The Influence of the Microbiota on Brain Structure and Function: Implications for Stress-Related Neuropsychiatric Disorders

John D. Sterrett, Nathan D. Andersen, and Christopher A. Lowry

Abstract Based on research conducted during the last decade, it is becoming clear that the human microbiota plays an important role in the maintenance of human health. Recently, it has become clear that the human microbiota plays a role not only in physical health but also in mental health, which will be the focus of this chapter. Data suggest that, depending on the diversity and community composition of the human microbiota, the microbiota can either contribute to negative mental health outcomes or promote stress resilience. Here we will focus on the mechanisms through which the human microbiota influences mental health outcomes, with a focus on impacts on brain structure and function. In the context of these mechanisms, we will consider the consequences in humans of the large-scale transition from a hunter-gatherer existence or rural lifestyle to an urban lifestyle and the implications for functioning of the microbiota-gut-brain axis, brain structure and function, and mental health. Finally, we will consider the role of the human microbiota in vulnerability and resilience to stress-related psychiatric disorders, including anxiety disorders, affective disorders, and trauma- and stressor-related disorders, including posttraumatic stress disorder, and the mechanisms involved.

Keywords Anxiety · Depression · Gut-brain axis · Microbiota · Microbiota-gut-brain axis · Posttraumatic stress disorder

1 Introduction

The human body harbors communities of microorganisms at many locations including all mucosal and epithelial linings that cover the body's internal and external surfaces [1, 2]. These communities of organisms have been termed microbiota, and they are known to play a role in regulating many facets of host health. Where the

J. D. Sterrett · N. D. Andersen · C. A. Lowry (✉)
Department of Integrative Physiology, University of Colorado Boulder, Boulder, CO, USA
e-mail: John.Sterrett@colorado.edu; Nathan.D.Anderson@colorado.edu; Christopher.Lowry@colorado.edu

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term microbiota is used to describe the organisms making up the community, the term microbiome refers to the entire "theater of activity" from microorganisms, including genetic material and metabolites [3]. Due to the inability of many microorganisms to be cultured, many microbiota are typically assessed via the microbiome through whole genome shotgun sequencing or sequencing of the 16S ribosomal RNA gene region, while the molecular products of these microorganisms are assessed using metabolomics, proteomics, and transcriptomics. Mammals have historically coexisted symbiotically with their microbiota, forming the "holobiont," or the combination of a eukaryotic organism with its microbial colonies [4, 5]. However, due to increased sanitization and urbanization, altered dietary patterns, use/overuse of antibiotics, and lifestyle changes, human microbiota have experienced disruptions characterized by decreased biodiversity and a loss of contact with specific immunoregulatory organisms with which humans coevolved [6–10]. These immunoregulatory organisms, such as the saprophytic soil bacterium *Mycobacterium vaccae* NCTC 11659, the unique human milk oligosaccharide degrader *Bifidobacterium longum* subspecies *infantis* (*B. infantis*), and even the parasitic helminth *Schistosoma mansoni*, modulate the host immune system in order to coexist, which is proposed to be important for the maintenance of health under the Old Friends hypothesis [11–14].

With reduced exposure to immunoregulatory organisms, we have seen an increased prevalence of immune, allergic, and inflammatory disorders, and an increasing body of research suggests a causal link [12, 15]. Importantly, the heightened prevalence of chronic low-grade immune activation, as well as immune and inflammatory disorders, has contributed to increased rates of psychiatric conditions, as the physiological state of the body impacts brain neurophysiology, ultimately affecting behavior [7, 16]. The altered risk of psychiatric conditions is evident when studying stress responses from rural versus urban participants, as individuals who grow up in urban environments without daily close contact with animals have exaggerated immune and autonomic nervous system responses to psychosocial stressors, relative to the rural participants [17]. Microbiota-mediated modulation of psychiatric states occurs through a number of distinct mechanisms, including (1) afferent neural signaling; (2) altered immune signaling from the periphery to the brain; (3) humoral mechanisms involving effects of microbially derived metabolites, altered host metabolism, or altered host endocrine signaling; and (4) influencing the gut-blood and blood-brain barriers. Here we will discuss each of these mechanisms in turn, as well as our rapidly increasing understanding of their role in determining mental health outcomes. Figure 1 outlines mechanisms covered in this review.

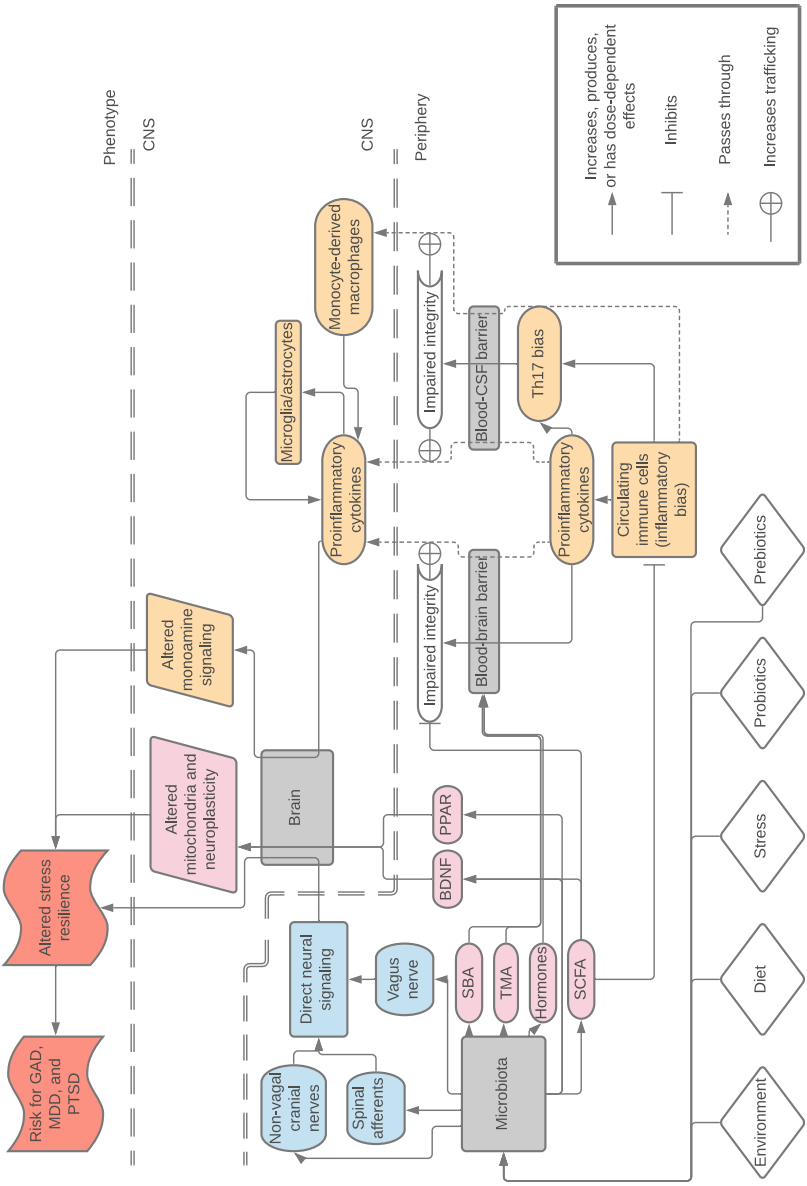

Fig. 1 Mechanisms contributing to modulation of neuropsychiatric outcomes by the microbiota. *BDNF* brain-derived neurotrophic factor, *CNS* central nervous system, *CSF* cerebrospinal fluid, *GAD* generalized anxiety disorder, *MDD* major depressive disorder, *PPAR* peroxisome proliferator-activated receptor, *PTSD* posttraumatic stress disorder, *SBA* secondary bile acids, *SCFA* short-chain fatty acids, *Th17* T helper 17 cells, *TMA* trimethylamine

2 Neural Signaling

The gut microbiota has been heavily implicated in the modulation of the central nervous system (CNS) structure and function. Given the speed of neural transmission, direct signaling to the CNS by nerves innervating mucosal surfaces that are in direct contact with microbiomes is the fastest means of microbiota-brain signaling. Though much research has focused on mediation of the gut-brain axis by the vagus nerve, methods for studying vagal signaling have unaddressed drawbacks, and other understudied neural pathways are also potentially important for microbiota-CNS signaling. Examples of non-vagal neural signaling include spinal afferents from areas such as the skin, gut, airways, and lungs and cranial nerve afferents from nasal and oral microbiota.

2.1 Vagal Afferents

The vagus nerve has long been implicated in communication from the gut microbiota to the brain [18]. The efferent arm of the vagus nerve, as a portion of the autonomic nervous system, controls heart rate, respiration, digestive tract function, as well as immune function [19]. Importantly, however, over 80% of vagus nerve fibers are afferent, transmitting information to the brain, whereas 10–20% are efferent [20]. Neurons from the vagal afferent pathway innervate much of the digestive system, including a large portion of the enteric nervous system (ENS) [21]. Additionally, they have receptors for many gut peptides and microbial metabolites. A prime example is the expression of toll-like receptor (TLR) 4 on vagal afferent neurons, allowing them to detect the common bacterial antigen lipopolysaccharide (LPS) [22]. Moreover, vagal afferent neurons also express TLR2 (which detects components of gram-positive bacteria such as acylated lipopeptides, peptidoglycan, and lipoteichoic acids) and TLRs 3 and 7 (which detect viral mRNA) [23–25]. Afferent vagal fibers terminate almost exclusively in the brainstem nucleus of the solitary tract, which can relay signals to neural systems within the brain. The afferent vagal fibers originating in different organ systems innervate different subregions of the nucleus of the solitary tract, suggesting that different organ systems, i.e., the large intestine versus the bronchopulmonary system, can have different effects on brain structure and function [26].

2.2 What Have We Learned from Vagotomies?

Vagotomies, or surgical procedures that cut or remove portions of the vagus nerve, date back to 1814, when Benjamin Brodie observed that a vagotomy prevented mucous secretion in the stomach after arsenic insertion into a thigh wound of a dog

[27]. In the years since, vagotomies have seen widespread use in clinical practice and are presently being phased out due to the creation of therapeutic interventions with fewer side effects [28]. Currently, vagotomies are often used in animal models to study vagus-mediated aspects of the periphery-brain axis signaling [28].

Notably, Konsman et al. [29] demonstrated that vagotomy blocks behavioral depression in response to peripheral inflammation in rats. Vagotomies in mice prevented a broad spectrum of neurophysiological, endocrine, and behavioral responses following 28 days of chronic oral *Lactobacillus rhamnosus* JB-1 supplementation, including (a) decreased gamma aminobutyric acid $(GABA)_{B1b}$ mRNA expression in the cingulate cortex and prelimbic cortex; (b) increased $GABA_{B1b}$ mRNA expression in the hippocampus, amygdala, and locus coeruleus; (c) reduced $GABA_{A\alpha2}$ mRNA expression in the prefrontal cortex and amygdala; (d) increased $GABA_{A\alpha2}$ mRNA expression in the hippocampus; (e) blunted stress-induced increases in plasma corticosterone concentrations; and (f) reduced anxious and depressive behavior [30]. Similarly, Sgritta et al. [31] showed that vagotomies prevented the stress resilience effects of 28 days of oral *L. reuteri* MM4-1A (ATCC-PTA-6475) in mice.

Vagotomies also have been shown to blunt neuroactive cytokine signaling and alter behavior following experimentally induced peripheral inflammation. For example, Laye et al. [32] demonstrated that a vagotomy blocks interleukin (IL)-1β mRNA expression in the hypothalamus and hippocampus (but not the pituitary gland) in mice in response to peripheral LPS injection. Luheshi et al. [33] also demonstrated that vagotomy in mice blocks decreased social exploration but does not prevent fever following intraperitoneal IL-1β injection. Wieczorek et al. [34] showed that the effects of peripheral IL-1β and LPS injection in mice (including decreased appetite and locomotor activity, increased plasma adrenocorticotropic hormone and corticosterone concentrations, and altered serotonin and tryptophan metabolism in the brain) were somewhat attenuated by vagotomy. However, the attenuation was "marginally significant," leaving room for other mechanisms, such as immune activity. This is supported by Van Dam et al. [35], who showed that vagotomy in rats did not block the LPS injection-induced increase of IL-1β-immunoreactive cells in areas where the blood-brain barrier (BBB) and blood-cerebrospinal fluid barrier (BCSFB) are weak, such as the circumventricular organs and choroid plexus, respectively. Ji et al. [36] additionally demonstrated that vagotomy in rats increased monocyte chemoattractant protein 1 (MCP-1; also known as C-C motif chemokine ligand 2 [CCL2]) in the dorsal motor nucleus of the vagus nerve, suggesting that the vagotomies also impact monocyte chemotaxis. Overall, vagotomies have demonstrated that the vagus nerve is involved in signaling from the peripheral nervous system (PNS) to the brain, and it is involved in altering behavior and neuroinflammation, but it does not fully control all relevant immune responses.

2.3 What Have We Learned from Vagal Stimulation Studies?

Vagal stimulation methods (vagal nerve stimulation, VNS), in contrast to vagotomies, have initially been studied as tools for altering brain structure and function in the context of neurological disorders such as epilepsy [37]. The observed effects of VNS on monoamines in the brains of individuals and animals with epilepsy prompted more research on the effects of VNS on anxiety, affective disorders, and trauma- and stressor-related disorders. Overall, it has been found that in animal models VNS decreases anxious and depressive behavior and increases extinction of conditioned fear (a hallmark of resilience to trauma and stress) partially via peripheral muscarinic receptor activity. Vagal activity can be modulated by certain microbes; for example, intestinal injection of *Lactobacillus johnsonii* La1 in rats increases gastric vagal nerve activity [38]. Given that the vagus nerve innervates the gut and the vagus nerve can be stimulated by microbes, it follows that stimulation of the vagus nerve by the microbiota could modulate physiological and behavioral responses relevant to psychiatric disorders.

Noble et al. [39] demonstrated that VNS generally reduces anxious behavior in rats exposed to 2 days of auditory fear conditioning, as evaluated by elevated plus-maze behavior. Furmaga et al. [40] found that the anxiolytic effects of VNS in rats require activation of serotonergic and noradrenergic neurons, as administration of 5,7-dihydroxytryptamine and 6-hydroxydopamine (serotonergic and noradrenergic neuron neurotoxins, respectively) to the lateral ventricles blocked the anxiolytic effects of 2 weeks of VNS. Additionally, Noble et al. [41] demonstrated that blocking peripheral muscarinic receptors (of the parasympathetic nervous system) via intraperitoneal administration of the muscarinic receptor antagonist methyl scopolamine reverses the anxiolytic effects of 2 weeks of VNS in rats, indicating a role of peripheral signaling via the parasympathetic nervous system in VNS's anxiolytic effects. When combined with the facts that VNS attenuates the systemic inflammatory response to endotoxin in rats and that VNS attenuates neuroinflammation in response to LPS in mice, the necessity of parasympathetic nervous system activation for anxiolytic effects demonstrates that VNS's effects are at least partially dependent on peripheral inflammatory responses, not solely direct afferent signaling [42, 43].

VNS has also been found to exhibit antidepressant-like effects in rats undergoing chronic stress. Two weeks of VNS in rats increased the expression of 5-hydroxytryptamine (5-HT) receptor 1A in the dorsal raphe nucleus and nucleus tractus solitarius, along with the expression of 5-HT_{1B} receptor and brain-derived neurotrophic factor (BDNF) in the hippocampus, and it prevented decreases in expression of hippocampal 5-HT_{1B} receptor and BDNF induced by 2 weeks of chronic restraint stress [44, 45]. Notably, the modulation of hippocampal 5-HT_{1B} receptor and BDNF expression by VNS was accompanied by decreased depressive behavior in the rats who underwent chronic restraint stress [44, 45]. Moreover, the increase in hippocampal BDNF expression was blocked by injection of 5,7-dihydroxytryptamine into the dorsal raphe nucleus, demonstrating that effects

of VNS on BDNF expression are dependent on 5-HT signaling in the dorsal raphe nucleus [44, 45]. Furmaga [40] also found that 5,7-dihydroxytryptamine administration to the lateral ventricles blocked the antidepressant effects of VNS, but 6-hydroxydopamine administration did not, indicating involvement of serotonergic but not noradrenergic neurons in VNS's antidepressant-like behavioral responses, as assessed by forced swim test performance.

In addition to the ability of VNS to decrease anxiety-like behaviors and induce antidepressant-like behavioral responses, VNS has been shown to enhance fear extinction in mice and rats. For example, Noble et al. [46] found that VNS every other day for 12 days during the extinction phase of a posttraumatic stress disorder (PTSD) model (involving a single prolonged stressor followed by auditory fear conditioning) enhanced fear extinction and decreased PTSD-like symptoms. Furthermore, Souza et al. [47] showed that the effects of 5 days of VNS in rats follow an inverse U-shaped curve, where 0.4 and 0.8 mA VNS enhance fear extinction, but efficacy declines at 1.6 mA. Moreover, Noble et al. [41] demonstrated that 2 weeks of VNS in rats enhances fear extinction, and this was not blocked by intraperitoneal administration of the muscarinic receptor antagonist methyl scopolamine, indicating that peripheral signaling of the parasympathetic nervous system is involved in VNS's anxiolytic effects, as described above [39–41], but not its effects on fear extinction. Overall, VNS in animal models has shown to affect anxious, depressive, and PTSD-like behavior in a dose-dependent manner, via both serotonergic signaling and modulation of peripheral muscarinic receptor activity.

2.4 Epistemology of Vagal Signaling in the Microbiota-Brain Axis

Though vagotomies and vagal stimulation studies inform researchers about the relevance of the vagus nerve in the gut-brain axis, we must be critical of how they actually affect host physiology. Importantly, the vagus nerve is not solely composed of afferent fibers: up to 20% of vagal nerve fibers are efferent [20]. Thus, cutting the vagus nerve will indisputably have effects on non-CNS host physiology via altered efferent signaling. For example, Kessler et al. [48] demonstrated that a vagotomy modulates the immune system of septic mice, increasing the risk of death and elevating serum concentrations of tumor necrosis factor (TNF) and IL-6. Moreover, Di Giovangiulio et al. [49] demonstrated that vagotomized mice have increased susceptibility to dextran sulfate sodium (DSS)-induced colitis, along with decreased colonic lamina propria and mesenteric lymph node regulatory T cell (Treg) populations, indicating that vagotomies decrease peripheral immune regulation in mice. This suggests that previously mentioned immune changes in neural tissue are not purely a result of afferent signaling; vagal efferent modulation of the peripheral immune system is involved in these changes as well. Previously discussed research on vagal stimulation complements this, as it was demonstrated that VNS's anxiolytic

effects are dependent on peripheral signaling of the parasympathetic nervous system, and VNS suppresses immune and inflammatory responses to endotoxin and LPS exposure.

Due to the technological limitations of vagotomies (which affect both afferent and efferent fibers), it cannot be concluded from vagotomy or VNS studies that the results are solely due to afferent vagal signaling. To elucidate the roles of afferent and efferent vagal signaling, techniques such as selective optogenetic stimulation of afferent vagal fibers as demonstrated by Booth et al. [50] and efferent vagal fibers as demonstrated by Fontaine et al. [51] must be utilized in mechanistic studies. In conclusion, one should consider that the vagus nerve contains both afferent and efferent fibers before deriving causality from vagotomy- and vagal stimulation-based studies.

2.5 Non-vagal Afferents

Though the vagus nerve is perhaps the most studied direct afferent pathway relaying signals from the microbiota to the brain, it is not the only one. Other pathways include cutaneous spinal afferents, the remaining cranial nerves, and interoceptive afferent signals that travel in sympathetic nerve bundles. More thorough discussion of the effects of interoceptive signaling can be found in the human research section of this chapter.

2.5.1 Spinal Afferents from the Skin and Bronchopulmonary System

Emerging research in preclinical and human studies supports the hypothesis that activation of afferent spinoparabrachial and spinothalamic pathways from the skin and bronchopulmonary system, including activation by microbial inputs, modulates serotonergic signaling in the brain [11, 52]. For example, subcutaneous injection of *M. vaccae* NCTC 11659, which has been shown to alter serotonergic signaling in the dorsal raphe nucleus of the brainstem and to prevent stress-induced anxiety-like defensive behavioral responses, is hypothesized to exert these effects via spinoparabrachial and spinothalamic pathways, though the direct neural mechanisms involved have yet to be determined [11, 53–55]. Kim and Yosipovitch [56] review the ability of the skin microbiota to contribute to interoceptive stimuli (particularly itch) that are likely relayed to the brain via spinoparabrachial and spinothalamic pathways and are also modulated by the amygdala, which is sensitized by chronic stress and hyperactive in germ-free (GF) mice. However, direct mechanistic studies on these pathways from the skin microbiota to the amygdala are lacking.

Additionally, Hale et al. [26] identified that bronchopulmonary inflammation in mice (which is linked to the lung microbiome [57]) activated both spinal and vagal pathways. Given that bronchopulmonary microbiome literature is still in a nascent stage, it's not yet possible to draw a clear link between bronchopulmonary

microbiome and psychiatric outcomes via interoceptive signaling, but this should be considered a target for future research.

2.5.2 Cranial Nerve Signaling

Cranial nerves innervating the oral, nasal, and skin microbiota have the ability to impact neuropsychiatric outcomes. However, like other afferent signaling, the mechanisms of non-vagal cranial nerve afferents' effects on brain structure and function are understudied in animal models. This is despite the fact that some microbial taxa that are thought to be relevant to mental health, e.g., mycobacteria, appear to be restricted, or at least highly overrepresented, in oral and nasal compartments, relative to the gut microbiota [58, 59]. Although trigeminal nerve stimulation has been studied as a treatment modality for reducing major depressive disorder (MDD) symptoms in humans, the mechanisms through which trigeminal nerve stimulation affects behavior have not been thoroughly evaluated in animal models [60]. Additionally, studying trigeminal nerve activity and stimulation faces similar challenges as studying the vagus nerve since it is a combination of afferent and efferent fibers. Studies in animals have shown that microbes can traffic to the brain via the trigeminal nerve, as Riviere et al. [61] demonstrated trafficking of *Treponema*, a spirochaete bacterium with various subspecies that cause the diseases syphilis, bejel, and yaws, to the brain via the trigeminal nerve in mice.

The trigeminal nerve is not the only direct microbial trafficking pathway to the CNS; the olfactory nerves also allow the spread of herpes simplex virus 1 from the nasal mucosa to the CNS in rodent models [62]. Olfactory bulbectomy is a mouse model of depression that results in similar immunologic changes seen in MDD-, PTSD-, and anxiety-vulnerable populations, along with alterations to neuronal signaling [63]. Importantly, Ozcan et al. [64] demonstrated that olfactory bulbectomy in mice causes neuronal loss and morphological changes in the dorsal raphe nucleus, a major source of serotonergic innervation of forebrain circuits controlling stress-related behaviors and stress resilience. Of note, microbes can activate painful stimuli via olfactory sensory neurons. For example, mouse olfactory sensory neurons express the formyl peptide receptors (FPRs) that detect *n*-formyl peptides, which are produced by bacteria such as *Escherichia coli* [65, 66]. Importantly, FPRs activate nociceptive neurons during infection with *E. coli* or *Staphylococcus aureus* in mice [67]. Microfold cells in the nasal epithelium and upper respiratory tract sense microbial antigens and could also be responsible for triggering immune responses that activate cranial or spinal afferents [68]. Overall, the convergence of multiple pathways by which microorganisms in the skin, mouth, lungs, and gut activate spinal afferents with integration in somatosensory and affective CNS regions suggests that multiple microbiota act on non-vagal cranial or spinal afferent nerves to impact neuropsychiatric outcomes. The paucity of research in these areas, however, must be addressed by future studies.

3 The Microbiota-Immune Axis Modulates Brain Structure and Function

3.1 The Microbiota Modulates the Immune System

Multiple microbial ecosystems such as the gut and skin microbiota are known to modulate the immune system, which, in turn, plays a role in stress resilience and the risk of development and persistence of symptoms of stress-related psychiatric disorders, including anxiety disorders, affective disorders, and trauma- and stressor-related disorders such as PTSD [15, 69–75]. Immune-mediated effects on brain structure and function can occur in various ways, such as by cytokines from the periphery passing into the CNS; by immune cells passing through the BBB, BCSFB, or circumventricular organs into the CNS; or by neural afferents in the periphery relaying signals to the CNS [76]. It should also be noted that the effects of microbial exposure on the immune system do not require the microbes to be alive or to colonize the microbiota. Pseudocommensals, as they have been termed, are organisms that pass through the gut without colonizing it and exhibit strong immunomodulatory effects [77]. Exposure to living, dead, and even partial microbes has strong roles for regulation of the immune-brain axis in the context of mental health.

3.2 Sickness Behavior: An Insight into the Immune-Behavior Axis

Most who have dealt with infections, vaccines, broken bones, or other physical trauma are familiar with the associated psychological symptoms, such as reduced appetite, malaise, increased pain sensitivity, social withdrawal, and difficulty concentrating. These symptoms are collectively known as sickness behavior and, interestingly, overlap heavily with the symptoms of MDD [71, 78]. Generally, sickness behavior is induced by physiological or psychological stressors, ranging from chronic psychosocial stress to broken bones or signals of infection, such as elevated LPS [55, 79–81]. The stressors trigger a systemic immune response in both humans and rodents, notably including the systemic release of the proinflammatory milieu, IL-6, IL-1β, and TNF, along with interferons (IFN) such as IFN-γ [79, 82]. A portion of these proinflammatory cytokines and the immune cells they prime pass into the CNS and activate microglia (the brain's resident immune cells) and astrocytes to alter tryptophan-serotonin pathways, increase reactive oxygen species/reactive nitrogen species ROS/RNS concentrations, decrease BDNF, and contribute to excitotoxicity via altered glutamate signaling [76]. Additionally, increased BBB permeability by the proinflammatory state further allows the trafficking to occur. Together, these changes elicit increased anxious or depressive symptoms and decreased neuroplasticity and stress resilience, providing a window into how cytokines impact mental health and behavior [76]. Research suggests that sickness

behavior via the outlined immune response was evolutionarily advantageous to prevent the spread of diseases and to support healing [83]. However, in modern societies where psychological stressors are much more common than predator attacks, we may often be at odds with sickness behavior, with chronic low-grade inflammation and immune activation likely contributing to the increase in mental health disorders seen globally [84].

3.3 *Cytokines and Brain Structure and Function*

A large body of research demonstrates associations between altered proinflammatory cytokines, including elevated circulating IL-6, C-reactive protein (CRP), TNF, and IL-1β, and impaired stress resilience, as reviewed by Raison et al. [83] and by Maier and Watkins [85]. In addition to being able to alter BBB permeability, IL-6, IL-1β, and TNF can pass into the CNS through saturable transport mechanisms or through gaps in the BBB [86]. Once in the brain, IFNs, IL-1β, and TNF affect monoamine signaling, including serotonin, noradrenaline, and dopamine, as well as glutamate in humans and rodents (for review, see [76]). Serotonin signaling is altered by induction of indoleamine 2,3-dioxygenase (IDO), which is upregulated by IFN-γ, IL-1β, and TNF [87]. In both humans and rodents, IDO diverts the metabolism of tryptophan to kynurenine, decreasing the production of serotonin and potentially increasing the production of neurotoxic quinolinic acid [76, 88]. In rats, quinolinic acid activates *N*-methyl-D-aspartate (NMDA) receptors (a subset of glutamate receptor) while also stimulating astrocyte glutamate release and inhibiting reuptake [89]. These effects are further amplified by proinflammatory cytokines directly decreasing astrocyte glutamate reuptake and increasing glutamate release, which contributes to excitotoxicity in human cell lines and in vivo in rats [76, 90, 91].

IL-1β and TNF additionally activate p38 mitogen-activated protein kinase (p38 MAPK) in mice, increasing expression and function of serotonin reuptake transporters [92]. Furthermore, elevated proinflammatory cytokines can decrease serotonin, norepinephrine, and dopamine synthesis via destruction of tetrahydrobiopterin, a cofactor for tryptophan hydroxylase and tyrosine hydroxylase, by ROS [93, 94]. Under the monoamine hypothesis of MDD, increased serotonin reuptake and decreased serotonin, norepinephrine, and dopamine synthesis contribute to MDD. Overall, elevated proinflammatory cytokine concentrations in the periphery and CNS alter monoamine signaling and contribute to excitotoxicity, altering brain structure and function, which modulates stress resilience. Given that microbial exposure can alter circulating cytokine concentrations (see Sect. 3.5) and chronic low-grade inflammation is a risk factor for stress-related psychiatric disorders, it is evident that cytokines are a potential mechanism by which microbiota modulate stress resilience [16].

3.4 Cellular Access to the Brain

3.4.1 Stress Creates a Proinflammatory Repertoire of Circulating Immune Cells

Upon being exposed to a psychological or physiological stressor, the immune cell profile of the body is shifted toward a proinflammatory state, generally increasing the quantities of proinflammatory cytokines produced in response to exposure to proinflammatory microbial antigens such as LPS. Additionally, chronic, lower-grade stress also pushes the immune cell repertoire toward a proinflammatory state characterized by resistance to glucocorticoids (GCs). Of note, repeated social defeat in mice increases CD14 and CD86 expression on macrophages [95], and the chronic subordinate colony housing model induces GC resistance of Th2 lymphocytes and a decrease in Tregs [55, 81, 96].

Psychosocial stressors also lead to upregulated expression of TLR4 in mice, increasing the likelihood of nuclear factor-kappa B (NF-κB) priming of peripheral immune cells, including macrophages and monocytes [97, 98]. Wan et al. [99] demonstrated a positive feedback cycle, where NF-κB increases TLR4 expression, increasing sensitivity to LPS and further upregulating NF-κB in THP-1 cells (a human monocytic cell line). Activation of TLR4 and NF-κB during this cycle primes monocytes to a proinflammatory state, characterized by increased IL-6, pro-IL-1β, and TNF production [76]. Additionally, in mice, repeated sympathetic nervous system activation by stressors increases systemic norepinephrine and encourages myelopoiesis, resulting in a less mature and more inflammatory population of immune cells (particularly bone marrow-derived monocytes) in circulation [100, 101]. Due to the shift of the circulating immune cell repertoire to a proinflammatory state that is induced by stress, chronically stressed individuals may exhibit heightened sensitivity to disrupted microbial communities and increased epithelial permeability at locations such as the gut mucosa. Thus, interventions focused on increasing microbiota community health to improve the functioning of the microbiota host-epithelia barrier may prevent or attenuate activation of the immune system that can contribute to impaired stress resilience.

3.4.2 Stress and Microbes Modulate the Inflammatory State of Microglia in the Central Nervous System

Thion et al. [102] showed that absence of a microbiota during development or disrupted microbiota community structure by antibiotic exposure results in altered microglia transcriptome in a sex-specific manner in mice. Notably, mice with GF mothers have altered expression of microglial immune response genes indicative of immaturity beginning in utero, with an increased expression of the genes *Ly86* and *Aoah*, which are involved in the response to LPS [102]. These changes affected males more strongly in utero but had more lasting effects into adulthood in female

mice, highlighting sex-specific modulation of behavior-relevant immune activity [102]. Moreover, treatment with an antibiotic cocktail containing ampicillin, streptomycin, colistin, and amphotericin for 1 week induced changes in microglial gene expression, including decreased concentrations of the anti-inflammatory and immunosuppressive genes *Nfkbia* (NF-κB inhibitor 1 alpha), *Tsd22d3* (glucocorticoid-induced leucine zipper, GILZ), and *Ddit4* (DNA damage inducible transcript 4) in both male and female adult mice [102]. Moreover, Boehme et al. [103] found that 12 weeks of consumption of a fructooligosaccharide-enriched inulin prebiotic alters microbiome composition and prevents an age-related increase in the fraction of activated microglia in mice. Together, these data demonstrate that (1) the lack of a microbiota impairs microglia development; (2) disruption of the microbiota alters the inflammatory reactivity of microglia; and (3) microbiota-bolstering techniques such as prebiotic administration are able to attenuate microglial reactivity.

Additionally, psychosocial stress can alter microglial gene expression; Wohleb et al. [95] demonstrated that repeated social defeat in mice increases CD14, CD86, and TLR4 expression on microglia. Moreover, Frank et al. [104] demonstrated that inescapable shock (an acute stress model) induces microglial priming in rats. Rats exposed to inescapable shock had increased concentrations of major histocompatibility complex II (MHCII) and decreased neuronal glycoprotein CD200 in vivo, along with heightened production of IL-1β in response to stimulation with LPS ex vivo 24 h after inescapable shock exposure [104]. Increased MHCII and decreased CD200 contributing to microglial reactivity and increased IL-1β after LPS challenge demonstrate this ex vivo. Stress-induced microglial priming and stress-induced increases in anxiety-like defensive behavioral responses, assessed 24 h following stress exposure, are prevented by prior immunization with *M. vaccae* NCTC 11659 [104], demonstrating that microbial exposures have the potential to increase stress resilience. Overall, microbial exposure modulates the state of microglia in the murine brain, conferring resilience against stress-induced microglial changes and resulting neuroinflammation and altered behavior.

3.4.3 Stress Causes Immune Cell Trafficking into the Brain

Multiple lines of evidence suggest that exposure to chronic stressors can increase immune cell trafficking into the brain. Repeated social defeat stress causes an increase in brain chemoattractant production in mice, causing GC-insensitive monocytes from the bone marrow to traffic to the brain [83, 105]. To elaborate, repeated social defeat causes the release of C-C motif chemokine ligand (CCL) 2 from cytokine-stimulated astrocytes in the brain, attracting CCL2 receptor $(CCR2)^{+}$/ $CX3CR1^{+}$ monocytes; consistent with a significant role for CCL2 signaling, this monocyte trafficking to the brain is largely blocked in CCR2 knockout mice [76, 106]. Furthermore, monocytes trafficking into the brain due to peripheral inflammation produce TNF upon arrival, increasing the proinflammatory cytokine load in the brain [107, 108]. Upon arrival in the brain, monocytes differentiate into brain resident macrophages, which are capable of proinflammatory responses

stronger than those from microglia [105, 109]. Overall, systemic inflammation from psychosocial or physiological stressors primes the circulating immune cell repertoire to a proinflammatory state and induces trafficking to the brain, resulting in impaired stress resilience and anxious behavior via cytokine release.

3.4.4 The Choroid Plexus Is a Gatekeeper of Immune Cell Access to the Brain

The brain is enveloped by three layers of meninges, the dura mater, arachnoid mater, and the pia mater. The choroid plexus resides in the innermost layer of the meninges (pia mater), which is in close contact with the cerebral cortex and spinal cord. Within the choroid plexus (CP), the blood-cerebrospinal fluid barrier (BCSFB) is characterized by fenestrated capillaries [110]. Upon passing through the fenestrated capillaries into the parenchyma of the CP, circulating lymphocytes, accompanied by (antigen-presenting) dendritic cells (DCs), await translocation into the CSF [110]. This exposure of lymphocytes to DCs immediately before crossing into the CSF can be critical for encouraging a proinflammatory lymphocyte bias if the DCs are presenting antigens that promote proinflammatory responses [111]. In cases of infection or hyperpermeable host-microbiota epithelia (at any location harboring a microbiota), high relative abundances of microbial antigens presented by DC could prime lymphocytes to a proinflammatory state prior to entering the CNS. Notably, Th17 lymphocytes, increased by IL-1β, are a chink in the BCSFB's armor, which is particularly important given that microbial exposure alters Th17 lymphocyte concentrations through multiple mechanisms [84, 112]. Even in an uninflamed brain, $CCR6^+$ Th17 lymphocytes can cross the BCSFB at the CP [113]. After crossing, their interactions with DC in the subarachnoid space activate a proinflammatory cascade that can damage BCSFB tight junction integrity [113]. This proinflammatory cascade is associated with the release of vascular cell adhesion molecule (VCAM) 1, a driver of lymphocyte trafficking [114, 115]. Thus, a Th17 lymphocyte bias from systemic or peripheral inflammation characterized by increased IL-1β can result in a permeabilized BCSFB at the CP and further lymphocyte trafficking into the CNS. Moreover, Kertser et al. [116, 117] demonstrated that severe psychological stress in mice impairs CP BCSFB function, allowing increased leukocyte trafficking in a manner dependent on GC signaling. Blocking GC receptors restores BCSFB immune surveillance by increasing Treg trafficking and attenuates posttraumatic behavioral deficits. When combined with Baruch and Schwartz's [118] review of how CNS-specific $CD4^+$ T cells shape brain function via the CP, this research suggests a role of the Th17/Treg balance (an identified therapeutic target in autoimmune conditions) in maintaining the BCSFB for proper stress resilience [119]. Notably, exposure to microbial old friends, such as the helminth *S. mansoni*, regulates the Th17/Treg balance, highlighting the importance of microorganisms in protecting the BCSFB to prevent proinflammatory lymphocyte trafficking, which can impair stress resilience downstream [84].

Pathogens (naked or attached to or inside immune cells) can trigger cells in the CP to relay inflammatory signals to the brain or even cross the CP and enter the CNS. For example, *Listeria monocytogenes* enters the CNS via a "Trojan horse" method, passing across the BCSFB inside peripheral mononuclear phagocytes [120]. Likewise, *Streptococcus suis* can enter the CNS via a "Trojan horse" method inside polymorphonuclear neutrophils [121]. Another example is that death following infection with SARS-CoV-2 is associated with CP inflammation, increased CCL2 and CXCL2 expression in the brain, and increased CP to cortex proinflammatory signaling associated with microglial activation [122]. These proinflammatory responses occur via SARS-CoV-2 binding at the CP but without SARS-CoV-2 actually entering the brain [122], but antigens including the M1 spike protein from SARS-CoV-2 have been shown to cross the BBB in mice, outlining a potential mechanism by which proinflammatory cascades could be triggered from within the CNS [123]. Though it is impossible to know how SARS-CoV-2 infection alters CP inflammation and CCL2 and CXCL2 expression in individuals who survive the infection, this suggests that viral exposure can modulate the inflammatory state of the CP and that infection may confer long-term risk for impaired cognition and depression. Schwerk et al. [124] have reviewed the evidence that because some pathogens can cross the BCSFB at the CP, the CP responds to pathogen challenge by increasing cytokine and chemokine production and BCSFB permeability to encourage leukocyte trafficking into the brain. In the case of pathogens in the brain, the response of the CP to increase leukocyte trafficking is protective against the pathogens, but it also has the unfortunate "side effects" of impairing cognition and decreasing stress resilience by encouraging proinflammatory cytokine production in the CNS [124]. Notably, exposure to dysbiotic microbiota with overgrowth of pathogens or pathobionts such as *Neisseria meningitidis* or *E. coli* or disruption of the host-microbiota epithelial barriers has the potential to trigger these "side effects," highlighting the importance of maintaining diverse microbiota that are resilient to pathogen overgrowth and microbiota that support healthy epithelial barriers [124]. The CP serves as a gatekeeper of immune access to the brain, but modulation of immunophenotypes by a microbiota encouraging inflammation and a Th17-dominant lymphocyte repertoire as well as pathogen infection (which could be somewhat prevented by a diverse microbiota) can impair the BCSFB, resulting in decreased stress resilience.

3.5 Impacts of Microbial Exposure on the Immune-Brain Axis

The ability of stressors to modulate the immune-brain axis raises the question of what can be done to intervene. One potential means of regulating the immune system to confer stress resilience is through microbial exposure. It's important to note that effects of microbe-immune system interactions on brain structure and function do

not rely on microbe colonization or even live/whole microbes. Prime examples of this include the ability of immune stimulation by LPS injection or by subcutaneous or intratracheal administration of heat-killed *M. vaccae* to activate serotonergic neurons in the dorsal raphe nucleus, conferring stress resilience in mice [125]. Initial research demonstrated that subcutaneous injection with heat-killed *M. vaccae* NCTC 11659 activated a subset of serotonergic neurons in the dorsal raphe nucleus in mice, improving performance in the forced swim test [11]. Since then, a series of follow-up studies has demonstrated immunoregulatory effects of *M. vaccae* NCTC 11659. For example, Reber et al. [55] demonstrated that *M. vaccae* NCTC 11659 immunization prevents stress-induced colitis and anxiety in response to the chronic subordinate colony (CSC) housing model, a validated model of PTSD [81]. Additionally, Amoroso et al. [58] demonstrated that *M. vaccae* NCTC 11659 prevents stress-induced aggravation of dextran sulfate sodium-induced colitis in mice, likely through the induction of Tregs [126]. Moreover, *M. vaccae* NCTC 11659 improved stress resilience, stabilized the gut microbiome, and attenuated proinflammatory physiological responses to a "two-hit" stress exposure mouse model of circadian disruption followed by acute social defeat [54]. Further research demonstrated the ability of a novel lipid derived from *M. vaccae* NCTC 11659, 10(*Z*)-hexadecenoic acid, to act on peroxisome proliferator-activated receptor alpha (PPARα) to decrease IL-6 mRNA and protein expression following LPS challenge in freshly isolated murine peritoneal macrophages [127]. In this research, 10(*Z*)-hexadecenoic acid also attenuated LPS activation of TLR4, resulting in less NF-κB downstream signaling.

Similarly, exposure to other microbe-derived lipids, such as conjugated linoleic acids (CLAs) from *Lactobacillus* spp. and *Bifidobacterium* spp., can be immunomodulatory. For example, Miyamoto et al. [128] demonstrated that 10-hydroxy-cis-12-octadecenoic acid prevents TNF-induced gut epithelial dysfunction. Additionally, oral supplementation of CLA has been shown to prevent age-related deficits in BDNF and synaptic function in an aged mouse model of depression risk [129]. The attenuation of hallmarks of age-related depression pathophysiology was found to be mediated by nuclear erythroid-related factor 2 (NRF2), a transcription factor important for anti-inflammatory response regulation [130]. Due to NRF2's roles, including inhibition of NF-κB, NRF2 and NRF2-modulating phytochemicals have been identified as a potential pharmacological target for inflammatory disorders [130]. Hashimoto [131] reviews the role of NRF2 in affective disorders, including evidence such as (a) lower NRF2 expression in the prefrontal cortex (PFC) and CA3 and dentate gyrus (DG) regions of the hippocampus in mouse models of depression, (b) depressive-like behavior in NRF2 knockout mice, and (c) decreased BDNF in the PFC, CA3, and DG. Overall, a variety of living and dead microbes (i.e., postbiotics, see Salminen et al. [132] for elaboration) as well as their metabolites can activate the host immune system to confer stress resilience.

4 The Microbiome, the Blood-Brain Barrier, and Neuropsychiatric Outcomes

4.1 Blood-Brain Barrier Integrity Influences Neuropsychiatric Outcomes

The BBB is an important component of the CNS in maintaining proper cognitive and behavioral function. The BBB functions as a primary gatekeeper, controlling which molecules pass between the circulatory system and the CNS [133]. Though the BBB was initially described as a static barrier, current research has characterized it as a highly dynamic and sensitive system of inter-woven brain microvascular endothelial cells (BMECs), neurons, pericytes, astrocytes, and smooth muscle cells stitched together by protein complexes [134]. These components, combined with circulating blood cells, comprise neurovascular units (NVU), which are responsible for maintaining hemodynamic homeostasis in response to cerebral hypo- or hyperemia and for the regulation of molecular and cellular transport into the brain [135].

It is becoming clear that the gut microbiota influences BBB structure and function. Although not all of the underlying mechanisms are fully understood, evidence suggests a number of distinct mechanisms are involved. For example, there are many microbial metabolites that can affect BBB permeability including bacterial metabolites such as short-chain fatty acids (SCFAs), trimethylamine *n*-oxide (TMAO), and modified bile acids, along with host-derived signaling molecules induced by the microbiota, such as cytokines, hormones, and ROS. Notably, there is complex interplay between the host and microbiota for the production of these molecules, as some (e.g., SCFA) are purely microbe-derived; some (e.g., TMAO) are microbe-derived and host-altered, meaning the host modifies the structure of the molecule to convert them to a bioactive form (e.g., oxidizing TMA to form TMAO). Some (e.g., secondary bile acids) are host-derived but microbe-altered, meaning that the microbiota is involved in converting them to their bioactive form; and others (e.g., cytokines, hormones, ROS) are host-derived and structurally unaltered by microbes, but their quantities in the host are altered by microbes.

Allostatic load placed on the BBB by a dysbiotic microbiota, trauma, or sickness across a lifetime can lead to BBB dysfunction, which is associated with increased risk for affective and stress-related disorders in humans or anxiety-like/depressive-like behavior in murine models [136, 137]. Additionally, chronic psychosocial stress can cause BBB disruption in mice, and the resulting molecular changes to the BBB further contribute to decreased stress resilience [136, 138]. Upon BBB disruption by stress and/or peripheral inflammation, macrophages and monocytes primed to a proinflammatory state by microbial antigens and proinflammatory cytokines in circulation can more easily traffic into the CNS, contributing to anxious and depressive-like behavior [76, 105, 139]. Thus, maintenance of the BBB by a variety of host- and microbe-derived metabolites is important for maintaining stress resilience.

4.2 *Bacterial Metabolites Influence Blood-Brain Barrier Integrity*

4.2.1 Short-Chain Fatty Acids (SCFA)

The human digestive system lacks many enzymes that are required to break down complex plant fibers, and transit time in the gastrointestinal tract is too short to allow the complete breakdown of resistant starches. These fibers and resistant starches pass through the small intestine into the colon (or large intestine), where they are fermented by the members of the gut microbiota. One major product of this fermentation is a class of molecules known as short-chain fatty acids: fatty acids up to six carbons (C) in length. Ninety-five percent of SCFAs produced are acetate (2C), propionate (3C), and *n*-butyrate (4C), which generally exist in a ratio of 60:20:20, respectively, in the stool [140, 141].

As the major energy substrate for the cecocolonic epithelium, butyrate has been the subject of much research, which has uncovered important roles in maintaining host health [142]. One important mechanism by which butyrate maintains host health is through regulating epithelial function, which has historically been primarily studied at the gut epithelium. Decreased butyrate concentration in the gut results in changes to intermediary metabolism (decreased NADH/NAD(+), oxidative phosphorylation, and ATP) within colonocytes that confer catabolic processes, leading to poor colonocyte health [142]. Furthermore, butyrate's mechanisms for modulating epithelial function include non-energetic mechanisms. For example, it acts as a histone deacetylase (HDAC) inhibitor throughout the body, regulating cell proliferation and resistance to oxidative stress, and also acts through its binding to immunomodulatory G protein-coupled receptor (GPR) 41 and GPR43 expressed on enteroendocrine cells in the gut [143–145]. GPR41 and GPR43 are also referred to as free fatty acid receptor (FFAR) 2 and FFAR3, respectively. They have high affinity for butyrate and propionate but low affinity for acetate [145].

The benefits of SCFAs for epithelial function are not localized exclusively to the gut. FFAR3, found on vascular endothelial cells in the brain, responds to physiologically relevant quantities of propionate to protect the BBB from lipopolysaccharide (LPS)-induced tight junction disruption and damage from oxidative stress in human cell lines in vitro [146]. Braniste et al. [147] demonstrated that oral butyrate administration in GF mice decreased BBB permeability to the same extent that exposure to a pathogen-free microbiota did. Additionally, this decrease in BBB permeability was thought to be mediated by increased expression of occludin proteins, which also mediate the effects of the microbiota on epithelial function in the gut and testis and are known as key modulators of tight junction function in the BBB [148–150]. Moreover, butyrate exerts protective effects on the BBB via the immune system, as it induces Treg proliferation and inhibits NF-κB production [151, 152]. Tregs are associated with protection against BBB damage following stroke and traumatic brain injury in mice [153, 154], and inhibition of NF-κB blocks

a proinflammatory cascade of cytokines that disrupts BBB integrity (discussed in cytokine section below).

Although acetate is known to readily cross the BBB in humans, not much is known about its direct actions on the BBB in humans or mice [155]. Additionally, the effects of other less abundant SCFAs on the BBB are not well characterized, though they are known to have effects in other areas of the body. For example, similar to butyrate, valerate (5C, also referred to as pentanoate) has demonstrated activity as a HDAC inhibitor in lymphocytes in mice, assessed both in vivo and in vitro, yet its direct impacts on the BBB remain unknown [156]. Future studies should further evaluate the mechanisms through which other SCFAs act on immune and BBB function.

4.2.2 Trimethylamine N-Oxide

Another class of molecule known to modulate the BBB is TMAO. TMAOs are derived from quaternary amines such as choline, carnitine, and lecithin sourced from the diet [157]. Such amines are converted to trimethylamine (TMA) in the gut by *Anaerococcus*, *Clostridium*, *Escherichia*, *Proteus*, *Providencia*, and *Edwardsiella* and then absorbed and oxidized to form TMAO in the liver [158, 159]. TMAO has been studied for its impact on endothelial function in humans and animal models, as reviewed by Naghipour et al. [160] and Tang et al. [161], and recently some studies have uncovered roles of TMAO in modifying the BBB. Hoyles et al. [162] and McArthur et al. [163] have shown that low doses of TMAO exert protective effects on the BBB in in vitro human cell culture and in vivo animal models, likely through effects on actin cytoskeletons and tight junctions. However, Liu and Huang [164] demonstrated that chronically elevated TMAO concentrations in the plasma of poststroke patients were associated with the development of impaired cerebrovascular function, and their follow-up rat model demonstrated an impaired BBB following high TMAO diets. Current research on TMAO's effects on the BBB cannot draw a full story of dose responsiveness but, to date, suggests the potential of a U-shaped dose response curve of TMAO-BBB interactions.

4.2.3 Secondary Bile Acids

For years, bile acids, synthesized from cholesterol in the liver, were primarily considered as facilitators of lipid digestion and absorption in the gut. However, research emerging over the past two decades has demonstrated their function as signaling molecules throughout the body, with receptors in endocrine glands, adipocytes, skeletal muscles, immune organs, and the nervous system [165]. Additionally, when passing through the digestive tract, bile acids can be deconjugated and decarboxylated by specific gut bacteria to form secondary bile acids, increasing the diversity of the bile acid repertoire [166]. These unconjugated and uncharged bile acids can be passively absorbed in the colon, where they are directed toward hepatic

portal circulation [166]. In humans, less than 10% of absorbed bile acids make it past enterohepatic circulation to systemic circulation, resulting in a plasma concentration between 5 and 15μmol/L [166]. Highly elevated bile acid concentrations in the blood can result in disruptions of the BBB in rats and guinea pigs, likely due to cell membrane damage from the same detergent properties that make bile acids useful in digestion [167, 168]. The effects of lower concentrations of bile acids, however, may not be generalizable across all types of bile acids. For example, in rats, the unconjugated secondary bile acids chenodeoxycholic acid and deoxycholic acid at low relative abundances increase phosphorylation of occludin tight junction proteins, disrupting barrier function, whereas other secondary bile acids, ursodeoxycholic acid and glycol-ursodeoxycholic acid, exert protective effects on the cerebrovascular epithelium in human cell lines [166, 169]. It is important to consider that the beneficial effects of certain secondary bile acids on the BBB could be mediated by a hormetic response. That is, secondary bile acids that improve BBB integrity could do so by causing acute physiological damage that induces BBB proliferation in response. Secondary bile acids that have been shown to exert protective effects at low concentrations may not be protective when chronically elevated or at high concentrations, but research to date has not fully elucidated these effects.

4.3 Host Signaling Molecules Whose Quantities Are Altered by the Microbiome Influence Blood-Brain Barrier Integrity

4.3.1 Cytokines

The gut, skin, and oral microbiota are well known to regulate immune function (as reviewed in Lowry et al. [7], Kau et al. [170], Park and Lee [171], and Idris et al. [172]), which affects the integrity of the BBB (as reviewed by Banks and Erickson [173]). While proper regulation of immune function can lead to maintenance of BBB integrity, immune dysregulation can lead to BBB disruption via increased proinflammatory cytokine production. Of note, dysbiotic gut microbiota states associated with inflamed gut mucosa can upregulate production of the cytokines TNF, IL-6, and IL-1β, leading to increased BMEC permeability [174, 175]. Likewise, dysbiotic states or the presence of extracellular RNA from pathogens in the oral mucosa can increase TNF, IL-6, and IL-1β abundances in mice and human macrophages (in vitro), widening tight junctions of the BBB via decreasing claudin-5 protein expression [176, 177]. Moreover, TNF production in mice encourages neutrophil trafficking to the CNS, encouraging BBB permeability by releasing chemokine ligands (CXCL) 1, 2, 3, and 8 and other metabolites such as ROS [178, 179]. This breach further enables proinflammatory cytokine and immune cell trafficking into the brain [179]. However, the master regulator of proinflammatory cytokine production NF-κB, which upregulates IL-6, IL-1β, and TNF production, is

inhibited by butyrate, blunting the inflammatory milieu mentioned above [180]. Overall, the milieu of proinflammatory cytokines triggered by gut, oral, and skin inflammation impairs BBB integrity, but a diverse gut microbiota capable of promoting immunoregulation and producing SCFA can exert protective effects on the BBB, conferring stress resilience [181].

4.3.2 Hormones

In addition to cytokines, hormones also play a role in maintaining BBB integrity. Interest in the impacts of estrogen and testosterone on BBB integrity was sparked after a study showed sex differences in lateral striatal artery vulnerability mediated by estrogen and testosterone in mice [182]. Since then, research has shown that estrogen is a strong regulator of BBB integrity, protecting against tight junction disruption by inducing estrogen receptor α and nuclear receptor corepressor to downregulate matrix metalloproteinase (MMP) transcription in rats in vivo and in vitro [183, 184]. Thoroughly reviewed in Baker et al. [185], the gut microbiota is a primary modulator of circulating estrogen in animals and humans. Bacteria in the mammalian gut secrete β-glucuronidase, which deconjugates estrogens and phytoestrogens, conjugated in bile, to their active and absorbable forms [185]. Dysbiotic states of the gut microbiota with low richness and bacterial biomass decrease β-glucuronidase production, altering the estrobolome, which can exert direct effects on the BBB [185]. Wilson et al. [186] demonstrated that these effects may also be modulated by serum gonadotropins, which are dysregulated in GF mice [148].

Altered estrogen concentrations could also exert indirect effects on the BBB via the vaginal microbiome. Increased estrogen at puberty is associated with enhanced glycogen deposition at the vaginal mucosa, shifting the vaginal microbiome toward a *Lactobacillus*-dominated community [187]. As could occur with low estrogen concentrations, a non-*Lactobacillus*-dominated vaginal microbiome is associated with production of the previously mentioned proinflammatory milieu of IL-1β, IL-6, and TNF in humans, but this has not been studied thoroughly in murine models [188]. Notably, though diverse microbial exposure is important for training the immune system and protecting against infection in skin, oral, and gut microbiota, high vaginal microbiome diversity is associated with high pH and resultant pathogen susceptibility in humans [189, 190].

In addition to estrogen, testosterone is also modulated by the microbiota and has effects on the BBB. Chronically low testosterone concentrations in gonadectomized mice result in increased BBB permeability when compared to testosterone-supplemented gonadectomized mice roughly 2 months after castration [191]. The increase in BBB permeability was associated with astrocyte and microglia activation, along with increased hypothalamic expression of IL-1β and TNF, which were almost completely attenuated in testosterone-supplemented mice, suggesting indirect effects of testosterone on BBB function [191]. Notably, though testosterone often decreases with age, Poutahidis et al. [192] demonstrated that 3–9 months of

daily *Lactobacillus reuteri* ATCC PTA 6475 consumption prevented age-related decline of testosterone concentrations and testicular size in mice in an IL-17-dependent manner. Moreover, early life antibiotic exposure decreases Leydig cell testosterone function through both microbiome- and non-microbiome-mediated mechanisms [193, 194]. This is mirrored in humans as well, where microbiome diversity positively correlates with testosterone concentrations [44, 45].

Another hormone known to exert protective effects on the BBB and to be influenced by the microbiota is vitamin D (also known as 1,25-OH-cholecalciferol or calcitriol in its active form). This is important for BBB integrity because human BMECs express vitamin D receptors with detectable abundance of both mRNA and protein [195]. In vitro treatment with activated vitamin D prevents the decrease in occludens-1 and claudin-5 and the increase of intercellular adhesion molecule-1 and NF-κB caused by TNF exposure [195]. Direct binding to vitamin D receptors associated with the BBB is postulated to be a mechanism for this, as human BMECs express vitamin D receptors at both the mRNA and protein levels [195]. These findings suggest that vitamin D is another hormone mediating microbiota-BBB interactions.

4.3.3 Reactive Oxygen/Nitrogen Species

ROS and RNS are present in moderate concentration across most cells, acting as signaling molecules via oxidative modification of biological molecules [196]. However, high concentrations of ROS and RNS are associated with increased oxidative damage to tissues including the BBB [196, 197]. Specifically, MMPs, which act as proteolytic enzymes degrading extracellular proteins, are activated by high ROS and RNS concentrations in humans and animal models [197]. This is achieved directly via oxidation or S-nitrosylation of MMPs and indirectly by upregulation of the proinflammatory cytokine milieu IL-1β, IL-6, and TNF [197].

Mitochondria are major sources of ROS in the human body, as they produce ROS in the electron transport chain [198]. Microbial metabolites impact host mitochondrial function, resulting in altered ROS production, as reviewed by Ballard and Towarnicki [198]. A particular example from Wikoff et al. [199] demonstrated that GF mice have many dysregulated metabolic pathways, such as indole metabolism, which affect mitochondrial membrane potential, conferring altered organism-wide ROS concentrations [198].

Furthermore, SCFAs can alter ROS concentrations. Hoyles et al. [146] demonstrated that ROS production in response to proinflammatory stimuli in human BMECs in vitro was ameliorated by propionate treatment. Butyrate also exerts neuroprotective effects in vitro in human cell lines and in vivo in mice by stimulating mitochondrial biogenesis, which is widely associated with improved mitochondrial function, often defined as more efficient electron transport chain production of adenosine-5′-triphosphate and less aggressive production of ROS [200, 201]. Overall, since systemic ROS can lead to BBB damage, modulating mitochondrial biogenesis

may be another mechanism by which butyrate exerts protective effects on the BBB. Mitochondrial biogenesis will be revisited in more detail later in this chapter.

4.4 Circumventricular Organs

The third and fourth ventricles of the brain are associated with circumventricular organs (CVOs), including the subfornical organ, the area postrema, the organum vasculum of lamina terminalis (OVLT), the median eminence, the posterior pituitary, and the pineal gland, all of which lack a BBB. Because of their lack of a BBB, CVOs are particularly sensitive to and points of entry into the brain for contents of the circulatory system. This includes cells, cytokines, microorganisms, prions, and autoantibodies [202]. CVOs and disrupted (or "leaky") sections of the BBB allow humoral access for immune cells and cytokines to the CNS [76]. As a result of their access and sensitivity to circulatory system contents, CVOs play critical roles in regulation of immune access to the CNS and other processes that can affect mental health [7].

5 The Microbiota, Neuroplasticity, and Mitochondrial Function

It is important to note that neural architecture in the brain is not static; dynamic restructuring of neural connections throughout life occurs in normal, healthy humans [203]. The processes surrounding neuronal growth and restructuring are referred to as neuroplasticity and include neurogenesis, neuronal death, synapse formation and synaptic pruning, dendritic remodeling, and axonal sprouting and pruning [203]. Though most prevalent during early stages of life, neurogenesis occurs in healthy adults and is associated with learning and adaptation to new stimuli [203, 204]. In humans, neurogenesis is widely accepted to occur in two areas: the subgranular zone of the dentate gyrus with incorporation into the hippocampus and the subventricular zone with incorporation into the olfactory bulb [205–207].

Though olfactory bulb neurogenesis is not directly linked to psychiatric outcomes, one can reference the fact that olfactory bulb deficits, such as through olfactory bulbectomy (previously mentioned as an animal model for depression), downregulate hippocampal neurogenesis [208]. This is likely at least partially mediated by altered serotonin signaling, given that (a) neuronal death in the dorsal raphe nucleus following olfactory bulbectomy permanently impairs hippocampal serotonin signaling, (b) serotonin signaling encourages hippocampal neurogenesis, and (c) selective serotonin reuptake inhibitor (SSRI) treatment restores hippocampal neurogenesis following olfactory bulbectomy [209–212]. Activation of serotonergic neurons in the dorsal raphe nucleus via microbial exposure (e.g., as shown by

M. vaccae NCTC 11695 exposure [11]) may reduce stress susceptibility, but the effects of microbial exposure on neurogenesis via the dorsal raphe nucleus have not been studied directly [213].

5.1 Brain-Derived Neurotropic Factor as a Microbiota-Mediated Modulator of Neuroplasticity

BDNF is a key modulator of neuroplasticity in human and rodent brains, with roles in neuronal cell growth, survival, and function, conferring emotion and cognitive behavioral roles [214, 215]. Importantly, BDNF concentrations in regions of the brain including the hippocampus and brainstem can be altered by the gut microbiota. To establish a baseline, Sudo et al. [216] demonstrated that GF mice have decreased hippocampal BDNF receptor expression when evaluated following stressor exposure. Furthermore, Gareau et al. [217] showed that GF mice experience a reduction in BDNF and deficits in nonspatial and working memory after being stressed, which was mirrored in mice infected with *Citrobacter rodentium* and ameliorated upon 17 days of daily treatment with *L. rhamnosus* (R0011) and *L. helveticus* (R0052). In contrast to other GF studies, Neufeld et al. [218] found increased BDNF in the granule cell layer of dentate gyrus of the hippocampus of GF female mice, which was associated with anxiolytic behavior. Bercik et al. [219] showed that oral treatment with broad-spectrum antibiotics in nonstressed mice increased hippocampal BDNF protein expression and exploratory behavior, along with decreasing amygdala BDNF protein expression, changes that are associated with altered fear learning [220]. Notably, given that BDNF is released during and plays a critical role in the response to stressors, and given that the effects of BDNF are site-specific, a decrease in BDNF in stressed, GF mice does not necessarily contradict increased abundances of BDNF in the hippocampus of unstressed, GF or antibiotic-treated mice; there appears to be a complex interaction between microbial exposure and site-specific BDNF release in response to stress, and mechanisms have not been fully elucidated [221].

Gut mucosal infection from *Trichuris muris* was shown to increase peripheral inflammation, decrease hippocampal BDNF mRNA, and increase anxiety-like behaviors [222]. Notably, the decrease in BDNF was not attenuated by administration of anti-inflammatory agents; however, treatment with the probiotic *B. longum* NCC3001 (ATCC BAA-999) did attenuate BDNF expression and behavioral alterations without altering concentrations of proinflammatory cytokines. This suggests that BDNF expression is largely controlled by mechanisms unrelated to inflammation. Likewise, Savignac et al. [223] demonstrated that prebiotic feeding increases BDNF in central regions of the brain via gut hormones such as peptide YY in rats. Notably, the SCFA butyrate is another trigger for BDNF release, which has been shown to occur via both HDAC inhibition and decreased methylation of the *Bdnf*

gene [224, 225]. Overall, BDNF concentrations in the murine brain are altered by the microbiota through mechanisms separate from inflammatory cytokines.

5.2 *Microbiota-Immune Mediation of Neuroplasticity*

As previously discussed, microbiota alter the host immune system, which is important, as neuroplasticity is also regulated by immune mechanisms [226]. For example, in mice, low (physiological) concentrations of IL-1β are critical for long-term potentiation and memory formation, but excess IL-1β leads to impaired memory [227, 228]. Similar to the U-shaped effect of IL-1β, varying concentrations of IL-4, IL-6, and TNF appear to have differential effects on neuroplasticity under different conditions [226, 229]. Though chronically elevated IL-6 inhibits adult hippocampal neurogenesis, acute IL-6 responses are important for neuroplasticity in response to stressors, such as brain injury and ischemia in mice and gerbils [230, 231]. Likewise, TNF is involved in neurogenesis, but chronically elevated concentrations are not typically associated with increased cognitive function in animal models or humans [226, 232]. In addition to their direct effects on neurogenesis, IL-6 and TNF may play stronger roles by regulating inflammation in the CNS. For example, Cheng et al. [233] demonstrated that though chronic unpredictable mild stress decreases hippocampal BDNF and 5-hydroxytryptamine receptor 1 alpha, which is associated with increased hypothalamic IL-1β, IL-6, and TNF, along with depressive behavior, administration of Amuc_1100 (an outer membrane protein of the mucin degrader *Akkermansia muciniphila*) attenuates these changes. Amuc_1100 has been shown by Wang et al. [234] to act on TLR2, which Cheng et al. [233] postulate to be the mechanism of its effects on immune, serotonin, and BDNF signaling in the brain. Generally speaking, chronic elevation of proinflammatory cytokines in the CNS—which can be caused by microbiota-induced immunodysregulation discussed in the immune section of this chapter—is associated with decreased neuroplasticity, as the proinflammatory cytokines downregulate BDNF production in both animal models and humans [83, 235].

5.3 *Mitochondrial Health and Neuroplasticity*

It should be noted that the microbiota alters mitochondrial biogenesis, structure, and function [236–238], that mitochondria are involved in neuroplasticity, and that mitochondrial dysfunction is seen in multiple psychiatric disorders, including anxiety disorders, MDD, bipolar disorder, and PTSD as well as in rodent models designed to model endophenotypes of these conditions [239–243]. Regulation of key transcription factors for mitochondrial biogenesis by the gut microbiota (reviewed by Clark and Mach [236]) can modulate cellular differentiation in the CNS as well as axon outgrowth and synaptic plasticity. Undifferentiated human

senescent-induced pluripotent stem cells and embryonic stem cells exhibit an anaerobic state characterized by oxidative damage, low mitochondrial ATP abundance, and low mitochondrial biomass [244]. However, in these human cell lines, as the cells differentiate, mitochondrial biomass increases, and the cells shift toward a more aerobic state [244].

Increased mitochondrial mass not only supports neuron growth and cell differentiation via higher ATP concentration in the cell but also through the production of mitochondrial uncoupling protein 4, which decreases ROS production and mitochondrial calcium accumulation in rats [245]. Moreover, mitochondria are necessary for axon outgrowth. In rat hippocampal cell lines, depletion of mitochondria prevents axon growth even when ATP concentrations are maintained, suggesting an importance of mitochondrial function and mitochondrial biogenesis in neural remodeling [246].

Additionally, BDNF stimulates mitochondrial mobilization in neurons, which is crucial for synaptic plasticity and axon growth in rat hippocampal cell lines [247]. BDNF is stimulated by peroxisomal proliferator-activating receptor (PPAR) α and γ [248, 249]. Moreover, PPARs are postulated to have a role in the prevention of anxious and depressive behaviors through neuroplasticity-, mitochondria-, and inflammation-mediated mechanisms, as PPARs are major negative regulators of NF-κB expression [250, 251]. Notably, PPARγ has been identified as a therapeutic target for neurological diseases in which mitochondrial dysfunction is implicated, but much of the research to date has focused on animal models, and in humans, it has focused on other diseases [252].

Intriguingly, Loupy et al. [253] demonstrated that subcutaneous injection of *M. vaccae* NCTC 11659 in rats prevents stress-induced downregulation of PPARγ in the liver, which can potentially attenuate negative downstream impacts of stress exposure on BDNF and neuroplasticity subsequent to induction of proinflammatory cascades. Furthermore, Smith et al. [127] demonstrated that 10(*Z*)-hexadecenoic acid activates PPARα signaling in vitro, repressing the proinflammatory cascade that can prevent downstream neurogenesis and mitochondrial biogenesis. Additionally, in mice, intestinal PPAR signaling is also activated by SCFA produced by the gut microbiota, and it is upregulated upon 8 weeks of consumption of a prebiotic blend containing fructooligosaccharide, galactooligosaccharide, inulin, and anthocyanins in mice [254]. Moreover, *Lactobacillus* probiotics (8 weeks of *L. casei* Shirota in mice and 14 weeks of *L. reuteri* GMNL-263 in rats) attenuate the decreased PPAR expression seen in extremely high fructose-containing, nonalcoholic fatty liver disease-inducing diets in mice, highlighting another mechanism by which microbial exposure decreases risk of psychiatric conditions via inflammation, mitochondrial health, and neuroplasticity [255, 256]. Overall, the microbiota modulates mitochondrial structure and function via regulation of transcription factors, BDNF, and PPAR, conferring modulation of stress resilience via neuroplasticity.

6 Meningeal Immunity

Research suggests involvement of the meninges for maintenance of well-being and modulation of CNS inflammation, psychiatric diseases, and neurodegeneration. For in-depth reviews, see the studies by Kipnis and colleagues, including Norris and Kipnis [257]; Alves de Lima et al. [258]; and Kipnis [259]. Overall, the meninges contain a vast repertoire of CNS-privileged immune cells that participate in the neuroimmune response to injury as well as neurodegeneration and brain function. However, much research on meningeal immunity has focused on brain injury and neurodegenerative diseases, though some emerging research has connected meningeal immunity to social behavior in mice [260]. Thus, more research is needed on the interactions between meningeal immunity and anxiety disorders, affective disorders, and trauma- and stressor-related disorders.

7 Human Clinical Research

There is strong evidence for the impact of microbial exposure on psychiatric outcomes in human clinical studies. Many of these studies have demonstrated disrupted microbiota-brain axes (including neural and immune mechanisms), along with altered BBB integrity, brain structure, and neuroplasticity in individuals with psychiatric conditions. Additionally, they have found that microbiota-targeted interventions through modalities such as prebiotic/probiotic/postbiotic administration are feasible, tolerable, and safe, and many of these trials show that microbial exposure interventions are effective for ameliorating changes seen to the microbiota-brain pathways and for decreasing symptoms of psychiatric conditions. Studies investigating the microbiomes of persons with these disorders are outlined in Table 1.

7.1 Microbiota-Brain Signaling in Humans: Neural Signaling

Evidence suggests that interoceptive signals (including vagal and spinal afferents), to which microbes can contribute, play an important role in determining mental health outcomes in humans [274]. To note, interoceptive dysfunction is implicated in anxiety disorders; affective disorders, including MDD; and PTSD, and it is both an outcome of and a contributor to mental health conditions [274]. Additionally, the contributions of interoception to mental health conditions are not limited to painful interoception. Even non-painful interoception can contribute to behavior via vagal and spinal afferents with integration occurring in CNS regions including the autonomic ganglia, spinal cord, brainstem (including nucleus of the solitary tract, parabrachial nucleus, and periaqueductal gray), thalamus, hypothalamus, and

Table 1 Characterization of the gut microbiome in humans with generalized anxiety disorder, major depressive disorder, and posttraumatic stress disorder

Study	Participants and study design	Alpha diversity	Composition (beta diversity)	Altered taxa
Generalized anxiety disorder				
Jiang et al. [261]	$N = 76$	↓ (GAD)	Significant difference in unweighted UniFrac distance	**Phylum level**
	GAD ($n = 40$)			↑ Bacteroides
	vs.			↑ Fusobacteria
	healthy controls ($n = 36$)			↓ Firmicutes
				Genus level
				↑ *Bacteroidetes*
				↑ *Fusobacterium*
				↑ *Ruminococcus gnavus*
				↓ *Faecalibacterium*
				↓ *Eubacterium rectale*
				↓ *Sutterella*
				↓ *Lachnospira*
				↓ *Butyricicoccus*
Chen et al. [262]	$N = 60$	↓ (GAD)	Significant difference in unweighted and weighted UniFrac distances	**Phylum level**
	GAD ($n = 36$)			↑ Tenericutes
	vs.			↓ Firmicutes
	healthy controls ($n = 24$)			**Family level**
				↑ Bacteroidaceae
				↑ Enterobacteriaceae
				↑ Burkholderiaceae
				↓ Prevotellaceae
				Genus level
				↑ *Bacteroides*
				↑ *Escherichia-Shigella*
				↓ *Prevotella 9*
				↓ *Dialister*
				↓ *Subdoligranulum*
Major depressive disorder				
Mason et al. [263]	$N = 70$	No difference seen across any psychiatric conditions	No difference seen across any psychiatric conditions	↓ *Clostridium leptum* (MDD compared to healthy controls)
	Comorbid MDD + GAD ($n = 38$)			↓ Total bacterial load per gram of stool (comorbid MDD + GAD compared to healthy controls)
	vs.			
	MDD ($n = 14$)			
	vs.			
	GAD ($n = 8$)			
	vs.			
	healthy controls ($n = 10$)			

(continued)

Table 1 (continued)

Study	Participants and study design	Alpha diversity	Composition (beta diversity)	Altered taxa
Jiang et al. [264]	$N = 76$	↑ (active MDD)	No difference (both groups)	**Phylum level**
	Active MDD ($n = 29$)	No difference (responded MDD)		Active MDD:
	vs.			↑ Bacteroides
	treatment-responded MDD ($n = 17$)			↑ Proteobacteria
	vs.			↑ Fusobacteria
	healthy controls ($n = 30$)			↓ Firmicutes
				↓ Actinobacteria
				Responded MDD:
				↑ Bacteroides
				↑ Proteobacteria
				↓ Firmicutes
				↓ Fusobacteria
				↓ Actinobacteria
				Family level
				Active MDD:
				↑ Acidaminococcaceae
				↑ Enterobacteriaceae
				↑ Fusobacteriaceae
				↑ Porphyromonadaceae
				↑ Rikenellaceae
				↓ Bacteroidaceae
				↓ Erysipelotrichaceae
				↓ Lachnospiraceae
				↓ Prevotella
				↓ Ruminococcaceae
				↓ Veillonellaceae
				Responded MDD:
				↑ Acidaminococcaceae
				↑ Enterobacteriaceae
				↑ Porphyromonadaceae
				↑ Rikenellaceae
				↑ Bacteroidaceae
				↓ Ruminococcaceae
				↓ Veillonellaceae

(continued)

Table 1 (continued)

Study	Participants and study design	Alpha diversity	Composition (beta diversity)	Altered taxa
Huang et al. [265]	$N = 54$ MDD ($n = 27$) vs. healthy controls ($n = 27$)	↓ (MDD)	Difference evident from weighted UniFrac PCoA, no statistical testing performed	**Phylum level** ↓ Firmicutes
Lin et al. [266]	$N = 20$ 1 timepoint from drug-naïve MDD participants ($n = 10$) prior to receiving escitalopram followed by 2 timepoints while receiving escitalopram vs. healthy controls ($n = 10$)	Mentioned in methods, no results reported	Difference evident from weighted UniFrac PCoA, no statistical testing performed	**Phylum level** ↑ Firmicutes ↓ Bacteroides **Genus level** ↑ *Prevotella* ↑ *Klebsiella* ↑ *Streptococcus*
Aizawa et al. [267]	$N = 100$ MDD ($n = 43$) vs. healthy controls ($n = 57$)	N/A	N/A	↓ *Bifidobacterium* ↓ *Lactobacillus* (absolute cell counts, not relative abundances)
Kelly et al. [268]	$N = 77$ MDD ($n = 34$) vs. healthy controls ($n = 33$)	↓ (MDD)	Significant difference in Bray-Curtis, unweighted UniFrac, and weighted UniFrac distances	**Family level** ↑ Thermoanaerobacteraceae ↓ Prevotellaceae **Genus level** ↑ *Paraprevotella* ↓ *Prevotella* ↓ *Dialister*
Zheng et al. [269]	$N = 121$ MDD ($n = 58$; drug-naïve $n = 39$) vs. healthy controls ($n = 63$)	No difference	Difference evident from weighted and unweighted UniFrac PCoA, no statistical testing performed	**Phylum level** ↑ Actinobacteria ↓ Bacteroidetes

(continued)

Table 1 (continued)

Study	Participants and study design	Alpha diversity	Composition (beta diversity)	Altered taxa
Naseribafrouei et al. [270]	$N = 55$	No difference	N/A	**Order level**
	MDD ($n = 37$)			↓ *Bacteroidales*
	vs.			
	healthy controls ($n = 18$)			
Yang et al. [271]	$N = 311$	Bacteria	Bacteria	**Bacteria (phylum level)**
	MDD ($n = 156$)	No difference	Significant difference in bacterial Bray-Curtis distance	↑ Bacteroidetes
	vs.			↓ Firmicutes
	healthy controls ($n = 155$)	Viruses	Viruses	**Viruses**
		↓ (MDD)	No significant difference in viral Bray-Curtis distance	↑ *Escherichia* phage ECBP5
				↓ *Clostridium* phage phi8074-B1
				↓ *Klebsiella* phage vB KpnP SU552A
Posttraumatic stress disorder				
Hemmings et al. [272]	$N = 30$	No difference	No difference	**Phylum level**
	PTSD ($n = 18$)			↓ Actinobacteria
	vs.			↓ Lentisphaerae
	trauma-exposed controls ($n = 12$)			↓ Verrucomicrobia
	Population: South African citizens			
Bajaj et al. [273]	$N = 93$	↓ (PTSD)	N/A	**Family level**
	PTSD ($n = 29$)			↓ Ruminococcaceae
	vs.			↓ Lachnospiraceae
	controls ($n = 64$)			
	Population: military Veterans with cirrhosis			

Notes: Due to the likelihood of false positives, taxa identified below the family level were not included in this table if authors did not correct for multiple testing or if those taxa made up <1% of the microbiome. Studies were only included if participants were grouped based on clinical diagnosis of the psychiatric condition. If studies included multiple timepoints during treatment, results reported in this table only include those from the timepoint(s) prior to treatment. "N/A" indicates that the study did not mention assessing the outcome

GAD generalized anxiety disorder, *MDD* major depressive disorder, *PTSD* posttraumatic stress disorder

somatosensory cortex [275, 276]. There is a strong overlap of interoceptive neural integration regions with affective regions, and, importantly, interoceptive feedback may confer psychological alterations to vigilant behavior, the magnitude of reactions to stressors, and perception of stress magnitude [274]. Over time, interoceptive overstimulation leads to altered physiological stress axes with effects such as hypersecretion of cortisol, reduced sensitivity of negative feedback by GC, and a sympathetic bias of the autonomic nervous system resulting in impaired stress resilience through constant activation of "fight or flight" systems [277]. Given that microbial organisms shape the host's interactions with the "outside" in locations including the skin, nasal cavity, mouth, lungs, and gut, microbiota surely impact interoceptive stimuli, conferring potential to alter mental health outcomes through this mechanism.

A prime example of interoceptive overstimulation from a microbiota is irritable bowel syndrome (IBS). In the case of dysbiotic microbiota associated with IBS, increased sensory input from the gut mucosa alters CNS structure. Labus et al. [278] found altered volume of somatosensory brain regions in participants with IBS. Of particular interest, they demonstrated that increased volumes of the somatosensory regions evaluated were observed with higher relative abundances of Clostridia and lower relative abundances of Bacteroidia, characteristic of the subgroup of IBS participants who experienced early life trauma. Additionally, Mayer et al. [279] characterized an increased viscerosensory input to the brain and sensitization of the dorsal horn of the spinal cord as contributors to altered brain structure in IBS patients. Together, the microbial alterations associated with dysbiosis contribute to decreased gray matter volume in the insula and prefrontal cortex and to altered white matter tracts in the thalamus and basal ganglia [280, 281]. These changes to brain structure confer increased risk of neurodegeneration and chronic pain, and they are associated with both childhood and adult onset of MDD [282–284].

Additionally, spinal afferents from mucosal and cutaneous surfaces, such as the gut, lungs, and skin, regulated by local microbiota, contribute to psychiatric disorder risk. Evidence suggests [285–288] that activation of spinothalamic and spinoparabrachial pathways from the skin in humans may have antidepressant effects [282, 283, 284, 285], but these pathways have not been studied in the context of the skin microbiota and should be considered a target for future research.

Vagal afferents, which can come from multiple locations including the gut and lungs, have also been shown to modulate brain structure and psychiatric symptoms in humans. For example, Tillisch et al. [289] demonstrated that consumption of a probiotic fermented milk product modifies resting state networks, likely via vagal afferents signaling to the nucleus tractus solitarius and spinal afferents ascending to the periaqueductal gray. This resulted in alterations to brain connectivity associated with improved responses to emotional stimuli and decreased chronic pain signaling, thus conferring stress resilience and decreased symptoms associated with MDD. This is complemented by multiple clinical trials demonstrating efficacy and tolerability of transcutaneous vagal stimulation in patients with MDD, as reviewed by Kong et al. [290].

Other cranial nerves, such as the trigeminal and olfactory nerves, have additionally been shown to modulate psychiatric outcomes in humans. Notably, trigeminal nerve stimulation has been demonstrated as a potential treatment modality for reducing MDD symptoms, and olfactory nerve dysfunction is implicated in MDD [60, 291, 292]. Given that these nerves innervate the oral and nasal mucosa and can be stimulated by microbes, it follows that the oral and nasal microbiota have the potential to modulate mental health outcomes via non-vagal cranial nerves as well. However, these pathways are understudied but could be a focus of future research and could be targeted for development of novel alternative treatments for psychiatric conditions.

Emerging research suggests that metabolites from the gut microbiome can activate neural circuits in the brain in humans. For example, Osadchiy et al. [293] showed that gut microbial indole metabolites (produced from tryptophan by genera such as *Clostridium*, *Burkholderia*, *Streptomyces*, *Pseudomonas*, and *Bacillus*), including indole, indoleacetic acid, and skatole, correlate with activity and connectivity in the extended reward network of the brain in healthy humans. They were notably associated with activation of and connections in the amygdala-nucleus accumbens and amygdala-anterior insula circuits, which are known to be altered in humans with treatment-resistant depression and PTSD [294, 295]. However, research on the ability of microbiome-derived indole metabolites to act on monoamine and reward circuit signaling in the brain is sparse, especially in the context of psychiatric disorders in humans, and this merits future research.

7.2 Microbiota-Brain Signaling in Humans: Immune-Brain Interactions

Similar to microbiota-neural signaling, microbiota-immune system signaling has also been shown to be involved in modulating brain structure and neuropsychiatric outcomes in humans. Again, immune modulation has been shown to be a contributor to development of the psychiatric conditions, an outcome of stressors, and a potential therapeutic for psychiatric conditions.

7.2.1 Cytokines

It has been demonstrated (and extensively covered in the preclinical section of this chapter) that microbial exposure can alter circulating cytokine concentrations. Modulation of cytokines is particularly notable, as circulating concentrations of proinflammatory cytokines are elevated in anxiety disorders, affective disorders, and PTSD in humans. Hou et al. [296] found that individuals with generalized anxiety disorder (GAD) have elevated serum concentrations of proinflammatory cytokines TNF and IFN-γ, as well as decreased IL-10. Additionally, Hou et al.

[193, 194] found that treatment with SSRIs lowers serum CRP, IL-1α, IL-6, IL-8, IL-12, and IFN-γ and that elevated baseline CRP and IL-6 are positive predictors of SSRI treatment responsivity.

Moreover, cytokine concentrations are disrupted in MDD. Zou et al. [297] found that antidepressant drug-naïve individuals with MDD have elevated serum IL-1β, IL-10, and TNF compared to nondepressed individuals and that IL-1β and TNF abundances positively correlate with the severity of depressive symptoms. Alesci et al. [298] found disruption of the circadian rhythm of plasma IL-6 in MDD patients. Furthermore, a genetic link can be drawn between proinflammatory cytokines and psychiatric outcomes, as polymorphisms of the IL-1β gene are associated with symptomatology and responsiveness to antidepressant treatment [299]. Additionally, immune reactivity is attenuated during MDD treatment, as Kéri et al. [300] found that decreasing symptoms during cognitive behavioral therapy were associated with decreased TLR4-dependent priming of peripheral blood mononuclear cells in depressed patients. On a predictive level, elevated serum concentrations of proinflammatory cytokines including IL-6 and CRP are predictive of development of depressive or common mental health symptoms over the course of 12 years in adults [301, 302] and over the course of 9 years (from age 9 to 18) in children [303, 304].

Individuals with PTSD also exhibit elevated concentrations of proinflammatory cytokines. Wang et al. [305] found that individuals with PTSD from a deadly earthquake event have elevated serum IL-1β and TNF concentrations, along with elevated total proinflammatory cytokine scores (based on serum concentrations of IL-1β, IL-2, IL-6, IL-8, IFN-γ, and TNF). Likewise, Lindqvist et al. [306] found that the proinflammatory cytokine milieu (including IFN-γ, TNF, and the sum of IL-1β, IL-6, CRP, IFN-γ, and TNF concentrations) is elevated in individuals with combat-related PTSD independent of depression symptoms and early life stress. Moreover, Gola et al. [307] demonstrated that ex vivo cultured peripheral blood mononuclear cells (PBMCs) of study participants with PTSD had increased spontaneous production of IL-1β, IL-6, and TNF.

Altered cytokine concentrations have been shown to change neurotransmitter activity in the brain, conferring behavioral deficits and impaired neuroplasticity. Magnetic resonance spectroscopy showed increased glutamate in the basal ganglia and dorsal anterior cingulate cortex (dACC) in individuals receiving IFN-α treatment and in depressed individuals; however, these changes are not conserved across all depressed individuals, likely due to the heterogeneity of the diagnosis [308–310]. Altered glutamate signaling in individuals diagnosed with MDD is well supported by preclinical studies and is thought to contribute to excitotoxicity and decreased BDNF, impairing neuroplasticity and neurogenesis [76]. Additionally, concentrations of plasma proinflammatory cytokines can be predictive of PTSD development. Prime examples include Schultebraucks et al. [75] demonstrating that blood CRP concentration prior to military deployment is one of the top predictors of PTSD development following deployment and Pervanidou et al. [311] demonstrating that elevated serum IL-6 concentrations the morning following a motor vehicle accident are predictive of PTSD development 6 months later. Overall, it is evident

that cytokine concentrations are altered in anxiety disorders, MDD, and PTSD, that proinflammatory cytokines can alter neural signaling, that higher concentrations of proinflammatory cytokines subside during treatment for anxiety disorders and MDD, and that levels of proinflammatory cytokines are predictive of the development of psychiatric symptoms and disorders.

7.2.2 Leukocyte Populations

Similar to cytokine concentrations, populations of circulating leukocytes can be altered by microbial exposure in humans, as has been thoroughly characterized through the study of the "farm effect," reviewed by Vercelli and colleagues [312, 313]. As can be seen in the meta-analysis by Segerstrom and Miller [314], acute psychological stressors additionally induce a plethora of changes to immune cell populations in humans. Many of these changes, such as increased neutrophils, natural killer cells (along with increased natural killer cell function), and large granular lymphocytes and T helper cells as a percentage of leukocytes, correlate with the duration of the acute stressor [314].

To complement knowledge of the impacts of stressors on leukocyte populations in humans and the associations between leukocyte populations and anxious behaviors in stressed animal models, military Veterans with anxiety (according to DSM-III criteria) have elevated lymphocyte and T cell counts [315]. Additionally, individuals with panic disorder have increased abundances of natural killer cells, B lymphocytes, human leukocyte antigen DR isotype-presenting cells, and B lymphocytes presenting human leukocyte antigen DR surface markers [316]. Leukocyte populations are also altered in individuals with MDD. Ekinci and Ekinci [317] found an elevated neutrophil to lymphocyte ratio in depressed individuals who had attempted suicide, compared to healthy controls. Likewise, Schleifer et al. [318] found a decreased number of lymphocytes, along with decreased reactivity of the lymphocytes, in hospitalized depressed individuals.

Schultebraucks et al. [75] demonstrated that plasma basophil (referred to as large granular lymphocytes by Segerstrom and Miller) and monocyte abundances prior to military deployment to Afghanistan are predictive of PTSD development. Additionally, Schultebraucks et al. [75] also found that eicosanoids, which promote neutrophil stimulation and chemotaxis, are significant predictors of PTSD development. Human research, however, has yet to establish a causal link between leukocyte populations and altered risk for the development of PTSD. Altered leukocyte populations could co-occur with a past history of psychological trauma and stressors (as demonstrated by Segerstrom and Miller [314]) modifying neural circuitry independent of the immune system. Due to the lack of human studies that assess the ability of immunoregulatory interventions to prevent development of PTSD in traumatized or stressed individuals, we must rely partially on preclinical research for our knowledge in this area. Given the alterations in leukocyte populations following acute stress and the ability of leukocyte abundances to predict PTSD development, along with the preclinical evidence that immunoregulation via

microbial exposure attenuates immune responses to stress and the resulting impaired stress resilience, it should be noted that leukocyte populations have potential to play a strong role in modulating neuropsychiatric outcomes. Future research should investigate the long-term effects of immunoregulatory interventions, such as probiotic trials or nature exposure, in preventing the development of psychiatric disorders.

7.2.3 Brain Barriers and Leukocyte Trafficking

The CP, a key point of cellular trafficking into the CNS, has been demonstrated to be disrupted in individuals with psychiatric disorders. This is relevant because, as discussed above, a proinflammatory state of the immune system has been shown to alter BCSFB integrity at the CP in preclinical models. Such proinflammatory immune states (such as an increased population of Th17 cells that impairs BCSFB integrity, encouraging lymphocyte trafficking to the brain) can be modulated by microbial exposure. Additionally, in individuals with poor BCSFB integrity, microbiota with low diversity and therefore low resistance to pathogen overgrowth could allow pathogen translocation across epithelial barriers (e.g., the gut mucosa) and invasion of the CNS, triggering neuroinflammation that impairs stress resilience.

Turner et al. [319] demonstrated a downregulation in mRNA transcripts related to cytoskeleton and extracellular matrix maintenance in the CP of individuals with MDD postmortem, suggesting impaired BCSFB function. Lizano et al. [320] found consistent enlargement of the CP across a spectrum of psychiatric illnesses. These changes to the CP are complemented by the findings of Schiweck et al. [321], who found elevated T helper cells, particularly Th17, in peripheral blood mononuclear cell suspensions from individuals with MDD and high suicide risk, demonstrating the Th17 bias that has been shown to drive BCSFB permeability in preclinical studies.

Additionally, BBB dysfunction is associated with increased risk for affective and stress-related disorders in humans [136, 137]. Reviewed by Patel and Frey [322], BBB disruption is implicated in multiple clinical studies of psychiatric conditions, and many metabolites altered by the microbiota can modulate BBB integrity.

Although preclinical studies have firmly established that disruptions to the BCSFB and BBB integrity allow leukocytes and cytokines to traffic into the CNS, this has not been studied in vivo in humans. Though the disruptions are associated with affective and stress-related disorders, no human interventions have investigated modulation of the BCSFB/BBB to decrease symptoms or risk of anxiety disorders, MDD, or PTSD. In fact, to date, no human trials have investigated microbiota-targeted interventions for improving the integrity of the BCSFB and BBB to modulate mental health outcomes.

Microbiota-mediated modulation of vitamin D, which exerts protective effects on the BBB in preclinical models, could prove a promising intervention in humans. Circulating vitamin D concentration is associated with gut microbiota composition and has been clearly demonstrated to affect microbiome composition via vitamin D receptors in the human gut [323, 324]. Interestingly, 9 weeks of daily *L. reuteri*

NCIMB 30242 supplementation increased circulating vitamin D concentrations, indicating a bidirectional relationship between the microbiome and vitamin D [325]. However, the effects of increased vitamin D concentration from probiotic supplementation on the BBB with implications for mental health outcomes have not been studied. Overall, studies investigating the microbiota-BBB axis as a modulator of mental health in humans are sparse and could be a direction for future research.

7.2.4 Probiotic Interventions

To date, many probiotic trials have been carried out to assess impacts on psychiatric outcomes, often involving strains of *Lactobacillus* or *Bifidobacterium* [326]. Based on the meta-analysis by Amirani et al. [326], these studies typically show decreased depressive symptoms and decreased markers of systemic inflammation, such as CRP. Additionally, probiotics have been shown to modulate immune activity and chemotaxis proteins in humans; a randomized control trial of 12 weeks of supplementation with *L. rhamnosus* strain GG and *Bifidobacterium animalis* subsp. *lactis* strain Bb12 found decreased acute-phase reactant protein von Willebrand factor (vWF) and increased abundances of monocyte chemotactic protein-1 (MCP-1; also known as CCL2) and BDNF, suggesting immunomodulatory properties [327].

Of note, immunomodulatory probiotic trials targeting stress resilience have been repeatedly shown to be safe, feasible, and tolerable. Probiotic supplementation with *L. reuteri* DSM 17938, a gut microbe capable of CLA biosynthesis, was demonstrated to be a safe, feasible, and potentially effective intervention for military Veterans with co-occurring PTSD and mild traumatic brain injury [328, 329]. Results from the pilot study by Brenner et al. [328] showed a trend for decreased CRP, along with attenuated autonomic nervous system responses to the Trier Social Stress Test after 8 weeks of supplementation with *L. reuteri* DSM 17938. Moreover, Browne et al. [330] showed that 4 weeks of daily supplementation with a multispecies probiotic with multiple immunomodulatory taxa (*B. bifidum* W23, *Bifidobacterium lactis* W51, *B. lactis* W52, *Lactobacillus acidophilus* W7, *Lactobacillus brevis* W63, *L. casei* W56, *Lactobacillus salivarius* W24, *Lactococcus lactis* W19, and *L. lactis* W58) geared toward reducing maternal anxiety symptoms was safe and tolerable in pregnant women. Wallace and Milev [331] also demonstrated that 8 weeks of daily supplementation with *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 in treatment-naïve individuals with MDD was safe, tolerable, and able to improve affective symptoms. However, most randomized, controlled clinical trials targeting affective and stress-related conditions in human participants to date (including the three above) have only been pilot studies. Though there will be more pilot studies in the future to determine the safety of new probiotics in humans, other studies need to build off of existing pilot studies to create clinical guidelines for microbe-based interventions in humans with psychiatric disorders. Table 2 outlines the outcomes of probiotic interventions in individuals with GAD, MDD, and PTSD.

Table 2 Outcomes of probiotic interventions for individuals with clinically diagnosed generalized anxiety disorder, major depressive disorder, and posttraumatic stress disorder

Study (design)	Participants	Probiotic and methods	Major outcomes assessed	Findings	Limitations
Generalized anxiety disorder					
Eskandarzadeh et al. [332]	48 antidepressant- and anxiolytic-free participants diagnosed with GAD	8 weeks of daily:	Anxiety severity	↓ Anxiety severity (Hamilton Rating Scale for Anxiety)	Probiotic strain designations are not given
(double-blinded, randomized, placebo-controlled)	Probiotic $n = 24$	*Bifidobacterium longum*, *Bifidobacterium bifidum*, *Bifidobacterium lactis*, *Lactobacillus acidophilus*	Quality of life	No significant decrease in Beck Anxiety Inventory	Participants were drug-free but not drug-naïve
	Placebo $n = 24$	Both groups received 25 mg sertraline daily		↓ State Anxiety Inventory	
				No significant decrease in Trait Anxiety Inventory	
				No significant change in quality of life	
Major depressive disorder					
Romijn et al. [333]	79 antidepressant-free participants scoring ≥11 on the Quick Inventory of Depressive Symptomatology or ≥14 on the depression scale of the Depression, Anxiety, and Stress Scale, characteristic of MDD	8 weeks of daily:	Mood, stress, and anxiety symptoms	No difference found between probiotic and placebo groups for any psychological outcomes or blood-based biomarkers	Participants were drug-free but not drug-naïve
(double-blinded,	Probiotic $n = 40$	*Lactobacillus helveticus* R0052	Irritable bowel syndrome symptoms	High baseline vitamin D was significantly	

randomized, placebo-controlled)				associated with greater improvement in self- and clinician-reported mood and functioning in the probiotic group but not the placebo group	
	Placebo $n = 39$	*Bifidobacterium longum* R0175	Blood concentration of proinflammatory cytokines and brain-derived neurotropic factor		
Rudzki et al. [334]	79 participants with clinically diagnosed DSM-IV MDD	8 weeks of twice daily:	Severity of depressive symptoms	↑ cognitive function (attention and perceptivity test, California Verbal Learning Test)	Some participants were taking SSRIs prior to the start of the study, though they were evenly distributed between groups
(double-blinded, randomized, placebo-controlled)	Probiotic $n = 40$	*Lactobacillus plantarum 299v* (DSM 9843)	Cognitive function	↓ serum kynurenine	Not all participants received the same SSRIs, and authors did not identify reasons for the differing SSRIs
	Placebo $n = 39$	Both groups received daily selective serotonin reuptake inhibitors (SSRIs) to be taken twice daily with the probiotic/placebo. Most participants received the SSRI escitalopram, and there were no significant differences in SSRI type during treatment	Blood concentration of biochemical markers of tryptophan metabolism and proinflammatory cytokines	↑ serum 3-hydroxykynurenine to kynurenine ratio	
				No significant changes in severity of depressive symptoms or proinflammatory cytokines	
Akkasheh et al. [335]	40 participants with clinically diagnosed DSM-IV MDD	8 weeks of daily:	Severity of depressive symptoms	↓ Beck Depression Inventory total scores	Probiotic strain designations are not given
(double-blinded, randomized, placebo-controlled)	Probiotic $n = 20$	*Lactobacillus acidophilus*, *Lactobacillus casei*, *Bifidobacterium bifidum*	Serum metabolome, C-reactive protein, and markers of oxidative stress	↓ serum insulin and markers of insulin resistance	
	Placebo $n = 20$			↓ serum C-reactive protein	

(continued)

Table 2 (continued)

Study (design)	Participants	Probiotic and methods	Major outcomes assessed	Findings	Limitations
				No significant change in serum lipid profiles or serum antioxidant capacity	
Kazemi et al. [336]	81 participants with MDD	8 weeks of daily:	Severity of depressive symptoms	↓ Beck Depression Inventory total scores (probiotic vs. placebo; probiotic vs. prebiotic)	Authors do not provide justification for adjusting for isoleucine concentration, and the difference in kynurenine to tryptophan ratio was not significant when not adjusting
(three-arm, double-blinded, randomized, placebo-controlled)	Probiotic $n = 28$	*Lactobacillus helveticus* R0052, *Bifidobacterium longum* R0175 (CNCM strain I-3470)	Serum tryptophan, kynurenine, and branch chain amino acids	↓ kynurenine to tryptophan ratio when adjusting for serum isoleucine concentration (probiotic vs. placebo)	
	Prebiotic $n = 27$	*or*		↑ tryptophan to isoleucine ratio (probiotic vs. placebo)	
	Placebo $n = 26$	8 weeks of daily:			
		5 grams galactooligosaccharide			
Miyaoka et al. [337]	40 participants with treatment-resistant MDD who had already been taking antidepressants for at least 1 month	8 weeks of daily:	Severity of depressive symptoms	↓ depressive symptoms (Beck Depression Inventory, Hamilton Depression Rating Scale)	Study was open-label. Treatment was determined to be safe and tolerable
(open-label, randomized, placebo-controlled)	Probiotic $n = 20$	*Clostridium butyricum* MIYAIRI 588 (CBM588)	Severity of anxiety symptoms	↓ anxiety symptoms (Beck Anxiety Inventory)	
	Placebo $n = 20$		Safety and tolerability of intervention	70% of participants responded to probiotic supplementation treatment	

Reininghaus et al. [338]	61 participants receiving inpatient care for MDD	4 weeks of daily:	Depressive symptom severity	No significant difference in depressive symptoms between groups, though both groups improved over time	Authors did not assess microbiome beta diversity from timepoint 0 to timepoints 1 and 2 within the probiotic group. Thus, it cannot be determined whether the differences between groups at 1 and 4 weeks are due to the probiotic group changing or the placebo group changing, or to both groups changing
(double-blinded, randomized, placebo-controlled)	Probiotic $n = 28$	*Bifidobacterium bifidum* W23, *Bifidobacterium lactis* W51, *Bifidobacterium lactis* W52, *Lactobacillus acidophilus* W22, *Lactobacillus casei* W56, *Lactobacillus paracasei* W20, *Lactobacillus plantarum* W62, *Lactobacillus salivarius* W24, *Lactobacillus lactis* W29	Intestinal barrier function, assessed via plasma zonulin concentration	No significant change in plasma zonulin concentration	
	Placebo $n = 33$	+<30 mg (exact quantity not specified) fructooligosaccharide	Gut microbiome diversity and composition	No significant change in alpha diversity	
		Both groups received daily vitamin B7 (biotin)		Significant difference in beta diversity between probiotic and placebo after 1 and 4 weeks but no significant difference prior to the start of the study	

(continued)

Table 2 (continued)

Study (design)	Participants	Probiotic and methods	Major outcomes assessed	Findings	Limitations
Posttraumatic stress disorder					
Brenner et al. [328]	31 US military Veterans with comorbid PTSD and persistent post-concussive (PPC) symptoms from mild traumatic brain injuries	8 weeks of daily: *Lactobacillus reuteri* DSM 17938	Reactivity to the Trier Social Stress Test (TSST)	No significant difference in perceived stress during the TSST	Study did not assess changes to PPC symptoms or PTSD symptoms with the probiotic intervention
(double-blinded, randomized, placebo-controlled)	Probiotic $n = 16$		Plasma concentration of C-reactive protein, biomarkers of inflammation, and biomarkers of intestinal permeability	↓ in TSST-induced increase in heart rate	
	Placebo $n = 15$		Gut microbiome diversity and composition	↓ C-reactive protein (approaching significance)	
			Feasibility, safety, and acceptability of intervention	No significant difference in biomarkers of inflammation or intestinal permeability	
				No significant differences in microbiome diversity or composition	
				Treatment was determined to be feasible, safe, and acceptable	

GAD generalized anxiety disorder, *MDD* major depressive disorder, *DSM-IV* Diagnostic and Statistical Manual of Mental Disorders 4th edition, *PPC* persistent post-concussive, *PTSD* posttraumatic stress disorder, *TSST* Trier Social Stress Test

7.3 *Neurogenesis and Mitochondrial Function in Humans*

As discussed in Sect. 5 of this chapter, microbes can modulate many host signaling molecules involved in neuroplasticity, such as cytokines, BDNF, and PPARs. This is particularly relevant for psychiatric disorders in humans, as decreased hippocampal neuroplasticity (typically evaluated under the assumption that increases in hippocampal volume indicate increases in neurogenesis) is generally associated with anxiety disorders, MDD, and PTSD [339, 340]. Particularly, though decreased neurogenesis does not have an incredibly strong relationship with the development of psychiatric disorders, it is strongly associated with the maintenance of psychiatric disorders in humans [340–342]. To elaborate, the ability to increase hippocampal volume via neurogenesis appears important in recovery from MDD and PTSD, as individuals who recover experience increases in hippocampal volume compared to those who do not recover.

Mitochondrial dysfunction, which can be modulated by the microbiota, is also seen in multiple psychiatric disorders, including anxiety disorders, MDD, bipolar disorder, and PTSD [239, 241–243]. Moreover, Shapira-Lichter et al. [343] demonstrated that IL-6 is responsible for changes in mood and memory following surgery, suggesting that cytokines, which are altered by microbial exposure, play roles in mood and memory formation.

However, few studies have investigated the impacts of probiotics on neurogenesis and brain mitochondrial function in humans in the context of stress-related psychiatric disorders. One randomized, double-blinded clinical trial by Haghighat et al. [344] did show that 12 weeks of consumption of a synbiotic (prebiotic/probiotic blend) containing fructooligosaccharides, galactooligosaccharides, inulin, *L. acidophilus* T16, *B. bifidum* BIA-6, *B. lactis* BIA-7, and *B. longum* BIA-8 decreased anxiety and depressive symptoms and increased BDNF in a subgroup of individuals with MDD, but further human research is lacking. Overall, neurogenesis and mitochondrial function have been found to be impaired in individuals with stress-related psychiatric disorders, but human research on microbiota modulation of these issues is sparse, and more research is highly warranted.

8 The Case for Non-probiotic Interventions Under the Old Friends and Biodiversity Hypotheses

Moving toward a larger-scale focus, Lowry et al. [7] outlined a case for reduced microbial exposure and environmental microbial diversity across modernized societies contributing to the increased global mental health burden via impaired immunoregulation. Throughout history, mammals have been in close contact with dirt and mud (and thus the microbes contained in them), but urban "concrete jungles" are far from ideal for growth of the microbes with which we evolved, such as environmental mycobacteria [345]. However, soil bacteria aren't the only important microbes.

Commensal microbes with immunoregulatory properties, such as *B. infantis*, *B. longus*, and *B. brevis*, have decreased in both urban and wealthy populations due to behavioral and dietary changes including cesarean section delivery, early-life antibiotic use, and increased formula feeding, resulting in increased immune-mediated inflammatory diseases [346–348]. Though probiotic-driven recolonization with these bacteria is possible under the proper conditions, such as colonization of *B. infantis* EVC001 probiotic colonization during breastfeeding as demonstrated by O'Brien et al. [349], most probiotics do not colonize and should not be treated as a permanent way to reintroduce bacteria [350]. This is not to say that probiotics are useless—in fact, as previously outlined, they have demonstrated therapeutic potential in psychiatric disorders. It is important to note, though, that they do not serve a permanent role replacing the taxa that have been lost due to urbanization.

Additionally, it should be noted that supplementation with one or even multiple microbes will not "solve" every host's dysbiotic microbiota and improve host health. Consistent with the Anna Karenina principle, which states that while "happy families are all alike," in this case, perhaps through high diversity and functional redundancy, "every unhappy family is unhappy in its own way," there are many ways that microbial community health can fall apart, resulting in immune dysregulation and impaired stress resilience [351, 352]. Unfortunately, the multitude of microbiota changes seen in disease and the difficulty of predicting community changes from interventions make individualized microbiome-targeted approaches largely prohibitive at present [353]. Thus, perhaps the best microbial approach to decrease immune-mediated psychiatric disorders is not through probiotic cocktails but through using environmental and lifestyle interventions to improve key components of microbiota stability, functional diversity, and functional redundancy [354]. Functional diversity (having microbes that perform many functions) and redundancy (multiple taxa perform the same function) make communities resilient to perturbations that would otherwise lead to disruption [354]. Establishing functional diversity and redundancy at an early age through increasing environmental microbe exposure, social interaction, and dietary diversity could create long-lasting benefits to microbiome stability with conferred enhancement of psychosocial stress resilience [355]. Interestingly, Bastiaanssen et al. [356] demonstrated that microbiome volatility, quantified as the magnitude of changes in community composition (beta diversity) over multiple sampling timepoints within the same individual, is associated with stress and altered behavior in both humans and murine models. Ten days of chronic social defeat stress increased microbiome volatility in mice, and in humans, microbiome volatility correlated with perceived stress. Though no causal link has yet been identified for microbiome volatility impairing psychosocial stress resilience, it could be hypothesized that stress-induced volatility puts microbiota at risk of community disruption, increasing likelihood of pathogen colonization or pathobiont overgrowth, such as the increase in *Helicobacter* spp. caused by the CSC paradigm in mice [96]. These changes could confer immunodysregulation, whereas increased community resilience (through functional diversity and redundancy) has the ability to prevent this disruption.

Over time, individuals' microbiota homogenize with the built environment and undergo microbial transfer via social interaction, emphasizing the importance of diverse environmental microbes and socialization [357, 358]. Additionally, nature exposure can play an important role in microbe-mediated immunoregulation, as Roslund et al. [359] demonstrated that transferring forest floor soil to a preschool playground increased microbiome diversity, Treg, and plasma IL-10:IL-17A ratios (see chapter on "Distortion of the Microbiota of the Natural Environment by Human Activities" in this volume). Moreover, diversity of plants in the diet is associated with gut microbiome richness and composition, as demonstrated by the American Gut Project [360]. Approaches to increase microbial diversity, and therefore stability, can result in sustained diverse microbial exposure and prevent pathogen-mediated immune disruption.

9 Nutritional Psychiatry

Nutritional psychiatry, or the field of modulating psychiatric disorder symptoms through dietary changes, is accumulating evidence supporting its use in clinical settings. Evidence supporting the link between diet and mental health has been found in both epidemiological studies and clinical interventions. Due to the ability of diet to modulate the gut microbiota and the ability of the gut microbiota to modulate mental health symptoms, the diet-mental health link may be at least partially mediated by the microbiome.

9.1 Epidemiological Data Link Poor Diet Quality to Poor Mental Health

Epidemiological studies have shown associations of anxiety and depression with proinflammatory diets, such as diets high in added sugar and saturated fats, and some evidence suggests associations between diet quality and PTSD. Masana et al. [361] demonstrated an association between high consumption of saturated fats and added sugars and anxiety symptoms in adults over 50 years of age with no underlying cardiovascular or chronic diseases. Likewise, Jacka et al. [362] found that low diet quality (constructed from dietary quantities of fried foods, refined grains, sugary products, and beer) was associated with low psychological well-being based on General Health Questionnaire-12 scores across 20- to 93-year-old women. Additionally, Jacka et al. [362] found that consumption of more traditional diets characterized by high consumption of fruits, vegetables, meats, fish, and whole grains was associated with lower rates of anxiety and depression. Westover and Marangell [363] found a strong and significant cross-nation correlation between kilocalories of sugar consumption per capita per day and annual rates of MDD (with a Pearson

correlation of 0.948). Moreover, the meta-analysis by Psaltopoulou et al. [364] showed an association between adherence to a Mediterranean diet (high in fruits, vegetables, whole grains, nuts, and unsaturated fatty acids) and lower risk of depression. High adherence to the Mediterranean diet touted a strong association with lower depression risk independent of age, but moderate adherence was associated with a decreased depression risk that was slightly attenuated as age increased. This is also supported by the systematic review and meta-analysis by Lassale et al. [365], which showed that Mediterranean diet adherence was associated with decreased risk of depression across four longitudinal studies, that a low Dietary Inflammatory Index was associated with decreased risk of depression across four longitudinal studies, and that higher Healthy Eating Index and Alternative Healthy Eating Index scores were associated with lower risk of depression. Additionally, a systematic review demonstrated an association between PTSD and lower diet quality, where individuals with PTSD were more likely to have low diet quality than individuals without PTSD [366].

However, epidemiological studies do have limitations and cannot be used to establish causality in the development of psychiatric disorders. This is highlighted by Kim et al. [367] demonstrating that in 51,965 female participants in the Nurses' Health Study II PTSD sub-study, after the onset of PTSD, participants had a lower improvement in dietary quality over a 20-year follow-up period compared to participants without PTSD symptoms. These changes to diet quality occurred after the onset of PTSD, suggesting that behavioral changes from PTSD symptoms, which overlap with the symptoms of GAD and MDD, may be impacting diet quality.

9.2 Whole Dietary Interventions Alter the Microbiome and Decrease Depressive Symptoms

Whole dietary patterns are associated with microbiome composition, which can impact risk for anxiety, depression, and PTSD through mechanisms previously outlined. Whole dietary interventions can improve symptoms of MDD as well. However, little clinical research has shown an effect of whole dietary interventions on anxiety, and few studies have evaluated the effects of whole dietary interventions on PTSD.

Cotillard et al. [368] found that unsupervised clustering of dietary data revealed wide-scale dietary patterns associated with large differences in microbiome composition, highlighting that dietary patterns may be more relevant for microbiome composition than quantities of individual foods in the diet. For example, following a flexitarian diet (with a flexible dietary pattern rich in various plants and occasionally including animal products) rather than a standard Western diet (with high consumption of processed and fried foods along with saturated fats and added sugars, with low diversity of plants consumed) may have larger effects on microbiome composition than meeting certain quantities of fiber. Likewise, Johnson

et al. [369] found that dietary choices, but not the individual quantities of conventional nutrients, were associated with microbiome composition. Additionally, the American Gut Project identified that individuals who consume more than 30 species of plants per week have altered gut microbiome composition, along with increased microbiome diversity and CLA (independent of estimated dietary CLA consumption) compared to those who consume less than 10 unique plant species per week, highlighting the importance of diversity in dietary patterns [360]. Across multiple other studies, habitual diet and vegetable intake is associated with gut microbiome composition, which was found to mediate changes to host leukocyte profiles [370–372] Thus, whole dietary interventions instead of specific nutrient-based interventions may be an effective microbiome-mediated means of improving mental health symptoms.

To complement the existing research on whole dietary patterns associating with microbiome composition, a randomized controlled trial of a 1-year Mediterranean diet intervention in individuals 65–79 years of age across multiple nations resulted in modified gut microbiome composition and metabolites such as SCFAs, and it decreased serum concentrations of CRP and IL-17 [373]. On a shorter scale, David et al. [374] demonstrated that just 4 days of whole dietary interventions (plant-based diets or animal-based diets) rapidly and reproducibly altered the gut microbiome, including its gene expression and production of metabolites such as SCFAs and the secondary bile acid deoxycholic acid.

In a randomized controlled trial of young adults with depressive symptoms and poor diet quality, Francis et al. [375] demonstrated that just a 3-week wide-scale dietary intervention developed by a dietitian (to encourage adherence to a Mediterranean-style diet; increase dietary consumption of anti-inflammatory dietary components such as omega-3 fatty acids, turmeric, and cinnamon; and to decrease consumption of refined carbohydrates, processed meats, and soft drinks) decreased self-reported depression symptoms compared to controls who did not receive the intervention. Notably, the decrease in self-reported depression symptoms was maintained 3 months after the intervention ended, suggesting long-term effects of short-term, whole dietary interventions. Moreover, the meta-analysis of 16 whole dietary interventions by Firth et al. [376] found that dietary interventions reduce depressive symptoms even in individuals who are not clinically depressed. These effects were conserved across studies that used active and inactive controls, and females tended to experience stronger improvements in depressive symptoms. However, no studies to date have evaluated microbiome changes that are associated with improved depressive symptoms during whole dietary interventions, which should be considered an important objective for future research.

The meta-analysis by Firth et al. [376] additionally investigated symptoms of anxiety, and they concluded that there was no significant effect across 11 studies, potentially due to the heterogeneity of studies and dietary interventions. No studies to date have evaluated the effects of whole dietary interventions on PTSD symptoms, suggesting a target for future research.

Overall, dietary patterns alter the microbiome and decrease symptoms of depression, but existing evidence does not support their efficacy for reducing symptoms of

anxiety and PTSD. Aside from any potential effects on the microbiome-gut-brain axis, at the very least, dietary interventions should be investigated for their ability to improve quality of life via decreasing the risk of chronic diseases such as cardiovascular disease in individuals with psychiatric conditions, given that individuals with psychiatric disorders have poorer diet quality than individuals without psychiatric disorders.

10 Clinical Implications and Conclusions

There is a plethora of ways that microbial exposures impact mental health, including (but not limited to) modulation of the gut mucosa, direct activation of neural afferents, and modulation of immune signaling, metabolic signaling, blood-brain barrier integrity, leukocyte trafficking, cytokine production, neuroplasticity, and neural circuits. These effects can be induced by host exposure to live microorganisms, dead microorganisms, and even metabolites of microorganisms at sites such as the lungs, mouth, nasal cavity, skin, and digestive tract. The list of mechanisms linking microbial exposures and neuropsychiatric outcomes is vast, and many studies to date have demonstrated portions of these mechanisms. The variety of mechanisms is complemented by a large number of studies showing altered microbiome-host pathways in individuals with mental health conditions.

Despite the strong promise of this field, due to study limitations evidence does not currently allow many of these mechanistic pathways to be traced from the point of microbial exposure to behavioral outcomes. Thus, an emphasis on study design that will inform mechanisms involved is an important objective for future studies. Given that the relationship between the microbiota and CNS is bidirectional, researchers should be cautious about implying causality, and it is essential that they design studies with the ability to assess vertical mechanisms from microbes to behavior, rather than a horizontal approach of identifying all altered microbes associated with a disease or all altered host metabolites associated with microbiome disruption.

Moreover, given the high dimensionality, multicollinearity, and compositionality of microbiome data, researchers should be cautious performing and interpreting single taxa hypothesis tests and differential abundance tests, which are often built upon unverifiable assumptions [377–379]. With these limitations, bias toward positive result reporting, and the speed at which microbiome data are being generated, it is likely that microbiota-gut-brain axis research is destined for a similar fate as nutritional epidemiology, where almost every identified food is associated with strong alterations to cancer risk in single studies but effect sizes shrink in meta-analyses [380]. Ratio-based biomarker use (see [379]) is a promising method, but researchers should also develop a thorough understanding (or work with researchers with a thorough understanding) of ecological theory to assess subcommunities and networks of microbes. That being said, network analyses based solely on co-occurrence pose their own issues, as spurious correlations do not imply relationships between microbes. Thus, multi-omics approaches using tools that incorporate

previous mechanistic knowledge, such as those outlined in Vehlow et al. [381], should be used to increase the number of lines of evidence supporting network-based analyses. Additionally, artificial gastric digestive systems are being developed and provide a means for larger system microbiome study in vivo. As this synthetic microbiome research (i.e., in vitro research utilizing artificial digestive systems, for review see Mabwi et al. [382]) improves, investigating direct relationships between previously unculturable microbes will improve our working knowledge of microbial community ecology. Finally, low-hanging fruit for improving study design includes working with biostatisticians to determine adequate sample size for clinical trials (despite challenges associated with conducting human subject research) and proper negative and positive controls.

Notably, many studies, including Wallace and Milev [331], Brenner et al. [328], and Browne et al. [330], have demonstrated that probiotic interventions targeting psychiatric outcomes are safe, feasible, and acceptable. Additionally, meta-analyses have concluded that randomized controlled trials of pre- and probiotic interventions targeting psychiatric outcomes are generally effective to a degree in patients both with and without psychiatric conditions [383–388]. However, results are inconsistent, likely due to low power, a lack of standardized methodology, and inconsistent reporting across studies to such a degree that some meta-analyses have concluded no effect from probiotic interventions [384, 389]. Some incongruencies include differential effects of probiotics in healthy individuals versus individuals with a diagnosis of anxiety disorder, affective disorder, or trauma- and stressor-related disorder, the ability of studies to improve mental health scores without crossing clinical cutoffs for diagnosis, and the presence of comorbid conditions (such as IBS) in participants [387, 388]. Though a great body of research supports their use, the field is not yet at a point where clinical protocols can be outlined for pre-/probiotic interventions targeting psychiatric outcomes.

Additionally, though probiotics increase exposure to specific microbes that have been identified as beneficial, they may not influence all factors that contribute to healthy microbiota. Alternative options such as nature exposure and dietary interventions increase microbiome diversity, alter microbiome composition, and are safe and feasible [359, 360, 373, 390]. Moreover, these changes are associated with increased microbiome stability and immunoregulation, both of which confer stress resilience. Furthermore, there are costs (money and time) associated with individual dietary and probiotic interventions. These costs pose barriers for marginalized groups, furthering public health disparities in underrepresented communities where trauma often already runs rampant [391]. Thus, environmental interventions should be framed as a necessary approach to improving public mental health and stress resilience; they would particularly benefit historically oppressed communities, who already experience higher rates of psychiatric conditions, immune dysregulation, and impaired microbial exposure [7, 391]. On a wide scale, public health interventions aimed at increasing exposure to nature and its abundance of microorganisms serve a role for improving population-wide stress resilience that consumer-available probiotics cannot fill.

The unseen cost of paving paradise is that modern housing, sanitization, and work environments have alienated a large portion of the population from the native biodiversity of microorganisms with which they would have historically had symbiotic contact. Given the evidence that microbial exposure heavily impacts psychiatric disorder risk through neural, immune, and metabolic mechanisms, interventions are necessary. Promising interventions include pre-/probiotics, dietary interventions, and nature exposure, and current research supports the strong promise of this field. However, the field is not yet at a point to establish clinical guidelines, and more research must be performed with the goal of translating the already outlined mechanisms to humans with the aim of prevention or treatment of stress-related psychiatric disorders.

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