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The Microbiota-Gut-Brain Axis: A New Direction in Research on Depression

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ABSTRACT

Depression is a global health crisis which becomes increasingly urgent as depression rates continue to rise. While impressive progress has been made in understanding and treating depression, we face many more unknowns, and existing treatment options are not effective for all patients and all depressive episodes. In recent years, the microbiota-gut-brain axis has emerged as a promising research direction as increasing evidence shows the gut microbiota and the brain to be closely linked. This review presents the most compelling evidence for a bidirectional relationship between the gut microbiota and the brain, zooms in to examine putative neural, neuroimmune, neuroendocrine, and neurometabolic mechanisms involved in bottom-up communication, reviews existing evidence for probiotics, and discusses challenges and future directions of the field. Further research on the microbiota-gut-brain axis could provide a better understanding of the pathogenesis of depression and reveal opportunities for novel preventions and treatments.

Introduction

Major depressive disorder is one of the leading causes of disability worldwide, affecting approximately 300 million people and accounting for approximately 10% of disability globally (Winter et al., 2018). Depression has significant adverse effects on quality of life and physical health. Meta-analytic data show that people with depression have a risk of mortality from all causes that is 1.86 times that of healthy persons (Walker et al., 2015).

Multiple theories have been put forward to explain the pathogenesis and pathophysiology of depression. The monoamine deficiency hypothesis states that depression stems from decreased levels of serotonin, dopamine, and/or norepinephrine, and is supported by studies in which tricyclic antidepressants and monoamine oxidase inhibitors (MAOIs) promoted monoamine neurotransmission and alleviated depressive symptoms (Krishnan and Nestler, 2008). Additionally, neurotransmitters gamma-aminobutyric acid (GABA) and acetylcholine have been shown to be dysregulated in depression (Krishnan and Nestler, 2008).

Another theory focuses on the increased plasma cortisol observed in depression patients and posits that a combination of excessive stress and dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis underlies depression (Krishnan and Nestler, 2008). Depressive episodes are often preceded by stressful life events, which could trigger HPA axis dysregulation. Furthermore, an association has been observed between failed HPA axis normalization and poor response to treatment (Nelson and Davis, 1997).

The neuroinflammation hypothesis suggests that CNS inflammation plays a role in the genesis and exacerbation of depression. Neuroinflammation is regulated by microglia, resident immune cells of the CNS. Once activated, microglia can release neurotoxins, compromise the blood-brain barrier (BBB), regulate monoamine neurotransmitters synthesis, and activate the HPA axis (Miller et al., 2009). In support of the neuroinflammation hypothesis, higher levels of inflammatory cytokines including interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor- α (TNF-α), and interferon-γ (IFN-γ), as well as evidence of microglial hyperactivation are found in patients with depression (Dowlati, 2010). Further support comes from the higher rates of depression in individuals with autoimmune disease and severe infections (Dowlati, 2010), and instances of depression following clinical cytokine administration

(Miller et al., 2009). Neuroinflammation and HPA axis dysregulation are closely tied together. Microglial neuroinflammatory signals regulate HPA axis activation (Wohleb et al., 2014), while in turn, microglia express a large number of glucocorticoid receptors responsive to HPA axis activity (Sierra et al., 2008).

Connected to HPA dysregulation and inflammation theories, decreased adult neurogenesis could be a causal factor in depression. Adult neurogenesis is the process by which functional neurons form from neural stem cells in the adult hippocampus. Neurogenesis is thought to be influenced by inflammation and HPA dysfunction and is controlled by regulatory protein brain-derived neurotrophic factor (BDNF) (Egeland et al., 2015). BDNF is shown to be decreased in depressed patients and restored after effective pharmaceutical or psychological therapy (Molendijk et al., 2014).

Finally, on a larger scale, studies have found that in depressed patients, the amygdala, insula, and dorsal anterior cingulate cortex display a greater response to negative stimuli while the dorsal striatum and dorsolateral prefrontal cortex show a lower response (Hamilton et al., 2012). In addition, postmortem and neuroimaging studies of depressed patients reported reduced grey-matter volume and glial density in the prefrontal cortex and hippocampus, which could be the pathophysiology underlying the cognitive aspects of depression (Krishnan and Nestler, 2018).

Although multiple theories had been established, no single theory adequately explains the disorder. Similarly, while many psychological and pharmaceutical therapies have been put forward, standard treatments, even when combined, are effective in only 74% of cases (Winter et al., 2018). Our current state requires a better understanding of depression, and one potential direction is to look beyond the brain. While depression is primarily a disease of the brain, the brain does not exist in isolation but instead has complex connections with the rest of the body.

In recent years, research on depression has turned its attention to the gut microbiota, the collection of bacteria, archaea, fungi, viruses, and protozoa that inhabit the digestive tract. The average human gut is inhabited by 10^14 microorganisms, which is more than ten times that of human cells in the body (Gills et al., 2006). Microbial colonization commences at birth, and microbiota composition continues to be altered by diet, physical activity, disease, and drug use throughout life (Cryan and Dinan, 2012). Although much remains unknown, evidence shows that there is bidirectional communication between the gut microbiota and the brain, mediated by neural, neuroimmune, neuroendocrine, and neurometabolic mechanisms. Since gut microbiota balance is a crucial component of human health, gut microbiota dysbiosis could be a causal factor of depression, and depression could be maintained through a vicious cycle of pathogenic gut microbiota and brain changes. Although we are only beginning to unravel the complex relationships between the gut microbiota and the brain, research in this area could inform our understanding of depression and provide novel treatments that target the gut microbiota.

Evidence for a bidirectional link between the gut microbiota and depression

Evidence for the existence of a link

There is an extremely high rate of comorbidity between psychiatric disorders and functional gastrointestinal (GI) disorders. Psychiatric disorders, including depression, were reported in up to 94% of patients with functional GI disorders in one systematic review (Whitehead et al., 2002). Meanwhile, roughly 20% of patients diagnosed with depression report GI symptoms (Mussell et al., 2008).

Moreover, patients with depression demonstrate altered gut microbiota composition. Although there is conflicting evidence, most research shows that patients with depression demonstrate an overall decrease in gut microbiota richness and diversity (Jiang et al., 2015). Conflicting evidence could result from phylum-dependent changes in different directions and differences in diagnostic criteria, grouping criteria, and fecal microbiota detection methods. Most importantly, conflicting evidence may be reflective of small sample sizes.

An important large-scale study examined the microbiota composition of >1000 individuals enrolled in the Flemish Gut Flora Project (Valles-Colomer et al., 2019). Individuals were clustered into four enterotypes, and

interestingly, those who had depression or lower quality of life scores were overrepresented in an enterotype with a low relative abundance of *Faecalibacterium.* Further analysis revealed that higher quality of life scores were associated with butyrate-producing bacteria, such as *Faecalibacterium* and *Coprococcus. Meanwhile, Coprococcus and Dialister were largely absent in patients with depression. These findings were largely replicated in the LifeLines DEEP cohort. Together, they provide population-scale evidence for a link between gut microbiota composition and mental health.*

Evidence for top-down influence

Persuasive evidence for top-down influence comes from studies in which psychological stress, including maternal separation, chronic social defeat, restraint conditions, crowding, heat stress, and acoustic stress, alters gut conditions, microbiota diversity, and microbiota composition (Winter et al., 2018). There is a consensus among animal studies that stress induces an increase in gut permeability and a decrease in microbiota diversity and richness (Collin et al., 2012), alterations which are consistent with characteristics observed in human patients with depression. However, findings on changes in relative abundances differ across studies, possibly due to the different mouse strains and stress models used (Foster et al., 2013).

Evidence for bottom-up influence

The most compelling evidence for bottom-up influence comes from studies of fecal microbiota transplantations (FMT) from depressed subjects to healthy subjects. Zheng et al. (2016) and Kelly et al. (2016) transferred fecal microbiota from depressed patients and healthy controls to germ-free (GF) mice and microbiota-depleted rats, respectively. Both found that recipients of microbiota from depressed patients displayed depressive behaviors, as measured by open-field test (decreased proportion of center motion indicative of stress), forced swimming test (increased duration of immobility indicative of despair), sucrose preference test (reduced sucrose preference ratio indicative of anhedonia), etc., as well as depression-associated molecular markers, such as an elevated kynurenine/tryptophan ratio (Zheng et al., 2016; Kelly et al., 2016).

Top-bottom and bottom-up signaling likely coexist in a bidirectional relationship between the gut microbiota and the brain. Due to its significance in understanding and treating depression and the impressive research advances made in this area, the next section of this review focuses on bottom-up mechanisms by which gut microbiota dysbiosis could lead to depression.

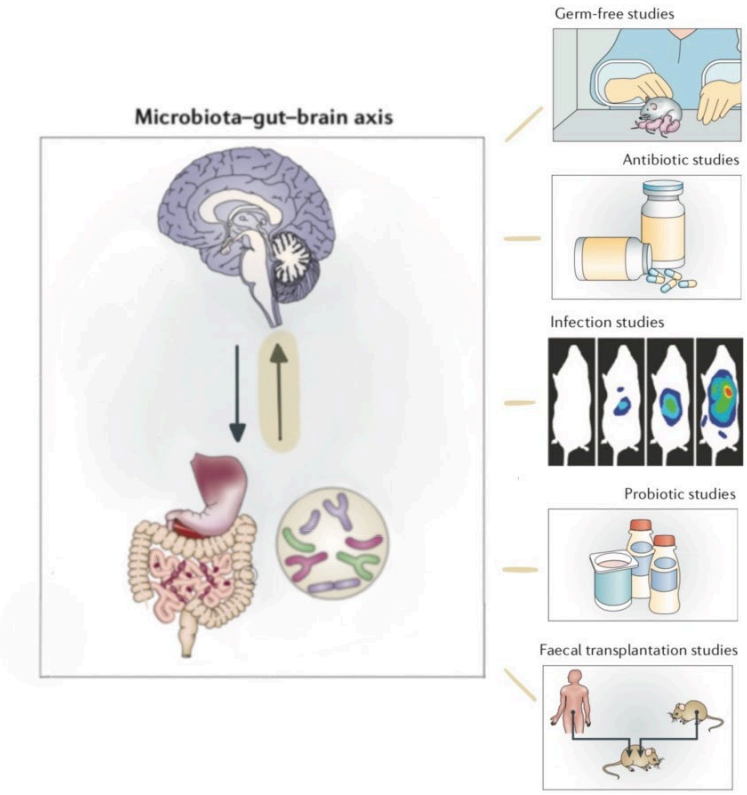

Figure 1. Different Types of Bottom-Up Studies. Adapted from "Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour," by J. F. Cryan and T. G. Dinan, 2012, *Nature Reviews Neuroscience*, 13(10), p. 701-712.

Mechanisms of bottom-up influence

Neural mechanisms

Enteric nervous system (ENS)

The enteric nervous system (ENS) contains about 500 million neurons and is the GI division of the peripheral nervous system (PNS) (Furness, 2012). With ENS sensory neurons synapsing onto ENS motor neurons, the ENS can act independently of the brain and spinal cord and is sometimes called the "second brain" because of this autonomy. However, the ENS can still interact with the central nervous system (CNS) via close contact of ENS sensory neurons with vagal afferent endings. Sensory neurons of the myenteric plexus of the ENS are the closest point of contact for microorganisms and their products in the gut lumen. Interestingly, these neurons are shown to be less excitable in GF mice, as measured by lower resting membrane potentials, slower afterhyperpolarizations, and fewer action potentials generated at 2x threshold (McVey Neufeld et al., 2013). McVey Neufeld et al. (2013) also showed that neuron excitability was normalized after conventionalizing GF adult mice. Additionally, sensory neuron excitability was increased after probiotic treatment with *Lactobacillus rhamnosus* (Kunze et al., 2009). These findings show that the gut microbiota plays a modulatory role in the ENS and suggests one way by which it could directly alter the local gut environment and indirectly affect the CNS.

Vagus nerve

As part of the autonomic nervous system's parasympathetic branch, the vagus nerve has both efferent and afferent roles. In the gut, efferent vagal fibers innervate the muscle and mucosal layers, while vagal afferents receive input from ENS sensory neurons and many endogenous chemicals; in the brain, vagal afferents project into the nucleus tractus solitarius, brainstem, and forebrain structures, including the amygdala, which is key in emotional processing, and the hypothalamus, which is part of the HPA axis (Berthoud and Neuhuber, 2000).

The vagus nerve's importance in bottom-up communication is shown in studies where gut microbiota manipulations only induced CNS and behavioral changes in vagus-intact animals. For instance, Bravo et al. (2011) observed neurochemical and behavioral changes (region-specific alterations of GABA receptor expression in the brain, reduced stress-induced corticosterone, and decreased depressive behavior) following chronic probiotic *Lactobacillus rhamnosus* str. JB1 treatment in only vagus-intact mice and not vagotomized mice. Similarly, in another study, probiotic *B. longum* str. NCC3001 normalized anxiety-like behavior in a chemical low-grade colonic inflammation mice model in a vagus nerve-dependent fashion (Bercik et al., 2011). Studies like these identify the vagus nerve as a major communication mechanism for the gut microbiota and brain.

Neuroimmune mechanisms: cytokines and neuroinflammation

Inflammation has long been believed to play a role in the induction of depression. As evidence for the importance of the gut microbiota in the postnatal development of the immune system, GF mice have an underdeveloped mucosal immune system (Mazmanian et al., 2015), fewer and impaired regulatory T cells for anti-inflammation (Ostman et al., 2006), and less effective immune responses upon inflammation (Inagaki et al., 1996). In regards specifically to neuroinflammation, GF mice's microglia exhibit an immature phenotype with atypical cell proportions and dysregulated gene expression (Thion et al., 2018). Since early life stress has been shown to perturb gut microbiota composition, these findings suggest one pathway by which early life stress could increase an individual's risk for depression throughout life (Thion et al., 2018).

The microbiota is also involved in triggering inflammation. The "leaky gut" hypothesis states that inflammation originates in the gut when pathogen-associated molecular patterns (PAMPs) "leak" through the gut lining (Kelly et al., 2015). According to the theory, psychological stress increases the epithelial barrier's permeability, allowing PAMPs to invade the body, bind to phagocytic cells, and trigger the production of proinflammatory cytokines. This peripheral inflammation can then be relayed to the CNS via afferent vagal fibers or via the direct passage of cytokines through permeable areas of the BBB. Importantly, after the inflammatory signal reaches the brain, hyperactivity of activated microglia could compromise the BBB, allowing the entrance of more cytokines into the brain.

The "leaky gut" theory is supported by studies in which the induction of inflammation led to depression-associated markers and behaviors. In one study, *Trichuris muris* administration led to increased circulating TNF-α and IFN-γ indicative of inflammation, decreased hippocampal BDNF mRNA associated with depression, and increased anxiety and depression-like behaviors in the light-dark test (shorter total time spent in light box and longer latency to re-enter into light box) and step-down test (longer latency to step down from elevated platform) (Bercik et al., 2010). Bercik et al. (2010) also showed that administration of cytokine antagonists etanercept, and to a lesser degree budesonide, normalized depressive behavior, highlighting the importance of the inflammatory response in the induction of the behavioral response. While Bercik et al. (2010) administered bacteria, direct injection of PAMPs or proinflammatory cytokines have also been shown to lead to depressive behavior, decreased hippocampal BDNF mRNA, and increased HPA axis activation (Bluthé et al., 1994; Bluthé et al., 1996). Interestingly, while both vagus-intact and vagotomized mice displayed a depressive behavioral response in the study done by Bercik et al. (2010), only vagus-intact mice demonstrated behavioral response in the studies by Bluthé et al. (1994), demonstrating the diversity of inflammatory signal transmission pathways.

Neuroinflammation and depressive behavior are also observed in previously healthy mice after FMT from mice exposed to chronic unpredictable mild stress (CUMS donors). Post FMT, recipient mice showed anxiety- and

depression-like behavior and significant elevations of IFN-γ and TNF-α in the hippocampus, indicating neuroinflammation (Li et al., 2019). Such FMT studies show that entire dysbiotic microbiota compositions, like single pathogenic strains, could play a causal role in neuroinflammation.

Neuroendocrine mechanisms: the hypothalamic-pituitary-adrenal (HPA) axis

Depression is associated with dysregulation of the HPA axis, the stress response center. The gut microbiota is important for normal programming of the HPA axis during postnatal development. In comparison to conventionally raised mice, GF mice display amplified adrenocorticotrophin and corticosterone levels in response to mild novel arena stressor (Clarke et al., 2013) and more intense restraint stress (Sudo et al., 2004). Surprisingly, while exaggerated HPA axis activation points to increased stress, GF mice are observed to show behaviors associated with decreased stress in the open-field test (increased exploration of center zone), light-dark test (increased exploration of light box), and elevated plus maze test (increased exploration of open arms) (Newfeld et al., 2011). The seemingly conflicting data may be due to contributions from other non-HPA pathways. Future research is needed to make sense of the data. Colonization with bacteria from control mice (Clarke et al., 2013) or with probiotic *Bifidobacterium infantis* (Sudo et al., 2004) at an early age, but not at a later stage, partially normalized HPA axis activity and stress behavior, suggesting that there is a critical time window for HPA programming.

The gut microbiota is not only involved in HPA programming, but it could also activate the HPA axis through two mechanisms, both of which are mentioned earlier (Winter et al., 2018). First, afferent vagal nerves could reach the hypothalamus and thus activate the HPA axis. Second, peripheral cytokines, certain PAMPs, and other bioactive bacterial products could reach the hypothalamus via circulation. Sudo et al. (2004) demonstrated that both neural and neuroimmune mechanisms could be involved in HPA axis activation. In their study, GF mice were orally administered probiotic *Bifidobacterium infantis*, pathogenic *Escherichia coli* (*E. coli*), or a mutated noninfectious strain of *E. coli*. They found that in comparison to those treated with nonpathogenic strain, mice treated with *E. coli* displayed both stronger neuronal activation in the paraventricular nucleus of the hypothalamus, as measured by immediate early gene and neuronal activation marker cFOS, and higher levels of circulating IL-1β and IL-6, Such infection studies provide evidence for bottom-up communication between pathogenic bacterial strains and the HPA axis.

Neurometabolic mechanisms: neuroactive host and microbial metabolic products

Neurotransmitters

Gut microorganisms respond to and produce a large number of neurotransmitters, including serotonin (e.g., *E. coli*, *Enterococcus*, and *Streptococcus*), dopamine (e.g., *Bacillus*), norepinephrine (*Bacillus*, *E. coli*, and *Saccharomyces*), GABA (*Lactobacillus* and *Bifidobacterium*), and acetylcholine (*Lactobacillus*) (Strandwitz, 2018).

In the gut, neurotransmitters play essential roles in regulating gut conditions and microbiota composition. For example, while serotonin is known for controlling mood and sleep in the CNS, it is a major regulator of peristalsis and secretion in the gut. Serotonin is mostly produced by host epithelial enterochromaffin cells, but its synthesis is controlled by small signaling molecules such as short-chain fatty acids (SCFAs) secreted by microorganisms (Strandwitz, 2018). The microbiota also regulates serotonin levels by controlling levels of its precursor, tryptophan. One species shown to elevate plasma levels of tryptophan is Bifidobacterium infantis (Desbonnet et al., 2010), which, interestingly, has been shown by many studies to be an effective probiotic (Desbonnet et al., 2008). Overall, neurotransmitter modulation by the gut microbiota could have a great local impact on host physiology.

However, the effect of gut neurotransmitter modulation on CNS neurotransmission is far from simple and still not fully understood. In GF animals, although serotonin turnover is increased (Diaz Heijtz et al., 2011), the level of serotonin in the hippocampus of male mice is also increased (Clark et al., 2013). Significantly, Bravo et al., (2011) demonstrated that microbiota alterations could directly impact the CNS receptor expression by showing that *in vivo* feeding of *L. rhamnosus* (JB-1) led to alterations of GABA receptor mRNA expression in a brain region-

dependent manner, accompanied by a reduction in depression-related behavior. It is interesting to note that pronounced alterations appeared in depression-associated regions like the prefrontal cortex, amygdala, and hippocampus. The findings are in line with previous studies showing that CNS alterations in GABA receptor expression are implicated in depression (Krishnan and Nestler, 2008).

Short-chain fatty acids (SCFAs)

In addition to neurotransmitters, many other microbial products have local and distal effects on the host. One important group of molecules is the short-chain fatty acids (SCFAs), products of bacterial fermentation of dietary fibers that cannot be broken down by host metabolism. More than 95% of SCFAs consists of acetate, propionate, and butyrate (Macfarlane and Macfarlane, 2012).

At high micromolar to millimolar concentration, SCFAs can activate several G protein-coupled receptors, with the most studied being free fatty acid receptor 2 (FFAR2/GPR43) and 3 (FFAR3/GPR41) (Le Poul et al., 2003). Activation of these receptors on local cells allows SCFAs to modulate the local gut environment, including the secretion of serotonin mentioned above. SCFAs could also indirectly modulate the CNS by activating FFAR3s on the vagus nerve (De Vadder et al., 2013). SCFAs could even directly influence the brain since they are able to pass into the bloodstream, cross the BBB via monocarboxylate transporters, and enter neurons and glia through these same monocarboxylate transporters (Pierre and Pellerin, 2005).

Interestingly, SCFAs have an overall anti-inflammatory effect by stimulating mucus production to enhance the mucosal barrier, stimulating tight junction assembly to repair damage to the epithelial barrier, regulating neutrophil chemotaxis, and suppressing cytokine production (Macfarlane and Macfarlane, 2012). This anti-inflammatory effect could explain the anti-depressive effects observed after peripheral and central SCFAs administration (Van de Wouw et al., 2018). Van de Wouw et al. (2018) treated the experimental group of male mice with acetate, propionate, and butyrate, then subjected control and experiment groups to three weeks of psychosocial stress. They found that the treatment of SCFAs alleviated anhedonia, heightened stress-responsiveness, and increased intestinal permeability induced by stress. This finding of SCFAs counteracting effects of stress is in line with the association made by Valles-Colomer et al. (2019) between higher quality of life scores and higher relative abundance of bacteria that produce butyrate.

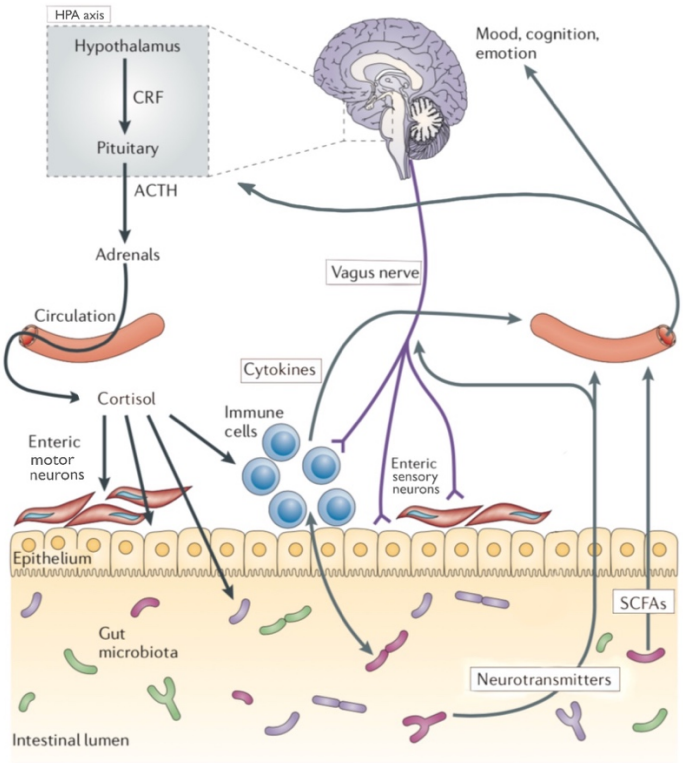

Figure 2. Pathways and Mechanisms of the Microbiota-Gut-Brain Axis. Adapted from "Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour," by J. F. Cryan and T. G. Dinan, 2012, *Nature Reviews Neuroscience*, 13(10), p. 701-712.

A novel treatment: probiotics

Probiotics are consumable microorganisms that promote gut microbiota balance. Through bottom-up pathways, probiotics could, in theory, confer health benefits on the host, including benefits regarding the brain and mental health. Although humans have long consumed fermented products for nutritional and therapeutic purposes, our progressing understanding of microorganisms and the microbiota-gut-brain axis allows us to be more intentional in our search for probiotics (Foster, J.A., & McVey Neufeld, 2013). Currently, probiotic strains shown to confer health benefits mainly come from the genera *Lactobacillus* and *Bifidobacterium*, as well as genera *Saccharomyces*, *Enterococcus*, *Streptococcus*, *Pediococcus*, *Leuconostoc*, *Bacillus*, *Escherichia coli* (Fijan, 2014).

There is considerable evidence from animal and human studies that show that probiotics induce cellular and molecular level changes associated with decreased risk of depression. Probiotics have been shown to increase the integrity of the intestinal epithelial barrier and decrease inflammation following stress. For instance, administering *Lactobacillus farciminis* reduced rat colon hyperpermeability and accompanying neuroinflammation induced by restraint stress (Da Silva et al., 2014) and water avoidance stress ((Da Silva et al., 2014). This benefit of ameliorated hyperpermeability has also been observed in humans. In a study with irritable bowel syndrome patients, small bowel permeability decreased after a four-week treatment with a probiotic-containing fermented milk product (Zeng et al., 2008). Probiotics have also been demonstrated to dampen the HPA axis stress response, measured through hormone levels, in both rats and humans. Gareau et al. (2007) showed that *Lactobacillus rhamnosus* significantly reduced corticosterone levels for rats subject to the EPM or maternal separation stress. Similarly, *Lactobacillus casei* alleviated academic stress-induced cortisol elevation in healthy medical students (Kato-Kataoka et al., 2016).

In addition, compelling evidence from human studies shows that probiotics can reduce depressive emotions. In a study with 20 healthy participants, subjects who have received a multispecies probiotic containing *Bifidobacterium bifidum*, *Bifidobacterium lactis*, *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Lactobacillus casei, Lactobacillus salivarius*, and *Lactococcus lactis* showed significantly reduced overall cognitive reactivity to sad, as measured by the Leiden index of depression sensitivity scale, compared to control (Steenbergen et al., 2015). In another study with healthy women, treatment with a probiotic-containing fermented milk product showed reduced responses to negative affect faces in an emotional recognition task compared to control groups with either nonfermented dairy product or no treatment (Tillisch et al., 2013). Notably, in both studies, brain activity changes were independent of self-reported GI symptoms. The strongest evidence comes from studies in which probiotics reduced depressive symptoms of diagnosed patients. For example, a double-blind, placebo-controlled study of 40 depressed patients saw that patients treated with a probiotic capsule containing *L. acidophilus, L. casei,* and *B. bifidum* for eight weeks had significantly decreased scores on the Beck Depression Inventory compared to control (Akkasheh et al., 2015).

The induction of both physiological and behavioral changes provides further support for hypothesized bottom-up mechanisms and suggest that probiotics could be an effective therapeutic option in the future. However, many questions remain. For instance, we must address the impacts the inherent host microbiota and diet could have on the incoming probiotic itself, as Crook et al. (2019) have observed cumulative genetic mutations in the probiotic candidate *E. coli* Nissle as it passed through the gut. Given the complexity and sensitivity of the microbiota-gut-brain axis, any small perturbation can have profound effects.

While there are many so-called probiotics in the current market, the efficacy of many is questionable. Many do not go through pre-market approvals and are commonly used for a wider range of scenarios than those for which their efficacy has been established (Fijan, 2014). As the probiotic market continues to grow, there is an urgent need for science-based clinical trials to prove safety and efficacy and ongoing scientific research to explore the underlying mechanisms of probiotic action.

Challenges and future directions

Transferring from mice to humans

A major challenge lies in transferring findings from animal studies to humans, as there are substantial differences between mice and human microbiomes. A few approaches can be taken to tackle this challenge. Microbiota transplantation studies from humans to mice made a promising start in showing areas of congruence. Another potential approach lies in using genetic editing technology to create chimeric animal models with knock-in human genes. These chimeric models would help illuminate the effects the microbiota has on human gene expression. While many questions remain in overcoming this challenge, meeting this challenge head-on will be crucial for understanding the role the gut microbiota plays in humans.

Sex differences

An important question to be addressed in future research is how sex comes into play. Depression is almost twice as common in women than in men over the lifespan due to environmental and biological factors. Interestingly, studies with both male and female animals note sexual dimorphic alterations in the microbiota-gut-brain axis. For instance, a decrease in hippocampal BDNF mRNA expression was observed in male GF mice compared to conventionally raised mice (Clark et al., 2013), while an increase was observed in female mice (Neufeld et al., 2011). Clark et al. (2013) also found a significant increase in plasma tryptophan availability in male but not female mice. Jaggar et al. (2020) noted significant "cross-talk" between the gut microbiota and sex hormones. These sex hormone-microbiota

interactions could underly the sexual dimorphic alterations observed along the microbiota-gut-brain axis and provide one biological explanation for the skew in depression prevalence.

Transcriptomic, proteomic, and metabolomic approaches

With Valles-Colomer et al.'s (2019) study as just one example, impressive advances have been made to profile gut microbiota species of healthy and depressed persons using DNA sequencing technology. Moving forward, genetic approaches need to be complemented with transcriptomic, proteomic, and metabolomic approaches, which would aid us in identifying the underlying mechanisms and achieving a better function understanding of the microbiota-gut-brain axis.

Conclusion

The continual global rise in depression and the current gaps in existing therapies call for a better understanding and novel treatments. The comorbidity between depression and GI disorders and the difference in microbiota diversity and composition between healthy and depressed persons suggests a connection between gut microbiota and depression. Studies of psychological stress-induced microbiota alterations indicate the existence of top-down influence; on the other hand, FMT, infection, probiotic, antibiotic, and GF studies show altered levels of depressive behavior and demonstrate bottom-up influence. Together, these studies suggest that the microbiota-gut-brain axis is bidirectional. This review examined putative bottom-up neural, neuroimmune, neuroendocrine, and neurometabolic mechanisms. Together, these mechanisms form our current understanding of how the gut microbiota is involved in the development and maintenance of depression.

In the past, treatments generally exclusively targeted the brain, as brain dysfunction was believed to be the sole causal and maintaining factor of depression's psychological and physiological symptoms. Now, with substantial evidence of bottom-up influence, probiotics offer a new solution. To confirm the safety and effectiveness of probiotics, there is a need for more large-scale, double-blind, placebo-controlled trials. Furthermore, we must address differences between animals and humans and between the sexes as we move forward in treatment development. We also need to supplement genetic approaches with transcriptomic, proteomic, and metabolic approaches to discern causations and not just correlations. Although many challenges and questions lie ahead, the microbiota-gut-brain axis continues to be an intriguing area of great scientific and clinical potential.

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