Gutted! Unraveling the Role of the Microbiome in Major Depressive Disorder

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Abstract: Microorganisms can be found in virtually any environment. In humans, the largest collection of microorganisms is found in the gut ecosystem. The adult gut microbiome consists of more genes than its human host and typically spans more than 60 genera from across the taxonomic tree. In addition, the gut contains the largest number of neurons in the body, after the brain. In recent years, it has become clear that the gut microbiome is in communication with the brain, through the gut–brain axis. A growing body of literature shows that the gut microbiome plays a shaping role in a variety of psychiatric disorders, including major depressive disorder (MDD). In this review, the interplay between the microbiome and MDD is discussed in three facets. First, we discuss factors that affect the onset/development of MDD that also greatly impinge on the composition of the gut microbiota—especially diet and stressful life events. We then examine the interplay between the microbiota and MDD. We examine evidence suggesting that the microbiota is altered in MDD, and we discuss why the microbiota should be considered during MDD treatment. Finally, we look toward the future and examine how the microbiota might become a therapeutic target for MDD. This review is intended to introduce those familiar with the neurological and psychiatric aspects of MDD to the microbiome and its potential role in the disorder. Although research is in its very early days, with much yet to be the understood, the microbiome is offering new avenues for developing potentially novel strategies for managing MDD.

Keywords: antidepressant, gut–brain axis, major depressive disorder, microbiome, psychobiotic

With the exception of a few notable instances, the fields of microbiology and psychiatry have not gone hand in hand. It is worth noting, however, that the syphilis-causing microbe *Treponema pallidum* was responsible for filling large parts of Victorian mental asylums. Also of note, the 1908 Nobel prize winner in Physiology or Medicine, Élie Metchnikoff, adhered to the idea that bacteria in fermented milk were beneficial against *autointoxication*, a term historically used to describe a wide range of symptoms such as fatigue and melancholia.[1] Building on Metchnikoff's ideas the psychiatrist Henry Cotton, medical director of New Jersey State Hospital at Trenton, was convinced that the bacteria on the teeth of his patients were the source of their psychiatric conditions. Infamously, he would have their teeth pulled as part of their treatment.[2] More recently, as the neurocognitive effects of HIV infection became evident, psychiatry once more saw a connection with microbiology. For the most part, however, in our heavily specialized medical and scientific training, the practitioners of these disciplines have rarely crossed paths.

This situation changed in tandem with the emergence of new technologies such as next-generation sequencing and the increase in processing power required to analyze large amounts of data. The microbes found in and on the human body have been mapped through projects like the Human Microbiome Project,[3] LifeLines-DEEP,[4] Flemish Gut,[5] TwinsUK,[6] MetaHIT,[7] and ELDERMET.[8] In humans, the greatest abundance of microbes is found in the gut. According to current estimates, the gut microbiome consists of around 3×10^{13} microbes from more than 60 genera and weighs approximately 200 grams.[9] Increasing efforts and research studies are currently investigating the microbiome–gut–brain axis (MGBA).[10–16] While the precise mechanisms involved in microbiome-to-brain dialogue are still an open question in the field, routes of communication include the immune system,[13,17] synthesis and metabolism of metabolites and neurotransmitters,[18] and activation of the vagus nerve.[19,20]

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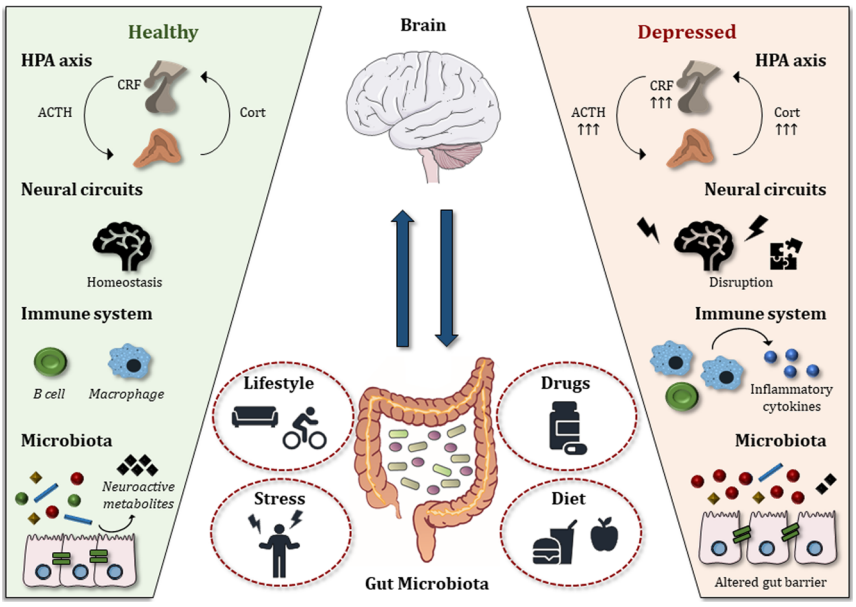

Figure 1. Impact of the gut microbiota on the gut–brain axis in health and depression. *Left panel:* A stable and balanced gut microbiota is essential for normal gut–brain axis signaling. *Right panel:* In major depressive disorder, alterations in the gut microbiota negatively affect the gut–brain axis at several levels. The HPA axis becomes hyperactivated; neural circuits and neurotransmitter levels are disrupted; the immune system produces excessive proinflammatory cytokines; and the intestinal barrier is disrupted. *Middle panel:* Factors that influence the microbiome and, in turn, the onset/development of depression include lifestyle, medications, stress, and dietary habits. ACTH, adrenocorticotropic hormone; Cort, corticosterone (rodents) or cortisol (humans); CRF, corticotropin-releasing factor; HPA axis, hypothalamus–pituitary–adrenal axis.

Over the years, it has become clear, especially from animal studies, that the gut microbiome can influence a broad range of factors, including the development of type 2 diabetes,[21] Alzheimer's disease,[22] and major depressive disorder (MDD).[18,23,24] Intriguingly, the gut microbiome has been linked to several physiological functions relevant to depression; these functions are described in Figure 1. From a clinical point of view, episodes of depression are associated with a dysregulated hypothalamic–pituitary–adrenal (HPA) axis,[25] and conversely, improved depressive symptoms are associated with stabilization of the HPA axis.[26,27] The gut microbiota plays a role in the programming and reactivity of the HPA axis. A direct link between the microbiota and HPA function was shown in a study from Sudo and colleagues,[28] who showed exaggerated corticosterone (CORT) and adrenocorticotrophin (ACTH) levels in germ-free mice in response to restraint stress when compared to conventionally housed, specific pathogen–free (SPF) mice. In rats, administration of probiotics (*Lactobacillus* sp.) during the early stress period was able to normalize basal CORT levels, which are increased following maternal separation.[29] In addition to affecting HPA-axis function, the microbiota could influence central nervous system function directly by means of neuronal activation of stress circuits. Studies involving the oral administration of the pathogenic bacteria *Citrobacter rodentium* and *Campylobacter jejuni* show that gut microbes can mediate the stress response by activating vagal pathways.[30,31] Alterations in the microbiome can also lead to hyperactivation of the immune system, with production of inflammatory cytokines typically observed in depression.[32] Finally, depression has been associated with impairment of the microbiome's ability to produce neuroactive metabolites and with disrupted intestinal barrier function (see Figure 1).[33]

Animal research has played an integral part in the development of the microbiome field. Numerous important findings with high potential translational value have been made in rodent models (see Text Box 1).[48,49] Because of the regular housing and feeding conditions for rodents, for example, the inter-individual variation in the microbiome is much lower than in humans, facilitating the detection of changes in the microbiome even in lower group sizes.[50] Furthermore, experimental manipulations such as germ-free environments can be applied in animals but are not feasible in humans. The use of germ-free models has been crucial in linking the microbiome to many key brain processes and behaviors,[51–53] despite the limitation of abnormal neurodevelopment present in germ-free animals.

In this review, we will first discuss factors that affect the onset/development of MDD that also affect the composition of the gut microbiome. Then we will examine the interplay between the microbiome and MDD. In particular, we will examine evidence suggesting that the microbiome is altered in MDD, and discuss why the microbiome should be considered during MDD treatment. Finally, we look toward the future and examine how the microbiome might become a therapeutic target in MDD, potentially affecting future clinical practice.

SCULPTING THE GUT MICROBIOME

The gut microbiome is a highly dynamic system, undergoing constant change over time. The degree and manner of change is thought to be determined by a vast combination of factors,

Text Box 1
What Have We Learned from Animal Research?

Animal research offers some unique advantages compared to human studies

It is possible to maintain a highly *controlled environment*, spanning from diet to housing conditions (temperature, humidity, air flow) and genetics—all factors that reduce the inter-individual variation of microbiome composition.

Moreover, compared to a human life expectancy of approximately 70 years, a mouse has a *lifespan* of 2–3 years, enabling researchers to study the entire life cycle.

Particularly important in the microbiome field, *germ-free rodents* are raised in germ-free isolators and represent a crucial tool in determining whether the microbiome plays a causal role in a given host function. In addition to the germ-free model, the effects of *perturbation or depletion of the microbiome* with unbalanced diets or antibiotics in animals allow for experimental setups that can provide important mechanistic insights.

Through animal research we are starting to delineate the routes of communication involved in the gut–brain signaling, including the vagus nerve, the production of microbial metabolites, and the involvement of the immune system.

Finally, the *access to the entire gastrointestinal tract* in animal models has been instrumental in studying mechanisms of digestive pathophysiology and in characterizing the microbiome along different sections of the gastrointestinal tract.

Nonetheless, there are limitations and challenges

One limitation of animal research relates to the *different anatomical structures* in rodents versus humans, including brain and gastrointestinal anatomy, which are particularly relevant in gut–brain research. Moreover, rodent brains, in addition to anatomical differences, undergo more postnatal development than humans.

Moreover, the *highly controlled conditions* previously mentioned as an advantage can also be construed as a disadvantage in that such regulated environments can be poorly translatable into human conditions.

Particularly when studying a heterogeneous disorder such as major depressive disorder, interpreting changes in *mood* and *behavior* in animals is challenging. Robust batteries of behavioral tests and paradigms have been set up to minimize variance in this regard,[34] but the specifics of these paradigms are outside of the scope of this review. Apart from changes in behavior, physiological readouts, such as blood levels of corticosterone, are often used.

Changes in animal studies linked to depression

It is worth mentioning the observed neurobiological changes that were reported in animals with an altered microbiome. For more than four decades, it has been known that stress can change the microbiome in mice, specifically decreasing *Lactobacillus*.[35] Since then, more preclinical studies have reported many similar changes in the composition of the microbiome.[36,37] Recently, chronic intermittent hypoxia was found to affect not only the physiology, including the autonomic nervous system, but also the microbiome in rodents.[38,39] The gut microbiome is also known to modulate the physiology and behavior of the animal. For instance, mice without a microbiome show a heightened myelination in the prefrontal cortex,[40] altered RNA-splicing in the amygdala in response to social interaction,[41] and an altered immune system.[42] Neurodevelopmental differences—including neurogenesis, a process that is dysregulated in depression—have been observed in mice with a humanized microbiome, where specific microbes seemed to be necessary for normal neurodevelopment.[43] Interventions targeting the microbiome have been found to protect against physiological and neuroimmune changes due to aging[44] and against the behavioral and cognitive effects of stress.[45–47]

ranging from stage of life to exercise. In the context of MDD and the microbiome, two such factors stand out especially—namely, diet and stress. Other factors, such as exercise and aging, that have been shown to affect microbiota composition will also be examined in the context of MDD.

Diet Alters the Gut Microbiome: Relevance for MDD

Though the precise mechanism is unknown at this point in time, diet is known to markedly shape the composition of the gut microbiome.[54–56] Furthermore, quality of diet is known to influence the severity of MDD. For instance, intake of the biologically related compounds folate and vitamin B12 were inversely correlated with severity of depression in a large cross-sectional study.[57] In a meta-analysis covering 16 studies, 15 of which were in non–clinically depressed populations, dietary intervention led to a significant improvement in mood but not in anxiety.[58] Notably, the SMILES trial, a 12-week dietary intervention using a modified Mediterranean diet to target MDD in adults, has shown convincingly that diet can be effective in alleviating MDD symptoms.[59,60] Recently, numerous researchers have called for further research to understand the interplay between diet, the microbiome, and MDD.[61–64] Along the same lines, one of the outcomes of the European Union's recent MyNewGut project—an initiative focused on understanding and promoting health by targeting the gut microbiome—was a dietary recommendation intended to improve MDD symptoms by targeting the gut microbiome through the increased consumption of fiber and fish.[64] These food groups, which are an important part of the Mediteranean diet, are associated with an increased abundance of bacteria with anti-inflammatory properties. That well-studied diet is known not only to affect the gut microbiome by increasing the abundance of microbes that produce short-chain fatty acids (SCFAs), but also to shorten episodes of depression.[65,66]

Stress Alters the Gut Microbiome: Relevance for MDD

Similar to the link between diet and the microbiome, the link between stress and MDD is known and well described.[67,68] Recently, it has become clear that stress influences the microbiome. Often, the reported changes are on the level of alpha diversity (defined as diversity within the ecosystem) or beta diversity (defined as difference between ecosystems, often in terms of composition), rather than involving the specific microbes being affected.[69–73] While human studies are

much needed in this area, the available evidence suggests that the human microbiome is similarly involved in maternal and early-life stress, with high-stress microbiomes featuring an increased diversity of *Clostridium* genera, which is generally associated with inflammation and disease.[74] Many studies exist in animal models showing the impact of stress on the microbiome—including rodents,[29,36,37,46,75,76] pigs,[77] and primates.[78,79] Generally, these studies report decreased alpha diversity in the stress group, reduced levels of *Lactobacillus*, a health-promoting genus that is abundant in early life, and reduced SCFA production. It is difficult, however, to identify specific trends between these studies. For one, the differing methods and databases used in the studies make it challenging to compare the specific microbes that have changed. Furthermore, functional analysis based on 16S sequencing (the most common method of sequencing) is limited by the quality of the tools and databases used, making it difficult to formulate mechanisms explaining the interactions between observed changes in the microbiome and the phenotype.

The gut microbiome also influences the response to stress. Relatively early in the microbiome field, Sudo and colleagues[28] discovered that germ-free mice show an exaggerated stress response as measured by CORT and ACTH. In the same study, this exaggerated response was found to be normalized after introducing a probiotic but worsened after introducing a pathogenic strain. More recently, the field has moved toward the notion that the microbiome plays an important role in resilience to stress, especially during early development. The adverse effects of stress have been found, in rodents, to be ameliorated by fiber-rich or milk-associated oligosaccharides, which are preferentially metabolized by certain gut microbes but are difficult to metabolize for the host.[45,71–73,80,81] This finding is itself supported by the finding that there is selectivity in favor of strains found in the mother's microbiome in the microbiome of breast-fed infants.[82]

It must be noted here that several intervention studies targeting the microbiome and reporting improvements in MDD symptoms and mood have been published. The nature of those interventions will be discussed later in this review.

Other Factors That Influence the Gut Microbiome: Relevance for MDD

In addition to dietary habits and stress—which, as discussed above, have been linked to changes in mood and behavior through alterations of the gut microbiome—other factors known to affect the gut microbiota could indirectly influence the onset or development of MDD. These factors include circadian rhythm, exercise, and aging.

It been shown not only that the microbiota modulates circadian rhythm[83,84] but that circadian disruptions can affect the intestinal microbiota.[85] Dysregulation of the peripheral or central clocks can lead to microbiome changes, as one recent study has demonstrated utilizing transgenic mice containing deletions of circadian clock genes.[85] These mice showed changes to the microbiome and a dampening or abolishment of microbiota compositional oscillations.[84,86,87] In one study, the dysregulation of the microbiome was rescued by specifically timed feeding, either exclusively during light or dark.[84] MDD can be associated with a dysregulation of the circadian clock;[88,89] more work is needed to understand the relationship between the microbiome, circadian rhythms, and brain health, including MDD.

A growing body of literature examines the effect of exercise on the gut microbiota and the gut–brain axis. In particular, moderate levels of exercise have been found to have positive effects on stress, immunity, and energy homeostasis.[90,91] Moreover, a case-control mouse study reports free access to exercise was significantly associated with an increase in the relative abundance of the genera *Bifidobacterium* and *Lactobacillus* and the species *Blautia coccoides* and *Eubacterium rectale*, as well as with an increase in microbiota diversity,[92] having potential positive effects on brain and behavior. Several studies in humans and mice have reported changes in the microbiome subsequent to exercise;[93–95] however, the magnitude and nature of microbiome-mediated positive effects of exercise on brain function and MDD remain to be investigated.[96]

During aging, the stability of the microbiome deteriorates.[97] It is worth noting, however, that we still lack an exact characterization of the aging gut microbiome. In humans, aging and age-related impairments such as frailty have been linked to a decrease in microbiota diversity.[8,98] Conversely, aged (24-month-old) mice exhibit increased microbial diversity than their younger counterparts.[99] Intriguingly, the aged gut microbiota composition can also contribute to *inflammaging*,[100] a term used to describe the heightened proinflammatory state and concordant decrease in adaptive immunity observed at older age.[101] Given the high prevalence of MDD in aging,[102,103] it is tempting to speculate that the microbiome might be at the intersection of aging and mood; this hypothesis needs to be further verified, however, in targeted and large population-based studies.[104]

CONSIDERING THE GUT MICROBIOME AS A PART OF THE DEPRESSED PATIENT

A wealth of studies, from different perspectives and experimental approaches, link the gut microbiome to MDD.[18,61,105] Not only is it now apparent that the gut microbiome is altered in MDD, but some studies have also shown that transferring the microbiome of a depressed individual into a healthy rodent can induce depressive-like behavior in the recipient. Such data suggest a causal role for the microbiota in depression pathophysiology (Figure 1). In this section we will discuss the evidence supporting the role of the microbiome in MDD. A summary of the studies investigating the microbiome composition in depressed patients can be found in Table 1.

Gut Microbiome Is Altered in Depression

In recent years, more and more studies have reported that MDD patients have an altered gut microbiome composition

Table 1

Human Studies Reporting Altered Microbial Composition in Depression

Cohort	Measures	Changes in the depressed cohort	Limitations	Study
MDD: n = 37, age[a] = 42.9 Controls: n = 18, age = 46.1	16S rRNA (Illumina)	↑ Bacteroidales at order level ↓ Lachnospiraceae at family level ↑*Oscillibacter* genus & *Alistipes* genus No differences in alpha diversity or richness	Medication use & diet could be confounding factors	Naseribafrouei et al. (2014)[106]
MDD: n = 46, age = 25.3 Controls: n = 18, age = 26.8	454 Life Sciences Genome Sequencer Serum cytokines Serum BDNF	↓ *Ruminococcus, Prevotella, Faecalibacterium, Dialister, Bacteroides* (A-MDD) ↑ *Bacteroides, Roseburia, Phascolarcto bacterium, Parabacteroides, Alistipes* (R-MDD) Shannon diversity significantly higher in A-MDD vs. HC No significant differences in TNF-a, IL-1b, & IL-6 levels BDNF significantly lower in A-MDD & R-MDD vs. HC	Intake of atypical antipsychotics may have affected the results	Jiang et al. (2015)[107]
MDD: n = 58, 39 drug-naive Controls: n = 63	16S rRNA	29 OTUs overrepresented in MDD subjects No significant differences in alpha diversity Beta diversity (PCoA of unweighted UniFrac): 19% difference between MDD & HC	Lack of detailed dietary information Ethnic biases in microbial phenotypes cannot be ruled out	Zheng et al. (2016)[108]
MDD: n = 34, age = 48 Controls: n = 33, age = 48	16S rRNA Fecal SCFAs Plasma inflammatory markers Kynurenine/tryptophan LBP	Genus level: ↑ *Eggerthella, Holdemania, Gelria, Turicibacter, Paraprevotella, Anaerofilum* ↓ *Prevotella, Dialister* ↓ Chao1 richness, total observed species, phylogenetic diversity ↑ levels of IL-6, IL-8, TNF-a, CRP & kynurenine/tryptophan ratio No significant differences in LBP No significant differences in fecal SCFAs (acetate, propionate, iso-butyrate, or butyrate)	The majority of patients were on ADTs	Kelly et al. (2016)[109]
Belgian Flemish Gut Flora Project population cohort: n = 1054[b]	16S rRNA Shotgun Annotation of 56 gut–brain modules Link between microbiota neuroactive capacity & QoL/ depression	QoL associated with the relative abundances of specific taxa, *Coprococcus* & *Dialister* ↓ in depression, & confirmation that the use of medications is a confounder Lower QoL in the *Bacteroides* enterotype 2 compared to *Prevotella, Bacteroides* enterotype 1 & Ruminococcaceae	QoL as a metric	Valles-Colomer et al. (2019)[33]

[a] Refers to average age.

[b] Results validated both in the LifeLines DEEP cohort and self-reported depression metadata (n = 1063).

A-MDD, active MDD; ADTs, antidepressants; BDNF, brain-derived neurotrophic factor; CRP, C-reactive protein; HC, health control; IL, interleukin; LBP, lipopolysaccharide binding protein; MDD, major depressive disorder; OTU, operational taxonomic unit; PCoA, principal coordinates analysis; QoL, quality of life; R-MDD, MDD/responded to treatment; rRNA, ribosomal RNA; SCFAs, short-chain fatty acids; TNF, tumor necrosis factor.

when compared to healthy controls, although the specific nature of the alterations can differ from study to study.[106–110] The alpha diversity of MDD patients tends to be lower overall, with a higher abundance of bacterial phyla generally associated with inflammation, like Bacteroidetes, and a reduction in phyla associated with a decrease in inflammation, like Firmicutes. The genera *Lactobacillus* and *Bifidobacterium* are sometimes reported as having a positive influence on mood. The variation in microbiome analysis methods, ranging from the use of different versions of reference databases or even entirely separate databases to differences in sampling methods, can account for discrepancies in findings between studies.[111]

An enterotype is, by definition, a classification of living organisms based on their bacteriological ecosystems in the gut microbiome. As part of the Flemish Gut Flora Project, a recently published study shows what might be the strongest available evidence for the existence of the gut–brain axis.[33] In that study, the authors found that in their cohort of more than 1000 participants, depressed individuals were more likely than their healthy counterparts to fall into a certain enterotype. The enterotype in question was defined by having a lower bacterial load and a relatively low abundance of the bacterial genus *Faecalibacterium*. Furthermore, participants who reported a lower quality of life were also more likely to belong to this enterotype,[33] which has been linked to inflammation in earlier research by the same group.[112] In addition to their finding concerning general composition, the Belgian group identified several features of the microbiome, such as the abundance of butyrate-producing bacteria in the gut, that were associated with a higher quality-of-life score.[33] In another important contribution to the field, this same article formulated what will likely become an invaluable tool for future research and understanding. The article identified 56 *gut–brain modules*, which are the pathways of metabolic function in the gut—for example, GABA metabolism—that potentially influence the brain. In looking at these modules, the authors found several to be altered in a subcohort of clinically depressed patients. A complication that the authors confronted in interpreting their data was that *enterotype* is a contested term in the microbiome field, and its definition and usefulness are subject to debate.[113,114] To address these issues, the Flemish study utilized a Bayesian multinomial-mixture model in assessing enterotype classifications—as opposed to the classical, contested definition of enterotypes within the microbiome, which relies on microbiome profiles falling into distinct clusters.

Gut Microbiome Can Transfer Depression

It must be noted that, even if the depressed microbiome is compositionally distinct from that of the general population, that in itself is not enough to conclude a causative role for the microbiome in the development of MDD. Two animal studies published in 2016 independently show in comparable, but distinct, manners that both mice and rats that received a fecal microbiome transplantation (FMT) from depressed humans displayed a heightened state of inflammation and increased anhedonia-like (as measured by the sucrose preference test) and anxiety-like behavior (as measured by the open field test and elevated plus maze test) compared to those who received FMT from healthy volunteers.[108,109] The forced swim test led to conflicting outcomes; the study in mice found an increased immobility time, associated with depressive-like behavior, whereas the rat study found no difference. Potential confounders of these studies include the fact that depressed donors were prescribed medication and that a low number of donors was used for a higher amount of recipients. While only shown in rodents, the behavioral outputs of these studies and their respective translational implications support the notion that certain compositions of the microbiome can affect behavior and mood. A general trend among the microbiomes of depressed patients and animals with increased depressive-like behavior is a drop in alpha diversity, an increase in relative abundance of microbes associated with a proinflammatory state, and a heightened state of inflammation in the host.

CONSIDERING THE GUT MICROBIOME DURING MDD TREATMENT

As previously discussed, alterations of the microbiome can affect the onset/development of MDD at different levels (see Figure 1). Furthermore, several drugs, including psychotropic drugs, can influence the composition of the intestinal microbiota.[115,116] Equally hard to confirm as to rule out, it has been speculated upon that the microbiome-targeted effects of psychotropic drugs might play a role in the mechanism of action or in the side effects of these medications. A recent study reports that the gut microbe *Ruminococcus flavefaciens* metabolizes fluoxetine and inhibits its mood-affecting effect.[117]

Psychotropic Drugs Influence the Gut Microbiome

In a large in vitro study, the growth of 40 microbes commonly found in the human gut was affected by supplementation of several commercial drugs, including psychotropic drugs.[118] While this study used bacterial monocultures and therefore did not account for the vast complexity of the microbiome, it suggests that the composition of the microbiome will be affected by the intake of these common drugs. Interestingly, nearly all subclasses of the antipsychotics with different chemical structure targeted a more similar pattern of species than that presumed from their chemical structure, suggesting that the antimicrobial action may not only express as a side effect of antipsychotics but also be part of their mode of action.[118] A hypothesized mechanism of action, for instance, would be that subpopulations of psychiatric patients (bearing microbiomes that are different from those of healthy individuals) might have beneficial treatment outcomes due to microbiome-targeting effects of such medications. In a recent study from our laboratory, chronic administration of psychotropic drugs

Table 2

Studies of Microbial Modulation of Mood by Probiotics

Cohort	Probiotic	Timespan	Effect	Study[a]
Men	*B. longum* 1714	4 weeks	Decreased cortisol response (vs. control, r = 0.45) Improved reported stress (STAI: effect vs. pre-stress, r = 0.12) after SECPT Decreased Cz theta power following probiotic administration (r = 0.57)	Allen et al. (2016)[132]
	L. rhamnosus (JB-1)™	4 weeks	Does not significantly impact HPA axis, stress, & cognition	Kelly et al. (2017)[131]
	B. longum 1714	4 weeks	Change in neural activity correlated with increased vitality (rho = 0.33)	Wang et al. (2019)[139]
Men & women	*L. casei* Shirota (Yakult)	3 weeks	Improvement in depression in POMS scale for people at the lowest end of the mood scale (F = 4.19), but not overall	Benton et al. (2007)[140]
	L. helveticus R0052 & *B. longum R0175*	30 days	Improved in HAD-A (z = 2.19) & HAD-D (z = 1.92) scores, indicating decrease in anxiety/depression Improved quality of life (z = 1.98) No changes in perceived stress (PSS)	Messaoudi et al. (2011)[141] Messaoudi et al. (2011)[142]
	Ecologic® Barrier: *B. bifidum, B. lactis, L. acidophilus, L. brevis, L. casei, L. salivarius, L. lactis*	4 weeks	Decreased aggression (ηp^2 = .115) & rumination (ηp^2 = .242) in response to depressive thoughts (LEIDS-r test)	Steenbergen et al. (2015)[143]
	Probiotic yogurt (group 1): *L. acidophilus* LA5, *B. lactis* BB12 Capsule (group 2): *L. casei, L. acidophilus, L. rhamnosus, L. bulgaricus, B. breve, B. longum, S. thermophilus,* FOS	6 weeks	Improvement in mental health in GHQ scale for the yoghurt (18.0±1.5 vs. 13.5±1.9) & capsules (16.9±1.8 vs. 9.8±1.9) but not the control yoghurt treatment	Mohammadi et al. (2016)[144]
	Ecologic® 825: *L. casei, L. paracasei, B. lactis, L. salivarius, L. plantarum, B. bifidum, L. acidophilus, L. lactis*	4 weeks	Improvements in depression as measured by LEIDS hopelessness (F = 6.3) & PANAS (F = 7.45) scores	Bagga et al. (2018)[145]
Chronic fatigue syndrome: men & women	*L. casei* strain Shirota	8 weeks	Improved anxiety symptoms (F = 8.415)	Rao et al. (2009)[146]
Irritable bowel syndrome: men & women	*L. paracasei, ssp. paracasei* F19, *L. acidophilus* La5, *B. lactis* Bb12	8 weeks	No significant psychological changes	Simrén et al. (2010)[147]
	B. longum NCC3001	6 weeks	Improved in HAD-D (RR = 1.98) scores indicating decrease in depression No changes in anxiety (HAD-A)	Pinto-Sanchez (2017)[148]
Aging (>60 years) men & women	*L. reuteri*	12 weeks	No persisting effects on depression, anxiety, or perceived stress	Östlund-Lagerström et al. (2016)[149]
MDD: men & women	Capsule: *L. acidophilus, L. casei & B. bifidum*	8 weeks	Improved depression by BDI score (−5.7 ± 6.4 vs. −1.5 ± 4.8 in placebo)	Akkasheh et al. (2016)[150]

Table 2
Continued

Cohort	Probiotic	Timespan	Effect	Study[a]
	L. helveticus R0052 & *B. longum* R0175 (CNCM strain I-3470)	8 weeks	Improved depression by BDI score ($\eta p^2 = 0.09$) Lowered kynurenine/tryptophan ratio ($\eta p^2 = 0.059$)	Kazemi et al. (2019)[151]
Pregnant women	*L. casei* strain Shirota (YIT 9029 (formulated in 100 ml of milk)	8 weeks	No change in psychological parameters (anxiety, depression scales)	Kato-Kataoka et al. (2016)[152]
	L. rhamnosus HN001	<6 months	Improved EPDS postpartum depression (effect size = −1.2) & anxiety (effect size = −1.1) scores	Slykerman et al. (2017)[153]
Low mood: men & women	*L. helveticus, B. longum*	8 weeks	No effect	Romijn et al. (2017)[154]
Multiple sclerosis: men & women	Capsule: *L. acidophilus, L. casei, B. bifidum, L. fermentum*	12 weeks	Improved depression by BDI score (−5.6 ± 4.9 vs. −1.1 ± 3.4)	Kouchaki et al. (2017)[155]
Obesity: men & women	*L. rhamnosus* CGMCC1.3724	24 weeks	Improved body esteem (3.6 ± 1.9) & depression scores in women (BDI, −1.5) compared to placebo	Sanchez et al. (2017)[156]
Type 2 diabetes	Capsule: *L. acidophilus, L. casei, B. Bifidum, L. fermentum,* vitamin D3	12 weeks	BDI (−2.8 ± 3.8 vs. −0.9 ± 2.1) BAI (−2.1 ± 2.3 vs. −0.8 ± 1.4) & GHQ (−3.9 ± 4.1 vs. −1.1 ± 3.4) scores improved compared to placebo	Raygan et al. (2018)[157]

[a] Studies targeting the microbiome and presenting mood-related readouts as an output were chosen.

BAI, Beck Anxiety Inventory; BDI, Beck Depression Inventory; EPDS, Edinburgh Postnatal Depression Scale; FOS, fructo-oligosaccharides; GHQ, General Health Questionnaire; HAD-A, Hospital Anxiety and Depression Scale–Anxiety; HAD-D, Hospital Anxiety and Depression Scale–Depression; LEIDS, Leiden Index of Depression Sensitivity; PANAS, Positive and Negative Affect Schedule; POMS, Profile of Mood States; PSS, Perceived Stress Scale; RR, risk ratio; SECPT, Socially Evaluated Cold-Pressor Test.

was shown to influence the microbiome composition and diversity in rats.[119] The authors point out that some drugs, including the selective serotonin reuptake inhibitor (SSRI) fluoxetine, specifically shape the microbiome in a distinct manner. What specific effects this compositional shift might have on the host mental well-being is still unknown. Many other studies have found antimicrobial activity in vitro with common SSRIs, reaffirming the idea that psychotropic drugs

Table 3
Studies of Microbial Modulation of Mood by Prebiotics

Cohort	Prebiotic	Timespan	Effect	Study[a]
Irritable bowel syndrome: men & women	Short-chain FOS	4 weeks	Improved HAD-A (anxiety) scores (effect size not given)	Azpiroz et al. (2017)[158]
Type 2 diabetes: women	Resistant dextrin (Nutriose®06)	8 weeks	Improved depression, anxiety, and stress (DASS, −38.4%) Lowered cortisol (−20.9%) & kynurenine/tryptophan ratio (29.1%) Altered peripheral immune markers	Farhangi et al. (2018)[159]

[a] Studies targeting the microbiome and presenting mood-related readouts as an output were chosen.

DASS, Depression Anxiety Stress Scales; FOS, fructo-oligosaccharides; HAD-A, Hospital Anxiety and Depression Scale–Anxiety; HAD-D, Hospital Anxiety and Depression Scale–Depression.

shape the gut microbiome.[120,121] In perhaps a whim of history, isoniazid and iproniazid, two of the first antidepressants ever developed, were originally classified and marketed as antibiotics.[122]

The Gut Microbiome Influences Drug Metabolism

The whole new field of *pharmacomicrobiomics* focuses on the role played by the gut microbiome in xenobiotic (typically synthetic molecules that are foreign to a biological system) metabolism.[123,124] Several drug classes, including cardiac glycosides, chemotherapeutics, and drugs used for immunotheraphy, are known to be metabolized by the gut microbiota,[125–127] but no evidence is currently available on how microbial perturbations influence the metabolism of psychotropic drugs. More research is now warranted, especially considering that several psychotropics have been shown to alter the gut microbiota composition both in vitro and in vivo.

LOOKING TOWARD THE FUTURE: POSSIBLE APPROACHES

After acknowledging the concept that the microbiome and the brain are in a constant bidirectional relationship with each other, it is logical to consider the microbiome as a therapeutic target for MDD. MDD is a complex disorder, and many patients fail to respond to antidepressant treatment, while others respond but do not fully remit. In the Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study, more than 40% of patients with MDD did not achieve remission, even after two optimally delivered trials of antidepressant medications.[128] Moreover, adjunctive treatments are commonly employed to improve therapeutic outcomes. Any alternative approach should be considered for its therapeutic value, either in tandem with traditional antidepressant treatment or as a stand-alone treatment. Therefore, the microbiome represents a potential target in treating of MDD. It is easily modifiable, and its manipulations do not cause adverse effects when using safe microbes.

Supplementing the Gut Microbiome to Improve Depression

Apart from dietary interventions, treatments meant to alter the composition of the microbiome can be split into two categories: *prebiotics* and *probiotics*. Prebiotics are defined as foods that are not digestible by humans (such as fibers) and that have a beneficial effect on the host's microbiome.[129] Probiotics are live microbes that have a beneficial effect on the host (provided, of course, that they are ingested in adequate quantities).[130–132] When both pre- and probiotics are coadministered, which happens increasingly, the term *synbiotic* is used. Microbiome interventions specifically designed to improve mental health are termed *psychobiotics*.[133–135] It should be noted that psychobiotics do not necessarily have to target a clinical population but may also be intended for general use. Indeed, numerous studies are available reporting the beneficial effects of specific probiotics on mood.[136–138] Human studies that involve probiotics or prebiotics and that have MDD symptoms or mood as an outcome measure are presented in Tables 2 and 3, respectively, with the reported effectiveness or lack thereof also reported. It must be stressed that, like any therapeutic, the type and quantity matter. Equally important, the beneficial effects observed in preclinical models need to be translated and confirmed in a human setting. In a relatively new field like the microbiome, a critical attitude and well-designed trials are not misplaced.

Fecal Microbiome Transplantation as a Therapy for MDD

Earlier in this review, studies were discussed where the microbiome was found to be a potential carrier of depressive mood.[108,109] When looking at potential therapies, a fecal microbiome transplantation from a healthy donor represents a feasible approach. As discussed earlier in the review, microbiome features such as an altered microbial composition, or alpha diversity, are often associated with MDD symptoms. FMT has been shown to transfer these features to the recipient.[160] While dedicated studies are not yet available, a recent study on the effect of FMT on inflammatory bowel disease has reported improved mood in recipients of a healthy microbiome (Table 4),[161] suggesting potential clinical implications for this technique. More research is also warranted to investigate whether it is the microbes or their metabolites that have a beneficial effect through the FMT technique. For example, FMT with sterile fecal filtrate rather than fecal microbiota was sufficient to induce a therapeutic effect in patients with *Clostridium difficile* infection.[162] This finding

Table 4

Studies of Microbial Modulation of Mood by Fecal Microbiome Transplantation

Cohort	FMT	Timespan	Effect	Study[a]
Irritable bowel syndrome: men and women	FMT from healthy donors	Single FMT	Improved depression & anxiety (HAM-D, 4.71 ± 5.38 vs. baseline; QIDS, −4.00 ± 4.62 vs. baseline; HAM-A, 5.18 ± 6.44 vs. baseline)	Kurokawa et al. (2018)[161]

[a] Studies targeting the microbiome and presenting mood-related readouts as an output were chosen.

FMT, fecal microbiome transplantation; HAM-A, Hamilton Anxiety Rating Scale; HAM-D, Hamilton Depression Rating Scale; QIDS: Quick Inventory of Depressive Symptomatology.

indicates that bacterial components, metabolites, or bacteriophages mediate some of the effects of FMT—which might represent an alternative approach to whole-microbiome FMT.[163]

CONCLUSIONS

In this review we have highlighted the role of the microbiome in MDD. Although this field of research is just emerging, accumulating evidence suggests that it deserves increasing attention in the biological psychiatry of MDD. The microbiome is altered in depressed patients, and common therapeutic drugs targeting MDD, such as SSRIs, affect the microbiome. The reverse is likely also true. Looking toward the future, the microbiome might be the place to probe when developing new treatments for MDD. An important future step in this field will be to translate the growing body of preclinical work into clinical practice, where the microbiome could be used as a tool to improve the patients' response to psychiatric drugs such as antidepressants. The addition of microbiota profiling to MDD biomarkers already in place may provide further diagnostic precision and potentially improve personalized treatment. It is important to remember that MDD is a highly heterogeneous disorder and bears a complex pathophysiology. Genetic predisposition, environmental factors, such as significant psychosocial stress, and biological systems all play a role in the onset of this disorder. We support the concept that the gut microbiota be added to this model.

Throughout the review, we have stressed the need for more dedicated human studies on depression and the microbiome. Many of the existing studies in humans feature low sample sizes, likely confounding factors such as irritable bowel syndrome, or both. Mechanistic studies in clinically relevant populations are urgently needed. In this regard, randomized, controlled trials with multiple timepoints of microbiome collection are essential to tease out cause and effect. Moreover, longitudinal studies with probiotics, prebiotics, and other microbiota-targeted interventions are required to validate the psychobiotic approach. Interestingly, it is over 100 years ago since George Porter Philips put forward the concept of treating melancholia with lactobacillus;[164] with more clinical research, we may be able to validate how much he was ahead of his time.

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