucine, isoleucine, and valine) was shown to improve repetitive self-grooming behavior in BTBR mice while also attenuating mammalian target of rapamycin (mTOR) signaling in the prefrontal cortex.[133] Currently, there is little information available as to whether the ketogenic diet or other diets may improve behavioral symptoms in autistic individuals. However, recently, it was demonstrated that a 6-week gluten-free diet intervention was beneficial in reducing gastrointestinal symptoms as well as modestly improving the behavioral symptoms of autism in children.[134] While these studies highlight the beneficial effects that diet may have upon behavior, a greater understanding of the mechanisms underlying how diet or dietary components improves social behavior and mood is required.

Moving toward mechanisms

In the last number of years, there has been a clear focus on understanding the mechanisms underlying how microbiota can influence gut–brain function (Fig. 3). It is clear that there are multiple mechanisms through which gut bacteria can signal to the brain, such as through the vagus nerve, immune system, production of microbial metabolites, and modulation of circulating tryptophan.[8,10,17,135] Understanding how the gut commensals modulate behavior remains one of the biggest unanswered questions in microbiota–gut–brain axis research. However, emerging studies are providing some insight into the mechanisms underlying how gut bacteria influence social behaviors. In a recent study from the Karolinska Institute, the authors identify a role for bacterial-sensing molecules in regulating social development.[136] Genetic knockdown of the peptidoglycan-sensing molecule PGLYRP2 was found to increase social behaviors in both male and female mice in the three-chamber test while also modulating the expression of genes related to synaptic plasticity.[136] The authors argue that bacterial components, such as peptidoglycan, cross the blood–brain barrier during postnatal development and bind to molecules, such as PGLYRP2, to influence neuronal circuits linked to social behaviors.[136]

Other biological mechanisms, such as the immune system and vagal signaling, are also likely to mediate the gut microbiota's influence on social behavior. D'Mello *et al.* demonstrated that peripheral inflammation, mediated by bile duct ligation, resulted in social withdrawal behavior in mice.[137] This behavioral deficit was ameliorated following treatment of mice with the probiotic mixture VSL#3, indicating that gut bacteria can influence how the immune system signals to the brain to modulate behaviors, such as sociability and anxiety.[137] In a separate study, vagotomy blocked the anxiogenic effects of intestinal colitis in mice, further corroborating that the vagal pathway is an important mediator for the gut microbiota to influence behavior.[138] Gut bacteria may also signal via the vagus nerve to influence social behaviors. Oral administration of the probiotic strain *L. reuteri* has been shown to increase the central expression and secretion of the prosocial hormone oxytocin in mice.[97,139] However, this effect of *L. reuteri* was lost following vagotomy, indicating that gut commensals signal via the vagus nerve to increase the synthesis and secretion of oxytocin from the brain. The production of neuroactive metabolites by gut bacteria may also influence social behaviors. Levels of the microbial metabolite cresol, were found to be elevated in the ceca of socially withdrawn mice.[61] Interestingly, *in vitro* experiments demonstrated that cresol was capable of

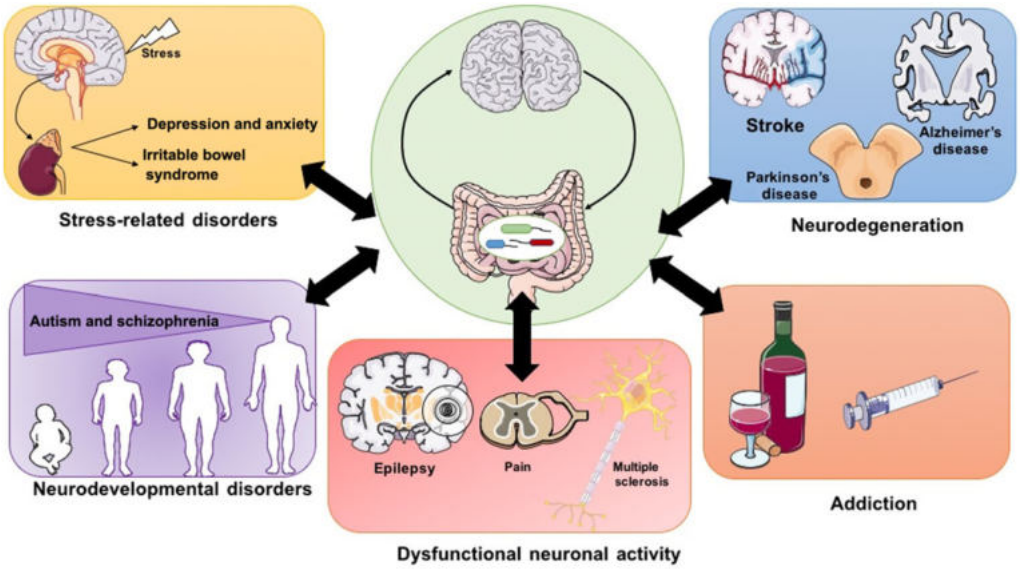

Figure 3. Overview of the gut–microbiota–brain axis in neurology and psychiatry. Evidence now suggests that the microbiota–gut–brain axis is involved in a variety of neurological and psychiatric conditions, such as depression, addiction, stroke, and Parkinson's disease. Moreover, modulating the microbiota in these conditions using probiotics, prebiotics, or through diet has displayed efficacy in preclinical studies, with some clinical studies also demonstrating efficacy. However, a greater understanding of how the intestinal commensals are affected in these various conditions will allow for the rational development of microbiota-based therapies in these various disorders.

inhibiting the expression of myelin genes and the differentiation of oligodendrocytes.[61] Thus, elevated levels of cresol may be driving social avoidance behaviors through modulating the expression of myelin-related genes. In support of this, we have recently demonstrated that the gut microbiota influences myelination in the prefrontal cortex of mice.[60] In addition to producing various bioactive metabolites, the gut microbiota also synthesize various neurotransmitters, such as noradrenaline and GABA.[140,141] Moreover, certain spore-forming bacteria have been shown to influence the secretion of serotonin from enterochromaffin cells, which subsequently influences local gut physiology.[142] Whether microbially derived neurotransmitters are capable of reaching the brain and influencing their specified circuitry and behavior is unknown. However, the microbiota has been shown to influence the level of some of these neurotransmitters in the brain. For instance, germ-free mice display increased hippocampal serotonin levels relative to conventionally colonized animals.[17,143] This is most likely due to the observed increased availability of circulating tryptophan in these animals owing to the absence of gut bacteria that utilize the amino acid.[17]

The gut microbiota is also capable of influencing anorexigenic signaling to the brain, thereby modulating feelings of satiety. Within the lumen of the gastrointestinal system, the gut microbiota is in close proximity to enteroendocrine cells, and evidence suggests that gut microbes are capable of modulating the secretion of satiety peptides from these cells. Breton *et al.* demonstrated that the *E. coli* chaperone protein caseinolytic protease (Clp) B is capable of increasing plasma levels of the satiety peptides glucagon-like peptide 1 (GLP-1) and peptide YY (PYY) while also suppressing food intake and increasing neuronal activation within hypothalamic nuclei that are associated with satiety.[144] ClpB likely mediates this increase in GLP-1 and PYY through activating melanocortin receptor 4 (MCR4) expressed on the cell surface of enteroendocrine cells. In support of this, ClpB was found to be an antigen mimetic of α-melanocyte–stimulating hormone (α-MSH), which binds to and activates MCR4.[145,146] In addition to indirectly activating satiety-related nuclei in the brain through increasing the secretion of GLP-1 and PYY, Breton *et al.* also demonstrated that ClpB was capable of activating proopiomelanocortin neurons when applied

directly to hypothalamic sections.[144] Thus, alterations to the level of *E. coli* in the gut microbiota are likely to have some bearing on satiety-related behaviors. In support of this, plasma levels of ClpB were found to be higher in female patients with eating disorders (anorexia nervosa, bulimia nervosa, and binge-eating disorder),[147] which suggests that dysregulation to the microbiota, observed as an increased production of ClpB, may be facilitating such deleterious behaviors.

While a great deal of attention has been given to understanding how the gut microbiota influences processes in the CNS, it is also important to consider the effect that gut commensals have on the ENS. Proper functioning of the ENS is required for physiological processes, such as gut motility, but also to ensure gut–brain communication. In the absence of a microbiota, germ-free mice display reduced excitability of after-hyperpolarization cells in the myenteric plexus.[148,149] A reduction in the neuronal firing rates of these cells within the ENS of germ-free mice is likely to affect proper signaling between the gut and the brain that may help to explain some of the behavioral deficits observed in these animals.[150] Moreover, bacterial strains that have demonstrated positive effects on behavior, such as *L. reuteri* and *L. rhamnosus* JB-1, have also been shown to modulate excitability of the ENS, which is likely to have some bearing on their psychobiotic effects.[151,152] The bacterial strain *B. fragilis*, which has demonstrated efficacy in improving autistic-related behaviors and ameliorating gastrointestinal permeability in the MIA model of autism,[103] has also been shown to increase excitability of intestinal primary afferent neurons.[153] This effect of *B. fragilis* was found to be dependent on its expression of the exopolysaccharide polysaccharide A.[153] The beneficial effects of probiotics on behavior may depend on their ability to modulate neurotransmission within the ENS, and future work should consider the role of the ENS in this regard.

Conclusions and future perspectives

Since the gut microbiota was first proposed to influence human health over 100 years ago, our understanding of such a role has increased immensely. Evidence now suggests that the microbiota, through the bidirectional communication axis known as the microbiota–gut–brain axis, is involved in physiological processes, such as mood and aging. Dysregulation of the microbiota–gut–brain axis is now emerging for a broad range of neurological and psychiatric conditions from Parkinson's disease to depression. Preclinical studies have been beneficial in elucidating how microbiota dysregulation is involved in such conditions. Moreover, preclinical studies have demonstrated the efficacy of modulators of the microbiota (i.e., probiotics, prebiotics, and diet), supporting their use as potential therapies for conditions such as depression and autism. However, caution must be taken with extrapolating these preclinical results to the clinical setting, given reports of probiotic strains displaying efficacy in mice but not in humans. Going forward, a greater emphasis should be placed on assessing the therapeutic efficacy of diet or probiotics in the clinical setting through conducting sufficiently powered, rigorous clinical trials.

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