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The Gut-Brain Axis: The effect of dysbiosis on mood disorders like anxiety and depression

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Abstract
The gut-brain axis (GBA) is a communication pathway between the central nervous system (CNS) and enteric nervous system (ENS), linking pathways between cognitive and emotional centers in the brain to the peripheral intestinal functions. The GBA is bidirectional due to the signaling from gut-microbiota to the brain and from the brain to the gut-microbiota through neural, neuroendocrine, immune and humoral pathways. Therefore disruptions in the gut microbiota through an imbalance of diversity or population reduction, known as dysbiosis, is linked to both central nervous system disorders and gastrointestinal disorders. This literature review focuses on the effect of dysbiosis on mood disorders like depression and anxiety, through the different GBA pathways. It also explores the possible probiotics, fecal transplants, and pharmacological interventions to treat mood disorders. The data discussed in this review has been collected using germ-free animal models, probiotics, antibiotics, fecal samples, MRI's, FMRI and experiments conducted on mice.

Keywords
Gut-Brain axis, Mood disorders, Microbiome, Dysbiosis, Gastroenterology, Anxiety, Depression, Mental health disorders, Neurotransmitters, Diet

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Introduction

The microbiota defines the microbe population in a specific ecosystem; this review will specifically focus on the gut microbiota. Within the gut, there are approximately 100 trillion bacteria that originate from different species and colonies (1). However, the gut microbiome is not limited to bacteria, but also includes fungi and viruses that have a symbiotic relationship with their human host. Bacteria chemically break down organic compounds in food, influencing nutrition intake in the body (2). Microbes also manufacture vitamins and synthesize chemicals to nourish and provide energy to cells in the colon which maintains colon health, influences mood, immunity, and other integral functions in the body (3).

Gut microbes are influenced by a mother's vaginal microbiota that is passed onto the child during birth. However, the main factor determining the gut microbiota is an individual's diet, lifestyle, and environment after birth. For example, processed foods, foods with high-fructose corn syrup, and hydrogenated fats are unhealthy for the gut microbes, often causing an imbalance in microbial diversity (4). This lack of diversity of gut microbiota has a profound influence on diseases, disorders, and the overall health of an individual. Having a healthy gut microbiota is generally recognized to be beneficial for the immune system, neural health, mood, and digestion. Fruits, vegetables, foods rich in fiber, and fermented foods promote the diversity and health of the microbiota. Alteration in the gut bacterial composition is termed dysbiosis and is associated with inflammatory diseases, allergies, autoimmune diseases, metabolic disorders, and neuropsychiatric disorders (5). Dysbiosis is often the result of an unbalanced and unhealthy diet and the ingestion of broad-spectrum antibiotics. Although each person's microbiota profile is different, the relative abundance and distribution of bacterial phylotypes are similar among healthy people. The most common phyla, which account for more than ¾ of the microbiome, are *Firmicutes* and *Bacteroides*. Specifically, the bacteria categorized as the *Firmicutes* phylum aid in immune regulation, digestion, and intestine nourishment while those associated with the *Bacteroides* phylum aid in digesting food (3).

The Gut-Brain Axis is the bidirectional communication between the central nervous system (CNS) and enteric nervous system (ENS), which links pathways between cognitive and emotional centers in the brain with intestinal functions. This axis controls gut function, intestinal permeability, immune activation, enteric reflexes such as peristalsis, and entero-endocrine signaling (3). The CNS consists of the brain and spinal cord and controls most functions of the body and mind. The ENS is a part of the peripheral nervous system (PNS) that controls gastrointestinal behavior independently of CNS input. This axis is part of a relatively new and upcoming stream in Neuroscience research, that explores the influence of gut microbiota on development, chronic diseases, and mood disorders like depression and anxiety. The interaction between the CNS and microbiota is through the vagus nerve, microbial metabolites, CNS-altering intestine permeability or *vice versa*, and other neuroendocrine and humoral signaling pathways (6).

Gut-Brain Axis pathways

In recent years there has been an increase in the number of of studies and published literature exploring the gut-microbiota and the GBA. The methods used to better understand this axis have primarily been germ-free animals, probiotics, antibiotics, fecal tests, and experiments on vagotomized mice(7).

A neural pathway connecting the gut and central nervous system is supported by the 10th cranial nerve or the vagus nerve. This nerve transmits information from the luminal environment of the intestine to the brainstem which then interacts with the hypothalamus in the limbic system. The hypothalamus regulates homeostasis and hormones in the body, an important part of the limbic system. The limbic system regulates behavior, memory and emotions in the body, therefore this cranial nerve links cognition and emotion to the gastrointestinal tract. The vagus nerve is the largest cranial nerve and sends signals from visceral organs such as the stomach, intestine and mouth to the brain *via* afferent nerve fibers. It regulates homeostasis, bowel movements, and also signals the brain if pathogens or toxins are detected, in order to generate an immune response (7). This anatomical connection was deciphered in an experiment in which vagotomized mice could not regulate food intake, intestinal homeostasis, satiety, and energy homeostasis, due to the abrogated bidirectional signaling through the vagus nerve (8).

Another GBA neural pathway is observed when external stress factors activate the Hypothalamic-pituitary-adrenal axis (HPA) in the brain, which in turn influences the functions of the intestine (3). The HPA axis is responsible for the interaction of the hypothalamus, pituitary gland, and the adrenal gland. The HPA modulates reactions to stress and regulates many processes. External stress factors or even strong emotions stimulate the HPA to signal the release of corticotropin-releasing factors from the hypothalamus in the brain. The corticotropin-releasing factor then stimulates the pituitary gland to secrete Adrenocorticotropic hormone (ACTH), which in turn stimulates the adrenal glands to release cortisol. Cortisol is a stress hormone that affects many organs including the intestine and helps mediate the fight or flight response of the sympathetic nervous system. Cortisol influences the secretions of the gut mucosa, influences intestinal motility, immunity, and gut permeability (9). Therefore stress factors influence the intestine and alter microbiota after HPA activation due to the connection between permeability of the intestine and intestinal mucus secretions, and microbes. Chronic stress increases cortisol levels in the body which consequently increases intestinal permeability. This increased permeability allows bacterial endotoxins like lipopolysaccharides into the bloodstream. These bacterial toxins often cause further psychiatric conditions like depression and anxiety and have been shown to enhance the symptoms of schizophrenia (10). This ‘leaky gut’ phenomenon is also theorized to contribute to depression, as depressed patients show elevated serum concentrations of immunoglobulin (Ig)-M and IgA (11). As stress and cortisol release is often a response to a fight-or-flight situation, it redirects blood from the intestine to other parts of the body like the limbs and brain, causing the

intestinal functions to slow down and become less efficient (3).

The gut-brain axis is also involved in neuroendocrine signaling. In this pathway microbiota interact with the ENS by producing microbial metabolites that act as local neurotransmitters. These neurotransmitters include gamma-aminobutyric acid (GABA), histamine, acetylcholine and catecholamines in the lumen of the gut (12). Many species of *Lactobacillus* and *Bifidobacterium* produce GABA, the main inhibitory neurotransmitter in the brain which aids in preventing anxiety, stress and fear. Other bacterial species produce neurotransmitters like serotonin, which stabilizes mood and contributes to emotions like happiness, and dopamine that mediates reward and movement in the brain (13). This provides a remarkable insight into the direct emotional impact of microbiota from the intestine excercized through the GBA to the CNS. Microbes can release metabolites that can signal enterochromaffin cells to synthesize neurotransmitters (2). The metabolites can also act as precursors to neurotransmitters or chemicals needed in the body. The neurotransmitter and chemicals formed are then released into the bloodstream, and some can cross the blood-brain barrier into the brain which can ultimately affect mood. Neuropod cells located in the intestinal epithelium synthesize and release neurotransmitters, which are then transmitted to the brain *via* the vagus (14).

Further neuroendocrine and metabolic pathways that affect the CNS are the production of short-chain fatty acids such as butyric acid and acetic acid by gut microbes from the fermentation of dietary fibers in food. Short-chain fatty acids are the main energy source for colon cells, aid in mucosal serotonin release, have antiinflammatory properties, prevent colon diseases, have several neuroprotective effects, and also support memory and learning processes (15). Short-chain fatty acids can cross the blood-brain barrier and regulate microglial cells in the nervous system, homeostasis, and brain tissue homeostasis which are required for proper development and behavior modulation such as increase in happiness levels and lesser anxiety-like behavior (16). Therefore a decrease in the consumption of foods rich in fiber, would decrease short-chain fatty acid levels in the intestine, thereby increasing the likelihood of inflammation, nutrient malabsorption, colon diseases, cancer and overall health (6).

The gut microbiota play a significant role in the nutrition intake of the body. This is because gut microbes produce certain vitamins and chemicals that cannot be naturally obtained from food digestion or synthesized in the body. The nutrition intake influences the release of biologically active peptides from enteroendocrine cells (17). Enteroendocrine cells are endocrine cells in the gastrointestinal tract that produce and secrete hormones based on the nutritional status stimuli. An example is the neuropeptide galanin which is involved in neurobiological functions like mood, circadian rhythm, blood pressure, and neurotrophic functions (18). Galanin stimulates the HPA and thus increases the cortisol secreted by the adrenal glands, thus regulating stress. This demonstrates the influence that gut microbes can have on stimulating parts of the central nervous

system through neurotransmitters and neuropeptides (19).

The role of the Gut-Brain Axis in gastrointestinal disorders

Functional gastrointestinal disorders are influenced by genetics and environmental factors, such as exposure to infections, antibiotics, mood disorders and psychological development. The disruptions in the gut-brain axis through an increase in cortisol secretion, dysbiosis, or bacterial metabolites causes visceral hypersensitivity in the intestine and alterations of the entero-endocrine and immune system (20). Irritable Bowel Syndrome (IBS) is the most common functional bowel disorder that affects 7 to 10% of the population. The visceral hypersensitivity experienced by IBS patients can be transferred from the microbiota of IBS patients to germ-free rats, demonstrating the role that the microbiota plays in the disease. The germ-free rats start to present with IBS related symptoms such as constipation, diarrhea, bloating, etc. (21). Fecal samples of IBS patients, showed an increased concentration of proteases that are associated with specific intestinal bacterial species such as the *Lactobacillales*, *Lachnospiraceae*, and *Streptococcaceae* groups (22). The alterations in their microbiota in terms of both stability and diversity causes an alteration in enzymes, such as proteases, that can deteriorate gut health. Recent studies suggest that IBS is caused when an abnormal gut-microbiota activates mucosal immune responses that increase the permeability of the intestine's epithelium layer, and subsequently activates nociceptive sensory pathways causing visceral pain (3).

The effect of the Gut-Brain Axis on mood disorders

The Gut-Brain Axis also impacts mood disorders like depression, anxiety and bipolar disorders, through the chemical, neuroendocrine, humoral and neural pathways. Depression is a prevalent neuropsychiatric disease with a high recurrence rate that affects over 350 million people worldwide. Depression is described as a mood disorder in which anhedonia and/or loss of interest or pleasure in life activities are present for a minimum of two weeks. The patient also presents with additional symptoms such as significant and unintentional weight loss or gain, insomnia or excessive sleeping, fatigue, recurring feelings of worthlessness, guilt, decreased ability to think or concentrate, and even suicidal thoughts (23). It is associated with the depletion or abnormal transmission of neurotransmitters such as serotonin, dopamine and norepinephrine. Even though depression is a complex chronic mood disorder that has both, genetic and environment drivers, it has recently been additionally linked to dysbiosis of the gut microbiota (24).

Another prevalent mood disorder is anxiety, categorized as frequent, excessive and persistent worrying and fear that escalates into panic attacks, increased heart rate and sweating. Even though mood disorders can be catalyzed by events or experiences in people's lives they are medical conditions that can be treated with consultation with mental health professionals, medication, and electroconvulsive therapy. New research and treatment options are being developed to combat these mood disorders (25).

Fecal microbiota transplantation (FMT) is a method of transferring fecal bacteria from a donor to a recipient and is widely applied in treatment and research. It has shown successful results in the treatment of microbial structural disorders, depression, and reprogramming the host's metabolism. The current treatment of fecal bacteria transplantation is typically conducted via endoscope, enema, and orally administered freeze-dried materials. Other types of transplants are being explored, such as Microbial Ecosystem Therapeutics-2 (MET-2). MET-2 is used to extract gut microbes from the stool samples of healthy people, which are then screened, cultured in a laboratory, and formulated before being administered to patients (11).

A study that examined microbiome alterations and depression by analyzing fecal microbiota of 37 patients diagnosed with clinical depression compared to 18 non-depressed controls found correlations between the samples of depressed patients. The analysis of fecal microbiota of depressed patients showed that bacteria from the phyla *Firmicutes* and *Bacteroidetes* were abundant in the microbiota of depressed patients (26). Hence, the central nervous system impairment caused by depression affects the microbial composition in the intestinal tract. Moreover, dysbiosis can also cause depressive symptoms because transplanting feces from depressed mice into normal mice could generate depression-like behaviors in the latter (11). Such studies demonstrate the bidirectionality between depression and dysbiosis; i.e. depression can cause dysbiosis and dysbiosis can worsen depressive symptoms. In another experiment, *Alistipes*, a genus in the phylum of *Bacteroides* was found in excess in depressed patients, and also in the samples of patients suffering from chronic fatigue syndrome and irritable bowel syndrome. *Alistipes* is associated with inflammation and therefore potentially linked to depression through inflammatory pathways (27).

Since *Alistipes* and other microbes can be modified through changes in the diet, it seems possible to treat mood and gastrointestinal disorders by changing the dietary composition. When *L.casei Shirota* was orally administered to patients with anxiety symptoms, caused by chronic fatigue syndrome, their anxiety-associated symptoms decreased significantly. The patients consumed either the probiotic or a placebo 3 times a day for 8 weeks, after which they experienced lesser anxiety and depression symptoms (28). Depression and anxiety are often comorbidities in individuals suffering from irritable bowel syndrome. In another similar study patients were administered prebiotic galactooligosaccharide; a form of dietary fiber that is a food source for gut bacteria and has shown to increase the growth of healthy bacteria in the gut. After 4 weeks of administration the patients' anxiety and mood scores improved significantly. To further test the link between a healthy microbiota and depression, a double blind randomized placebo study was conducted in which patients were adminstered a combination of *L acidophilus*, *L casei*, and *Bifidobacterium bifidum* for 8 weeks, which improved their scores on the Beck Depression Inventory (13).

Alterations in gut microbiota can increase the permeability of the gastrointestinal barrier, activate systemic inflammation and immune responses, and regulate the release of monoamine neurotransmitters. Monoamines refer to the neurotransmitters dopamine, noradrenaline and serotonin (29). Alterations in gut microbiota can also change the activity and function of the HPA and modify the levels of brain-derived neurotrophic factor (BDNF). Brain-derived neurotrophic factor influences cell differentiation, neuronal protection, synapses formation, neuroplasticity development; among other functions in the nervous system. Decreased levels of BDNF is a risk factor for impaired neuroplasticity and the development of depressive symptoms, leading to clinical depression (30). When stressed mice were administered probiotics (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) for two weeks, their hippocampal expression of BDNF was increased, therefore reversing the depressive symptoms and simultaneously improving learning and memory processes (31). This demonstrates the link that dysbiosis in the gut can have on creating a chain of events, beginning with the reduced release of monoamines, and BDNF, and eventually leading to mood disorders such as depression and anxiety. Additionally, such studies demonstrate the influence that the gut microbiota have on neurodevelopment, memory and learning processes. Most of the currently available antidepressants, despite favorably modulating the levels of monoamine neurotransmitters, do not satisfactorily treat depression and anxiety; an inadequate response occurs in approximately 30% of patients (32).

Another mechanism by the GBA influences mood disorders is through the metabolism of short-chain fatty acids, which are bacterial metabolites from digested food, which stimulate mucosal serotonin release (33). Mice that were fed 50%, lean ground beef had greater diversity in their microbiota than the mice that were fed rodent chow, and presented less anxiety-like behavior along with other health benefits (34). Similarly, it has been found that the diet and the resulting microbiota composition influence the tryptophan metabolism in the body and therefore the release of serotonin, thereby modulating the symptoms and likelihood of depression and anxiety (35). Studies have shown that depressed patients have lower levels of brain-derived neurotrophic factor and that this neurotropic factor can be upregulated by probiotics in murine models. The hyperactivity of the HPA axis which is indicated by the increased secretion of cortisol from adrenal glands can be reduced by altering the function of the microbiota-gut-brain axis *via* the gut microbiota (36).

An imbalance of cortisol is linked to significantly higher levels of depression and other mood disorders. Enterochromaffin cells contain more than 95% of the body's serotonin (5-HT). Serotonin synthesis in enterochromaffin cells is modulated by short-chain fatty acids (SCFAs) and secondary bile acids (2BAs) that are produced by spore-forming *Clostridiales*, by increasing the stimulation of these cells. Dietary tryptophan increases the SCFA and 2BA's, hence increasing serotonin release. Enterochromaffin cells communicate with afferent nerve fibers through synapse-like connections. The autonomic nervous system can activate enterochromaffin cells to

release serotonin into the gut lumen (37). Serotonin is involved in the regulation of gastrointestinal tract secretions, smooth muscle contractions, and pain perception, and the brain is involved in managing mood and cognition (38). The gut microbiota also metabolize tryptophan which is an essential amino acid that can only be obtained from the diet, as it is not synthesized by the human body. Tryptophan is an amino acid needed for growth in infants, and the maintenance of muscles, proteins and neurotransmitters. It is the precursor for serotonin production in the brain, therefore foods that metabolize into tryptophan will lead to increased serotonin in the body (37).

Table 1:Gut microbiota regulated neurotransmitter synthesis and some of their functions. This table illustrates the neurotransmitter, precursor, gut-microbiota, related intestinal cells and some of the functions of the neurotransmitters (13). Serotonin and glutamate are found in enterochromaffin cells while GABA and acetylcholine are found in myenteric neurons.

Neurotransmitters	Precursors	Gut Microbiota	Functions
Tyramine	Tyrosine	-*Staphylococcus* -*Providencia*	-Tyramine helps regulate blood pressure -Induce serotonin secretion by the enterochromaffin cells. -Promote gastrointestinal motility and colonic secretion
Tryptamine	Tryptophan	-*Staphylococcus* -*Ruminococcus Gnavus* -*Clostridium sporogenes*	-Tryptamine influences sensory perception, mood and emotions.
Serotonin	5-HTP Tryptophan	-*Staphylococcus* - *Clostridium*	-Serotonin stabilizes mood, feeling of well-being and happiness -Also influences sleeping, eating and intestinal motility
Glutamate	Glutamine	-*Lactobacillus plantarum* -*Bacteroides vulgatus* - *Campylobacter jejuni*	-Transfer signals from the intestinal to the brain through the vagus nerves. -Glutamate influences functions like learning and memory
GABA	Glutamine	-*Bifidobacterium* -*Bacteroides fragilis* - *Parabacteroides* -*Eubacterium*	-Modulate intestinal motility and inflammation -Main inhibitory neurotransmitter -Low GABA activity leads to anxiety, depression, insomnia, and mood disorders
Acetylcholine	Choline	-*Lactobacillus plantarum* -*Bacillus acetylcholine* - *Bacillus subtilis* -*Escherichia coli* -*Staphylococcus*	-Regulate intestinal motility and secretion -Main neurotransmitter of the parasympathetic nervous system.
Dopamine	Tyrosine l-DOPA	-*Staphylococcus*	-Influence gastric secretion, motility, and mucosal blood flow -Influence gastric tone and motility in a Parkinson's disease rat model

The immune pathway of the gut-brain axis that influences mucosal immune activation also has an effect on mood disorders. Mice that were treated with antimicrobials to induce dysbiosis experienced inflammation, and increased substance P expression in the ENS (39). Substance P is a neurotransmitter that plays a role in gastrointestinal functioning, memory, and vasodilation and modulates pain perception in the body (40). The influence of microbiota on immune activation is partly mediated by proteases produced by microbes. Therefore dysbiosis upregulates the levels of proteases in the gut and stimulates the immune system, thus causing inflammation. This constant inflammation can cause visceral pain, gastrointestinal disorders, and mood disorders. Inflammation in the body is mediated by the release of cytokines. Cytokines are proteins that help control the activity of immune system cells and erythrocytes, therefore controlling inflammation. Increased activation of cytokines is associated with depression-like symptoms. Increased cytokines cause dysregulation in the serotonergic and noradrenergic pathways in the brain by activating the HPA axis, or by decreasing the production of serotonin. This demonstrates the link between the gut microbiota, the immune system, and the central nervous system, therefore disruption in any one facet causes a myriad of diseases including mood disorders, gastrointestinal disorders, and autoimmune diseases (3).

The gut-brain axis is also linked to depression and anxiety through neuroendocrine pathways. In the gut, species of *Lactobacillus* and *Bifidobacterium* produce GABA, which, as mentioned earlier, aids in preventing anxiety, stress, and fear. Other bacterial species produce neurotransmitters like serotonin, dopamine, and catecholamines that mediate pleasure, reward, and mood (2).

Finding the appropriate doses of probiotics to modulate mood is challenging because high doses may counteract the benefits of that specific bacterial strain in the human body. Probiotic dosages are expressed in colony-forming units (CFU), which estimate the number of live microbes required to form a colony in the host. In clinical trials, the doses are determined by the amount of certain bacterial strains in the gut and the probiotic needed to maintain that composition, however, they vary from strain to strain; especially so when probiotics are mixed and matched. More research needs to be conducted on determining and optimizing the effect of probiotics so that the amount administered is most beneficial to ameliorate dysbiosis. The minimum dose requirement for probiotics that are not strain specific, such as *Bifidobacterium* (*adolescentis, animalis, bifidum, breve and longum*) and *Lactobacillus* (*acidophilus, casei, fermentum, gasseri, johnsonii, paracasei, plantarum, rhamnosus and salivarius*) is $1x10^9$ CFU. The CFU dose differs from bacterial strain to strain and also depends on the type and severity of dysbiosis (41).

Studies that test single-species probiotics (SSP) promote a better understanding of the function and influence of individual probiotics, which is difficult to measure in multiple species probiotics (MPP). However, multiple species probiotics may have higher potency in humans, as demonstrated in a study of depressed patients, where SSP (*L.*

plantarum) did not reduce depression symptoms but improved cognition , whereas MSPs showed antidepressant efficacy (42). The mixing of probiotics to deliver a combination of *Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactococcus lactis, Lactobacillus acidophilus, L. plantarum, Bifidobacterium animalis subsp. lactis* and *Limosilactobacillus reuteri* caused an anxiolytic response in patients. Another multi-species combination improved cognitive reactivity to a depressed mood in healthy participants. However, a later study using the same combination found that the benefits were only evident when participants were stressed, therefore demonstrating the need to carefully characterize populations. A multi-species combination which included *L. fermentum LF16*, *Lacticaseibacillus rhamnosus LR06*, *L. plantarum LP01*, and *B. longum BL04* caused significant improvements in mood, anger, fatigue and sleep quality in healthy participants (43). Hence, it appears that prophylactic treatement of healthy but at-risk depressive or anxiety populations must carefully consider probiotic composition.

Bacteria have recently been engineered for diagnosing disease. Studies have used the quorum-sensing abilities of bacteria to identify infections in the gut. For instance, the *L. lactis* species was engineered to secrete β-lactamase after detecting a signaling molecule produced by an infective *Vibrio cholerae*. Detection of the infective bacteria alters the color of the feces of the patient and helps diagnose the infection (44). Metabolic products of commensal gut bacteria (termed the metabolome) exert varied and important effects via the GBA and have recently been studied extensively. The microbiome and metabolome is hence inextricably linked so that tandem studies can be used to evaluate host-microbiome interactions. A study used 16S rRNA gene amplicon data to confirm that the gut microbiome is very metabolically active and that studying the fecal metabolome allows for the estimation of the gut microbiota's effect on health. However, 16S rRNA gene amplicon-sequencing does not fully capture the metabolic activity and newer methods such as whole metagenomic shotgun sequencing (WMGS) have emerged which are more accurate. Indeed, WMGS detects the taxonomic composition at higher resolution and provides more specific data that would allow for better diagnosis, and therefore a more precisely targeted probiotic composition. However, WMGS is currently considerably more expensive than 16S rRNA gene amplicon-sequencing and is therefore less used clinically (45).

Pathogenic bacteria like *Pseudomonas aeruginosa* use quorum sensing molecules, which are molecules used by bacteria to chemically communicate with cells in its proximity and sense the population density of similar cells. This pathway can be utilized by engineering commensal bacteria to cause bacteriostatic or bactericidal effects. An example is *E. coli* that was genetically engineered with synthetic quorum sensing, which was effective against a *Pseudomonas aeruginosa* infection, inhibiting the formation of the biofilm by nearly 90% (46).

Considering the essential role that the vagus nerve plays in the gut brain axis pathway, diseases affecting the vagus nerve, such as Gastroparesis, can cause GBA signaling disruptions. Gastroparesis patients have

higher levels of depression and anxiety, which may be due in part, to alteration of gut microbe composition. Despite GBA dysfunction, probiotics still retain their ability to improve the health and mood of these patients. This may be because of local effects, or communication with the CNS using extra-GBA pathways. Specific probiotics improve gastric emptying, and inflammation of the bowel, of gastroparesis patients (47).

Chronic HPA activation stimulates the inflammatory pathways and increases the formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), damaging DNA, lipids, and proteins in mitochondria and cell membranes. The prevalence of oxidative and nitrosative/nitrative stress is reflected by the elevated amounts of lipid peroxidation by-products, such as malondialdehyde and 4-hydroxynonenal, in depressed patients. Additional reports show significant decreases in endogenous antioxidants, such as melatonin, glutathione, glutathione peroxidase, etc, which are protective in the mitochondrial function and are involved in the regulation of cAMP/circadian genes, whose dysregulation may lead to behavioral disorders. Pathogenic taxa lead to depression either directly (by generating valeric acid, an inverse agonist at the adenosine A1 receptor) or indirectly (by increasing the synthesis of kynurenine from tryptophan). These pathogenic taxa or pathogens in gut dysbiosis increase gut leakiness and circulating lipopolysaccharide levels, which, in turn, trigger DAMP, TLR2/4 receptor, and inflammatory pathways (48).

Homocysteine (Hcy) is a non-essential, sulfur-containing, non-proteinogenic amino acid. It is synthetized by transmethylation of the essential, diet-derived amino acid methionine. Increased levels of homocysteine are associated with dysbiosis and with various neuropsychiatric disorders, which includes depression. Elevated levels of homocysteine can increase intestinal permeability and disrupt the epithelial barrier in rats by stimulating inflammatory and oxidative pathways. However, these effects were ameliorated by probiotic administration. Clinical studies report a high prevalence of homocystinuria in patients with psychiatric disorders. Vitamins B6, B12 and folate act as coenzymes to metabolize Homocysteine. Unsurprisingly, studies that treated homocystinuria patients with vitamin B6 resulted in an improvement in psychiatric conditions, including episodic depression; in part due to repopulating the dysbiotic microbiota in these vitamin-deficient patients with beneficial appropriate genera (49). Pathogenic bacteria such as *Subdoligranulum sp.*, *Eubacterium sp.*, and *Clostridiales family XIII* were found to be the main producers of homocysteine, supporting the pathogenic role of homocysteine in dysbiosis and depression. Vitamins and essential amino acids are a critical part of the healthy diet, and play a part in maintaining a healthy microbiome, which, in turn, ensures that the host receives a steady supply of proper, well-balanced nutrients (50).

Postbiotics are the inactivated microbial cells mixed with or without metabolites (e.g., lactic acid, proteins, vitamins, and SCFAs) or cell components (including pili, and cell wall components). Similar to probiotics, postbiotics also promote the integrity of the epithelial barrier function, restore the balance of the microbiota composition and diversity,

regulate the immune responses, and regulate the gut-brain axis signaling. In mice exposed to sub-chronic and mild social defeat stress, the administration of the heat-killed *Lactobacillus helveticus* strain MCC1848 reduced depression- and anxiety-like behaviors and upregulated the genes involved in neuron differentiation and development as well as signal transduction, in the nucleus accumbens (48).

Synbiotics are synergistic mixtures of prebiotics and probiotics that benefit the host by promoting beneficial microbial activity. A study reported that synbiotics (*Lactobacillus acidophilus T16, Bifidobacterium bifidum BIA-6, Bifidobacterium lactis BIA-7, and Bifidobacterium longum BIA-8*) increased the serum levels of BDNF and improved the depression symptoms in depressed patients compared to controls. Polyphenols, which are compounds found in plant-based foods, also maintain the populations of the immune-modulating bacteria, such as *Bifidobacteria* and *Lactobacilli*, as well as preventing colonization by pathobionts, despite the very low bioavailability of these phytochemicals. For example, blueberries contain polyphenol anthocyanins which significantly increase brain activity with improved working memory and reduce depression-like symptoms (48).

Even though human and mouse intestines share common bacteria, only ~ 4% of the bacterial genes are identical. Therefore this makes the translation of research done on the gut microbiota of mouse models to humans challenging. Furthermore, the translation of the dosage for a mouse into equivalent human doses does not consider the dosage of probiotic foods or supplements, where the dose is based on the number of live organisms present (43).

Recent studies show that Th17 cells which only present in the lamina propria of the small intestine increase the susceptibility to depressive-like behaviors in mice. These cells are pathogenic when they infiltrate the CNS, causing multiple sclerosis. Th17 cells are highly up-regulated by segmented filamentous bacteria (SFB), a nonculturable spore-forming Gram-positive bacterium that is closely related to the genus *Clostridium* and colonizes the intestines of humans and mice. Without SFB, Th17 cells are absent in the mouse small intestinal lamina propria, where they are usually abundant in rodents with healthy gut microbiota. SFB in the gut increases the bacterial quorum sensing molecules autoinducer-2 (AI-2) and the levels of SAA1 and SAA2, which then increase the production of Th17 cells and cause increased hippocampal Th17 cell accumulation. Disrupting this signaling pathway by blocking AI-2 with oleic acid provides antidepressant properties in the mice tested in the study. Therefore this study demonstrates a signaling mechanism by which changes in bacterial products (and/or in the number of bacteria producing these products) influence mood-relevant behaviors (51).

Certain types of diets are also associated with the improvement or deterioration of mental health, which can be correlated to the prevalence of mood disorders in certain ethnic populations. One example is the Mediterranean diet (52). Mediterranean diets consist of Oleic acid in olive oils and has been shown to have antidepressant properties. On the other hand, western diets contribute to dysbiotic conditions as the high-fat

composition of these diets causes widespread inflammation, and consequently, increases anxiety and depressive symptoms. Clinical trials showed improvement of depressive symptoms noted after switching from a western to a Mediterranean diet. However, these trials also considered the subjects' expectations and knowledge about how dietary changes could improve their health; thereby possibly confounding the results. The evidence that gut microbiota dysbiosis is the main causative factor for differences in the prevalence of mood disorders in different ethnic groups is still inadequate (53).

Research is ongoing to determine if and how telomere length (a proxy molecular marker of aging), inflammation, and the microbiota may interact and to find the mechanism(s) caused by such interactions on the development of mental disorders like schizophrenia (SCZ), bipolar disorder (BD), and major depressive disorder (MDD). Machia et. al. found that dysbiosis of the gut microbiota may be associated with accelerated aging in animal models as shown by a shortening of the telomere length. A greater number of short telomeres were present in the diagnostic groups (SCZ, MDD, and BD) as compared with healthy cells. The authors concluded that there was a plausible interaction between inflammation and telomere shortening in modulating the risk of severe psychiatric disorders, and this association might be reflected in specific detrimental alterations of the microbiota. However, the role of confounders could not be discarded due to the cross-sectional design of the study. Causality hence could not be determined. The authors suggested that the study be used as a hypothesis generator, to design the future research protocols that might be conducted in this field (54).

Cytosolic Forkhead box protein O1 (Foxo1) within goblet cells plays an essential role in promoting intestinal mucus layer formation by regulating mucin secretion in a process linked to autophagy (55). The formation and maintenance of mucus layer integrity is required for stabilization of the gut microbiota and maintenance of intestinal barrier integrity. Loss of Foxo1 in intestinal epithelial cells causes impaired mucus layer formation and dysbiosis in the microbiome, causing disrupted intestinal barrier integrity and therefore enhanced susceptibility to infection and tissue inflammation. Foxo transcription factors affect cell survival, cell division, and energy use, and play a critical role in T cell development that direct mucosal immune responses. A recent study identified a novel host–microbiota positive feedback loop that is critical for maintaining intestinal homeostasis and health of the tested mice. The disruption of intestinal barrier integrity and inflammation have been shown to amplify the symptoms of depression and anxiety (56).

Probiotics and FDA-approved antidepressants have potential synergistic effects and can be used in conjunction for the treatment of mood disorders. Several studies have shown that the clinical effect of FDA-approved antidepressants like monoamine oxidase inhibitors, and tricyclic antidepressants; when adjuvanted with probiotics; is greater than when administered without the addition of the probiotic. There are as yet no FDA-approved probiotics. Some antidepressants have antimicrobial properties that can lead to dysbiotic adaptive alterations in the gut

microbiota. Therefore it is possible that the probiotics allow for restoring microbial balance and diversity caused by the antidepressants, and hence they are beneficial to use in conjunction with one another (57). Since probiotics act *via* the GBA to reduce symptoms of mood disorders, this may represent an additional pathway when combined with approved antidepressants that act by other mechanisms.

Some studies that reported no significant difference in the change of depressive or anxiety symptoms of participants given probiotics as compared to the placebo group, achieved significance after sub-group analysis of separating the participants into mild and severe levels of depressive symptoms. Follow-up analysis showed that one month after completing the study, participants in the probiotics groups were more likely to shift from a subclinical diagnosis to a no depression diagnosis. Moreover, participants that were treated with larger doses of probiotics showed a significant improvement in depressive symptoms. The effect of probiotics may present over a longer time period than the 8-week intervention time period of such studies. However, there is still no consensus for how long each intervention will last as recovery of patients could also be attributed to spontaneous recovery over time (58).

Discussion

As research progresses in the area of the complex workings of the gut-brain axis, it can be concluded that gut-microbiota do have a significant effect on the health of individuals, including that of the CNS. The aforementioned studies and experiments conducted conclusively demonstrate the link between disturbances in gut microbiota composition and diversity and a myriad of diseases, disorders, and symptoms. In these studies, the strong data supporting the link between certain types of bacteria and the prevention of diseases could lead to safer and more effective treatments in the future. It is possible that in the future doctors prescribe bacteria such as *L.casei Shirota*, and *Bifidobacterium bifidum* to help treat mood disorders, as these bacteria species have been linked to improved mood due to their bacterial metabolites. The use of specific probiotics could increase a type of bacterial colony, decreasing the severity of a disease or eradicating symptoms. However, research for the use of probiotics is more established for diseases like Irritable Bowel Syndrome, which could be treated with a prescription of a variety of bacteria species to reintroduce diversity and health in the gut. Considering that most clinical studies have been conducted on mice, the dosages and types of bacteria will need to be altered for humans.

Even though in studies with mice, significant improvements in depression and anxiety symptoms have been obtained, clinical studies on humans have not been very successful, especially for patients with severe depression. This has prevented probiotics to become mainstream. With more research into specific strains for diseases, and titrated dosages, probiotics could be very beneficial to a subgroup of patients experiencing mood disorders. However, as depression has also been shown to cause dysbiosis, the effects of prescribing long-term probiotics to patients need to be explored in future studies. Such long-term studies should check for recurring symptoms and the effect of the treatment months after it was conducted. These patients

in the study could have their stool tested periodically to check for a recurrence of dysbiosis, thereby providing evidence that mood disorders can cause dysbiosis and that solving the dysbiosis may require prophylactic probiotic administration.

Through further research and experimentation, treatment options for mood disorders may take dysbiosis of intestinal bactaria into account. The use of probiotics synergistically with antidepressants has been shown to improve symptoms and can become more mainstream in the future. Conducting stool tests on patients could be used to determine their gut health and mood disorders, and diagnose irritable bowel syndrome and other neurological and gastrointestinal diseases. A stool test for microbiota would help diagnose disease and allow for treatment options specific to the patient based on the abundance or scarcity of a particular microbial genera or species. This would facilitate a patient-specific treatment course for these diseases. However, stool samples may not be representative of the entire gut populated bacteria. Therefore, more research needs to be conducted for this to be a viable option.

A limitation of this research is being able to conduct clinical experiments with controlled groups. This is due to the unethicality of creating germ-free humans . However, this limitation of not having a controlled group could be minimized by double-blinded placebo experiments with a large sample size over an extended period of time to observe if there is any substantial improvement in the condition of individuals suffering, due to the probiotics or placebo probiotic administered.

Conclusion

Research into the Gut-Brain Axis explores the profound influence that the gut microbiota has on the bidirectional communication between the gut and the nervous system. Dysbiosis in the gut microbiota can cause a myriad of health problems such as functional gastrointestinal disorders, barriers in neurodevelopment, and mood disorders due to the connection between the gut and brain. Some of the disorders and diseases whose pathogenesis originate from strain-specific microbes suggest a potential role of certain probiotics as treatments for neurologic, gastrointestinal and mood disorders. A new treatment option for patients with dysbiosis is fecal transplants from donors with healthy microbiota, probiotics, and a conjunction of antidepressants with probiotics. The research into the gut-brain axis emphasizes the importance of healthy and diverse gut microbiota through proper diet and lifestyle. This paper also provides insight into the neurological stimuli caused dysbiosis of the intestinal microbiota; which in turn; causes detrimental inflammatory and immunological downstream effects.

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