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Microbiota-Gut-Brain Axis: New Therapeutic Opportunities

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Abstract

The traditional fields of pharmacology and toxicology are beginning to consider the substantial impact our gut microbiota has on host physiology. The microbiota-gut-brain axis is emerging as a particular area of interest and a potential new therapeutic target for effective treatment of central nervous system disorders, in addition to being a potential cause of drug side effects. Microbiota-gut-brain axis signaling can occur via several pathways, including via the immune system, recruitment of host neurochemical signaling, direct enteric nervous system routes and the vagus nerve, and the production of bacterial metabolites. Altered gut microbial profiles have been described in several psychiatric and neurological disorders. Psychobiotics, live biotherapeutics or substances whose beneficial effects on the brain are bacterially mediated, are currently being investigated as direct and/or adjunctive therapies for psychiatric and neurodevelopmental disorders and possibly for neurodegenerative disease, and they may emerge as new therapeutic options in the clinical management of brain disorders.

INTRODUCTION

> A man's body and his mind, with the utmost reverence to both I speak it, are exactly like a jerkin, and a jerkin's lining;—rumple the one,—you rumple the other.
>
> —Laurence Sterne (1759), *The Life and Opinions of Tristram Shandy, Gentleman*

The concept of targeting the body rather than brain for treating psychiatric and neurological conditions may seem outlandish even now, 260 years since Sterne's great text. However, over the last decade it has received much attention. Indeed, it is becoming more and more apparent that biologists must include the impact of our microbiota in any consideration of human physiology, including pharmacokinetics and pharmacodynamics following drug administration. The microbiota inhabiting our bodies outnumbers our human cells by approximately 1.3:1 (1) and comprises a vast ecology of bacteria, yeasts, and parasites such as helminths, viruses, and protozoa. Currently, we know the most about the bacterial population present, particularly the bacteria within our gastrointestinal (GI) tract; however, we are very much in the dark when it comes to elucidating the precise impact of these microbes on host health. The exact composition of the gut microbiota present in each individual is different (2), and it is still unclear what definitively constitutes a healthy adult gut microbial profile other than one that exhibits both diversity and stability. These commensal gut microbes, microbes naturally present in the gut, largely have a more symbiotic relationship with their host than the term commensal suggests. They have several important roles in digestion, including fermentation of undigested carbohydrates, production of vitamins and secondary bile acids, and immune system development. However, it is clear that we need to consider the impact of our gut microbiota, particularly in the fields of pharmacology and toxicology.

These fields have traditionally focused on the direct absorption, distribution, metabolism, and excretion of drugs and the interaction of these drugs with their target receptors, transporters, or enzymes in the host to produce both benefits and undesirable side effects. We must now additionally consider that the gut microbiota, particularly in the case of drugs taken orally, can both impact the metabolism of the drugs and be a crucial effector/mediator of drug response. In addition to possessing poisonous qualities in its own right when present in unfavorable configurations, or in the case of invading pathogenic species, it is also possible that we could directly target the gut microbiota to treat an increasing number of diseases and that the gut microbiota represents an exciting reservoir of new therapeutic opportunities. In the present review, we focus on targeting bacterial gut microbiota in order to impact microbiota-gut-brain axis signaling for the effective treatment of central nervous system (CNS) disorders. It is important to note, however, that the virome (3) and mycobiome (4) also play a role in microbiota-gut-brain axis signaling.

MICROBIOTA-GUT-BRAIN AXIS

Gut Microbiota Profile

The presence of bidirectional gut-brain communication may seem obvious with respect to the central control of hunger and satiety signals and factors originating in the gut that regulate food intake in the short term (5). However, it is becoming more apparent that such communication is more widely implicated in cognition, social behavior, fear expression, and stress response. Changes in behavior are associated with both GI illness and functional GI disorders (disorders in which there is no overt GI pathology present but which have a high psychological component) and can result in severe GI discomfort. Furthermore, acute and chronic stress, anxiety, and depression are all impacted by the gut microbial profile (6), which is itself regulated by multiple factors. Colonization of the gut is considered by most researchers in the field to primarily occur at birth, and

the microbiome seeded in the infant initially resembles that of the maternal vagina (7). This occurs at the same time as a critical period in normal stress-response development: the development of the hypothalamic-pituitary-adrenal (HPA) axis (8). Birth via Caesarean section (C-section) results in a very different seeding of the gut microbiota than that from vaginal delivery, the former encompassing microbes present on the skin and from the delivery suite (7), and these infants have a lower colonization of *Bifidobacterium*, *Bacteroides*, and *Lactobacillus*. The latter profile more closely resembles the maternal vaginal microbiome (7). The gut microbiome profile of C-section-delivered infants becomes closer to that of vaginally delivered infants within the first two years of life (9); however, the functional effects of the differential seeding may endure (10) and have been postulated to be linked to the increased relative risk for childhood obesity (11) and asthma (12). Furthermore, the composition of the gut microbiota in early life is further influenced by a variety of factors, including the impact of prenatal antibiotic use, breastfeeding, growth in the first years of life, and geography (13). Throughout life, the composition of the gut microbiome is further affected by diet (14), associated geography (15), exercise levels (16–18), antibiotic usage (19), and the usage of other medications (20, 21) (**Figure 1**). Diet plays a huge role in the composition and diversity of the gut microbiome, and alterations in diet can cause rapid changes in gut microbial profiles (22). Consumption of a Mediterranean diet is associated with both a specific gut microbial profile (23) and a host of beneficial health effects (14). Detrimental effects of poor diet, including Western-style diets, upon the gut microbiome have been linked with the rise in the prevalence of metabolic syndromes (24).

Signaling Mechanisms Behind Microbiota-Gut-Brain Axis Communication

Signaling in the microbiota-gut-brain axis can occur through a variety of mechanisms. Gut microbes can produce bioactive peptides, including neurotransmitters, transformation of secondary bile acids, short-chain fatty acids (SCFAs), branched-chain amino acids, and gut hormones. SCFAs, including acetate, butyrate, propionate, and lactate, can enter the circulatory system, and it is plausible that they may signal to the brain via this route (25). Microbes can also mediate the metabolism of tryptophan, thus modulating serotonin signaling in some cases (26). Dopamine, noradrenaline, GABA, and acetylcholine can also be synthesized by gut microbes (27). The gut wall is richly innervated by the enteric nervous system (ENS), mainly responsible for gut motility, and this can be targeted by neurotransmitters and SCFAs. Furthermore, microbes contain microbe-associated molecular patterns, which can be recognized by toll-like receptors in the ENS. Indeed, immune signaling is an important mechanism by which microbiota-gut-brain signaling occurs (28). The gut also contains a dense concentration of immune cells, which provide a second line of defense to pathogens after the physical barrier of the mucous layer of the gut epithelium. Production of pro- and anti-inflammatory cytokines can be influenced by the gut microbiota, which can then signal to the brain via the circulatory system. Gut barrier permeability may also be negatively impacted during stress (29, 30).

The vagus nerve is heavily implicated in microbiota-gut-brain axis signaling and is one of the key modes of communication between the gut and the brain (31). Vagotomy, a surgical procedure that was commonly used to treat peptic ulcer disease prior to the now-widespread availability of pharmacological agents and the implication of *Helicobacter pylori* in the disease, has been associated with a decreased risk of developing Parkinson's disease (PD) (32). Conversely, many of the beneficial effects of *Lactobacillus rhamnosus* in a preclinical study were abolished following vagotomy (33), and vagal nerve stimulation is used for treatment of resistant depression (34–36). Recently, it was elegantly demonstrated using optogenetic stimulation that there is a vagal gut-to-brain axis pathway to CNS reward neurons (37). Glutamate released from enteroendocrine cells can activate these vagal afferents (38), while serotonin has also been implicated more generally (39).

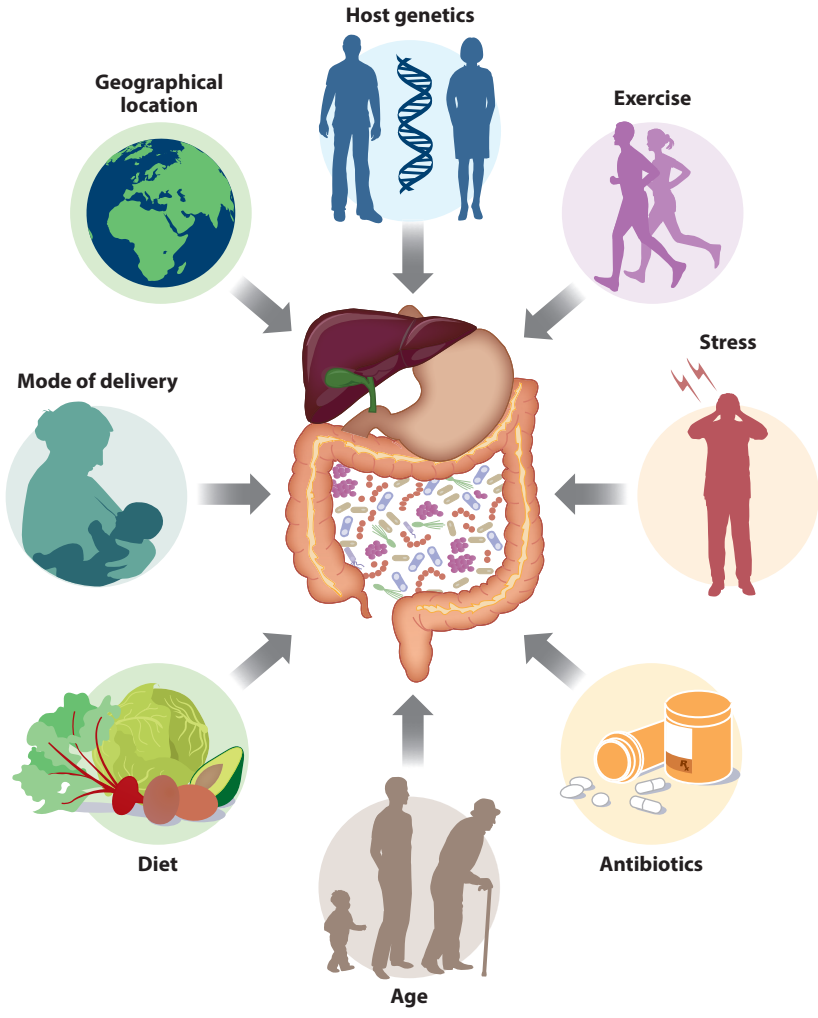

Figure 1

Factors affecting the gut microbiota profile. Multiple factors may impact, both positively and negatively, the composition and diversity of the gut microbiota profile throughout the life span.

ALTERED GUT MICROBIAL PROFILE IN BRAIN DISORDERS

Alterations in gut microbial profiles observed in preclinical models at least partially translate over to the human population. Differences in gut microbial profiles have been noted in Alzheimer's disease (AD) and PD patients and in patients with depression, autism, and post-traumatic stress disorder (PTSD), described in more detail in this section. Furthermore, fecal microbiota transplant (FMT) from patients with depression resulted in a transfer of the depressive phenotype from patient into preclinical models, reinforcing the concept that alterations in gut microbial profile in these disorders may have a direct impact on phenotypes (40).

Stress

Stress plays a significant role in altering the microbiota-gut-brain axis. Acute stress directly activates the HPA axis in order to respond appropriately to the stressor; however, prolonged, chronic

stress can cause heightened inappropriate HPA axis activation (41). There is an abundance of preclinical evidence describing stress-induced alterations in the microbiota-gut-brain axis in several hosts, including rodents (42–46) and nonhuman primates (47, 48). Furthermore, changing the early-life microbial profile through antibiotic exposure, lack of breastfeeding, birth by C-section, infection, exposure to stress, and other environmental factors, in addition to host genetics, can impact stress-related physiology and behavior (49), and the absence of a gut microbiota in germ-free animals results in aberrant stress responses (50, 51). In humans, there is a paucity of studies regarding specific stress-related alterations in the gut microbial profile. However, numerous studies have described an altered gut microbial profile in irritable bowel syndrome (IBS), a functional GI disorder (41, 52, 53). Prenatal stress exposure in women has also been shown to result in alterations to the gut microbial profile of the infants in addition to later infant GI symptoms and allergic reactions (54). Exposure to stress and anxiety through adverse life events can also impact stress-related depressive states, which are also currently under investigation with regard to gut microbiota profile.

Major Depressive Disorder

There are a number of studies describing gut microbial profile alterations in major depressive disorder (MDD) in comparison to healthy controls (40, 55–59), with differing findings of increased and decreased genera, and most describe decreased fecal microbial richness. Further evidence for a role of gut microbiota in MDD comes from two studies describing FMTs from patients with MDD into preclinical models, which transferred anxious and depressive phenotypes (40, 56). A recent large-scale study of the Flemish Gut Flora project also determined there were differences in the gut microbiota profile in the depressive cohort, differences that were still present after having taken into account the confounding effects of antidepressant medications (60). *Coprococcus* and *Dialister* species were not present in the depressed cohorts, and their presence in the nondepressed cohort was positively associated with quality of life scores (60), although it has been well noted that the latter scores are not equivalent to depression per se (61).

Parkinson's Disease

The presence of an altered gut microbial profile in PD has now been described in a variety of different studies (62–68). Prodromal gut-related symptoms, mainly constipation, have frequently been described in patients who go on to develop PD, and the initiation of PD etiology has been postulated to begin in the gut (69). Indeed, FMT from PD patients into mouse models of PD resulted in both motor deficits and neuroinflammation, providing further evidence that the gut microbiota is linked with PD (68). Epidemiological studies have shown that individuals who underwent a full truncal vagotomy for treatment of peptic ulcer disease have a diminished risk of PD as they age and provide some insight into the possible microbiota-gut-brain signaling involved (32, 70). The negative impact of *Helicobacter pylori* on the symptomatology of PD has been described (71, 72), and a recent meta-analysis has determined there is clear benefit for the eradication of *H. pylori* in ameliorating PD symptoms (73).

Alzheimer's Disease

AD has also been described as having an association with an altered gut microbial profile, with decreased richness and diversity found in very mild to mild AD (74) and differences in the Firmicutes:Bacteroidetes ratio (75), which was previously described in obesity and provides interesting

parallels into the link between AD and type 2 diabetes mellitus (T2DM) (76). Secondary bile acids, which are metabolized from primary bile acids by gut microbiota, were recently determined to be present at different levels in the serum of AD patients and patients with mild cognitive impairment compared with cognitively normal older adults, and this was linked with brain pathology (77). Furthermore, alterations in the gut microbial profile have been associated with cognitive impairment in otherwise healthy older adults (78) as well as with amyloid-β load (79).

Other Brain Disorders

With regard to schizophrenia, there are limited studies on the impact of gut microbiota in the disease, but schizophrenia does have a component of gut comorbidities (80). There is also preliminary evidence that the presence of *Candida albicans* is associated with worse psychiatric symptoms in males with schizophrenia (81). A recent study described specific taxa, including Veillonellaceae and Lachnospiraceae, which were associated with schizophrenia severity (82). Furthermore, FMT from these schizophrenia patients into germ-free mice caused a schizophrenic phenotype in the animals, in addition to altering glutamate signaling (82).

Autism spectrum disorder (ASD) patients frequently present with gut-related comorbidities (83), and indeed, unusual dietary patterns are a frequent occurrence in ASD patients. Several studies have described alterations in the gut microbial profile in children with ASD (84–86), and they particularly highlighted a reduced abundance of *Bifidobacterium*, considered to be a beneficial genus. Furthermore, the studies described an increased abundance of *Clostridia*, a potentially pathogenic genus. Treatment with vancomycin, a broad-spectrum antibiotic, resulted in positive alterations in behavioral symptoms in one short-term study, further highlighting the role of an aberrantly altered gut microbial profile in ASD (87). Furthermore, an FMT from healthy individuals into ASD patients in an open-label study resulted in improvements in both gut comorbidities and behaviors in the cohort (88).

A recent small study of the gut microbial profile in patients with bipolar disorder described limited differences in the profiles of these patients; however, there were some correlations between certain genera and sleep and stress states in the patients (89). Lastly, there is preliminary evidence that an altered gut microbial profile also exists in PTSD patients (90).

THERAPEUTICALLY TARGETING THE MICROBIOTA-GUT-BRAIN AXIS

Considering the emerging evidence for a role of the gut microbiota in these brain-related disorders, it is not surprising that the gut microbiota is now being targeted to mediate beneficial brain effects. The term psychobiotic was coined in 2013 by Dinan et al. (91, p. 720) as a novel class of psychotropic, defined as a "live organism that, when ingested in adequate amounts, produces a health benefit in patients suffering from psychiatric illness." This definition has since been expanded to include "any exogenous influence whose effect on the brain is bacterially-mediated" (25, p. 763). Psychobiotics, therefore, can include a range of substances that affect microbiota-gut-brain axis signaling, including probiotics, prebiotics, synbiotics, and postbiotics (**Figure 2**). These substances can be delivered through supplements, functional foods, and improvements to dietary intake. It is notable that the first antidepressants on the market were also antibiotics (92, 93).

Probiotics: Live Biotherapeutics

Probiotics are defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (9, p. 507). Probiotics were first described over 100 years ago in

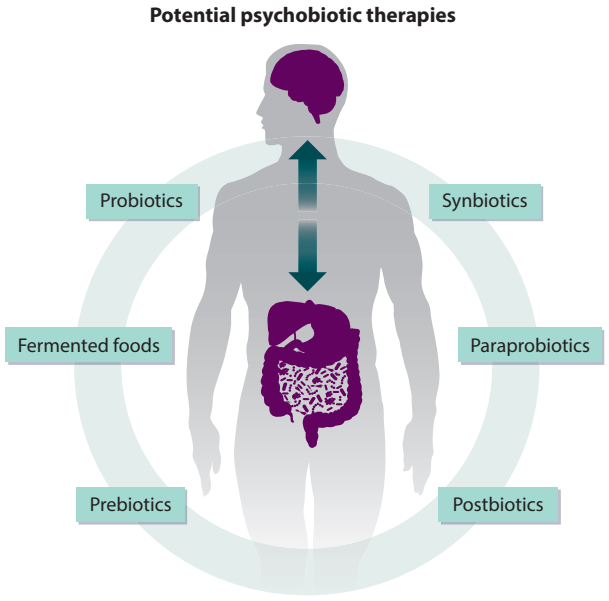

Figure 2

Potential psychobiotic therapies. These include probiotics, live biotherapeutics, prebiotics, synbiotics, paraprobiotics, postbiotics, and fermented foods.

the work of Elie Metchnikoff, who described health benefits associated with the consumption of lactic acid bacteria in fermented milk in a population of poor Bulgarians. There is now a plethora of studies showing benefits of a variety of probiotic strains in abrogating stress, depression, and anxiety-like behaviors in preclinical models in addition to a burgeoning number of successful human studies, albeit mostly indirectly, in nonpathological states or studies that were preliminary in nature (**Table 1**). What becomes immediately obvious is that the health benefits of putative probiotics are entirely strain-dependent, and not all probiotics have brain-beneficial effects (94–96). Strains of *Lactobacillus* and *Bifidobacteria*, which have well-defined safety profiles, are frequently used as probiotics, both genera generally being found to have beneficial health effects, but the individual strain differences are paramount in determining efficacy. Furthermore, the successful translation of promising probiotics, which have proven benefits in preclinical models, into human studies is not guaranteed (97). Probiotics generally do not become resident in the gut, that is, probiotic formulations require daily consumption to maintain positive effects. In general, probiotics are components of food products or are delivered as food supplements. Next-generation probiotics, including probiotics formally recognized as live biotherapeutics in the United States, encompass probiotics with no history of use but which may have health benefits, which may be delivered under more traditional pharmaceutical routes rather than through food delivery and are associated with a more stringent regulatory framework (98).

Psychiatry-related studies. Probiotics have proven to have some success in ameliorating mood and depression states in a number of human studies. Treatment with a milk drink containing *Lactobacillus casei* as part of a double-blind randomized controlled trial (RCT) resulted in mood score improvements in an elderly cohort, with the most benefit seen in those participants who scored the lowest in mood scores at baseline (99). A multibiotic of three strains (*Lactobacillus acidophilus*, *L. casei*, and *Bifidobacterium bifidum*) given over a course of 8 weeks to an MDD cohort in an RCT

Table 1 Probiotics studied with respect to microbiota-gut-brain axis signaling

Human cohort studied	Probiotic treatment	Treatment duration	Effect(s)	Reference
Healthy controls	Multibiotic: *S. thermophilus* CNCM I-1630, *L. bulgaricus* CNCM I-1632, *L. lactis* subsp. *lactis* CNCM I-1631, *L. acidophilus*, *S. thermophilus*, *L. plantarum*, *B. lactis* CNCM I-2494, *L. reuteri* DSM 17938	3 weeks	↓ anxiety scores (HAM-A)	105
	L. casei Shirota (YIT 9029)	8 weeks	No change in psychological parameters (anxiety, depression scales) ↓ stress-related physical symptoms	152
	Multibiotic: *L. casei* W56, *L. paracasei* W20, *B. lactis* W51 and W52, *L. salivarius* W24, *L. plantarum* W62, *B. bifidum* W23, *L. acidophilus* W22, *L. lactis* W19	4 weeks	Change in gut microbiota profile Improvements in some anxiety and depressive measures (LEIDS, PANAS) Improvements to emotional memory (fMRI tasks)	125
	Multibiotic: *B. bifidum* W23, *B. lactis* W52, *L. acidophilus* W37, *L. brevis* W63, *L. casei* W56, *L. salivarius* W24, *L. lactis* W19 and W58	4 weeks	↓ cognitive reactivity to sad moods (LEIDS-R test), particularly aggression and rumination thoughts No change to depression or anxiety	106
	B. longum 1714	4 weeks	↑ neurocognitive performance (PAL) Enhanced EEG mobility (frontal midline)	108
	L. rhamnosus (JB-1)^{TM}	4 weeks	No significant impact on HPA axis, stress, depression, anxiety, and cognition	94
	Fermented milk product: *B. animalis* subsp. *lactis* Multibiotic: *S. thermophilus*, *L. bulgaricus*, *L. lactis* subsp. *lactis*	4 weeks	Altered response to emotional processing (fMRI)	151
	Group 1: *L. acidophilus* LA5, *B. lactis* BB12 Group 2: *L. casei*, *L. acidophilus*, *L. rhamnosus*, *L. bulgaricus*, *B. breve*, *B. longum*, *S. thermophilus*, fructo-oligosaccharide	6 weeks	Improvement in GHQ scale with either probiotic treatment Improved depression and anxiety (DASS) No effect on HPA axis activity or kynurenine/tryptophan	112
	Multibiotic: *L. gasseri* SBT2055, *B. longum* SBT2928	12 weeks	↓ serum cortisol Improvements in anxiety measures (GHQ-28 subscale)	113
Low mood	Multibiotic: *L. helveticus* R0052, *B. longum* R0175	8 weeks	No effect on the mood, stress, and anxiety measures analyzed	120

(*Continued*)

Table 1 (*Continued*)

Human cohort studied	Probiotic treatment	Treatment duration	Effect(s)	Reference
Urinary-free cortisol 10–50 ng/mL (low)	Multibiotic: *L. helveticus* R0052, *B. longum* R0175	30 days	↓ perceived stress scores (PSS), depression and anxiety scores (HADS) ↓ HSCL-90 subscores, including somatization, obsessive-compulsive, anxiety, and depression scores	103
Stress	Multibiotic: *L. acidophilus* Rosell-52, *B. longum* Rosell-175	3 weeks	↓ stress-associated GI symptoms No effect on other symptoms (physical, psychological, sleep)	104
	L. plantarum DR7	12 weeks	↓ stress and anxiety measures Improvements in memory and cognition Enhanced serotonergic signaling ↓ plasma cortisol ↓ proinflammatory cytokines	114
Pregnancy	*L. rhamnosus* HN001	<6 months	↓ postpartum depression and anxiety scores (modified EPDS and STAI)	107
Students during exam stress	*L. casei* Shirota YIT 9029	8 weeks	↓ salivary cortisol levels ↓ physical symptoms of stress/anxiety	109
	L. plantarum 299v	14 days	No difference in perceived stress ↑ *L. plantarum* 299v and *Lactobacilli* on day 14 in saliva	111
Elderly	*L. casei* Shirota (Yakult)	3 weeks	Improvement in depression (POMS) for people with worst baseline mood scale No effect on cognition	99
	L. reuteri DSM 17938	12 weeks	No persisting effects on depression, anxiety, or perceived stress (HADS, PSS)	96
	L. helveticus IDCC3801	12 weeks	↑ cognitive performance in RVIP and Stroop Color-Word task (cognitively demanding tasks) No effect on stress levels (PSS) or depression levels (GDS-SF)	119
IBS patients	*B. longum* NCC3001	6 weeks	↓ in depression scores (HADS-D) No effect on anxiety	102
			↓ response to fearful stimuli ↑ quality of life	102
	Multibiotic: *L. paracasei* subsp. *paracasei* F19, *L. acidophilus* La5, *B. lactis* Bb12	8 weeks	No significant effects on anxiety or depression measures (HADS)	153

(*Continued*)

Table 1 (*Continued*)

Human cohort studied	Probiotic treatment	Treatment duration	Effect(s)	Reference
MDD patients	Multibiotic: *L. acidophilus*, *L. casei*, *B. bifidum*	8 weeks	↓ depression scores (BDI)	100
	Multibiotic: *L. helveticus* R0052, *B. longum* R0175 (CNCM strain I-3470)	8 weeks	↓ BDI score ↓ kynurenine:tryptophan ratio	101
	L. plantarum 299V (adjunctive to antidepressants)	8 weeks	Improved attention and verbal learning ↓ circulating kynurenine concentration	123
	C. butyricum MIYAIRI 588 (adjunctive to antidepressants)	8 weeks	Improvements in depression scores	115
Chronic fatigue syndrome	*L. casei* Shirota	8 weeks	↓ anxiety symptoms (BAI) No change in depression scores (BDI)	110
AD patients	Multibiotic: *L. acidophilus*, *L. casei*, *B. bifidum*, *L. fermentum*	12 weeks	↑ cognition (MMSE) Changes in blood lipid profile and carbohydrate metabolism factors	121
	Multibiotic: *L. fermentum*, *L. plantarum*, *B. lactis* or *L. acidophilus*, *B. bifidum*, *B. longum*	12 weeks	No effects on cognition	122
HIV patients	Multibiotic: *L. plantarum* DSM 24730, *S. thermophilus* DSM 24731, *B. breve* DSM 24732, *L. paracasei* DSM 24733, *L. delbrueckii* subsp. *bulgaricus* DSM 24734, *L. acidophilus* DSM 24735, *B. longum* DSM 24736, *B. infantis* DSM 24737	6 months	↑ neurocognitive performance in multiple measures	124
Schizophrenia patients	Multibiotic: *L. rhamnosus* GG, *B. animalis* subsp. *lactis* Bb12	14 weeks	No treatment effect in females Alteration of *C. albicans* antibody levels	81
	Multibiotic: *L. rhamnosus* GG, *B. animalis* subsp. *lactis* Bb12	14 weeks	No changes in frequency of psychotic symptoms ↓ incidence of severe bowel difficulties ↓ acute von Willebrand factor ↑ MCP-1, BDNF, RANTES, MIP-1	131
	Multibiotic: *L. rhamnosus* GG, *B. animalis* subsp. *lactis* Bb12	2 weeks	No differences in PANSS total symptom score ↓ incidence of severe bowel difficulties	130
	B. breve A-1	4 weeks	Improvements in anxiety and depressive scores	132

(*Continued*)

Table 1 (*Continued*)

Human cohort studied	Probiotic treatment	Treatment duration	Effect(s)	Reference
Schizophrenia patients/ Bipolar patients	Multibiotic: *L. rhamnosus* GG, *B. lactis* Bb12	14 weeks	Improvements in gut symptoms No effect on psychiatric symptoms	130
ASD	Multibiotic: *L. acidophilus*, *L. rhamnosus*, *B. longum*	3 months	Improvements in autism severity (ATEC)	129

↑ indicates an increase, and ↓ indicates a decrease. Abbreviations: AD, Alzheimer's disease; ASD, autism spectrum disorder; ATEC, Autism Treatment Evaluation Checklist; BAI, Beck's Anxiety Inventory; BDI, Beck's Depression Inventory; DASS, depression anxiety stress scales; EEG, electroencephalography; EPDS, Edinburgh Postnatal Depression Scale; GHQ, General Health Questionnaire; HADS, hospital anxiety and depression scale; HAM-A, Hamilton Anxiety Rating Scale; HPA, hypothalamic-pituitary-adrenal; HSCL, Hopkins Symptom Checklist; IBS, irritable bowel syndrome; MDD, major depressive disorder; MMSE, Mini–Mental State Examination; PAL, paired associates learning; PANAS, positive and negative affect schedule; PANSS, positive and negative syndrome scale; POMS, profile of mood states; PSS, perceived stress scale; RVIP, rapid visual information processing; STAI, State-Trait Anxiety Inventory.

resulted in ameliorations in scores of depression in self-report scales, compared with placebo (100), while a study on a *Lactobacillus helveticus* and a *Bifidobacterium longum* strain showed improvements in depression scores in a depressed cohort (101). A 6-week intervention with a *B. longum* strain in IBS patients resulted in an improvement in depression, but not anxiety, scores (102). *L. helveticus* and *B. longum* have also been described to benefit anxiety and stress responses (103), and an *L. acidophilus* and *B. longum* combination reduced stress-associated GI symptoms in stress-affected individuals (104). Additionally, a multistrain probiotic [*Streptococcus thermophilus* (2 strains), *Lactobacillus bulgaricus*, *Lactobacillus lactis*, *L. acidophilus*, *Lactobacillus plantarum*, *Bifidobacterium lactis*, and *Lactobacillus reuteri*] was recently described to have anxiolytic effects in healthy controls (105), while another multistrain probiotic (nine species, including *Lactobacilli* and *Bifidobacterium*) given to healthy controls improved cognitive reactivity to sad mood (106). An RCT of an *L. rhamnosus* strain significantly lowered measures of anxiety and postnatal depression when taken during pregnancy (107). A study from our lab outlined improvements in anxiety and cognition due to a *B. longum* strain intervention in healthy volunteers (108). Otherwise healthy medical students had reduced levels of stress related to university examinations following consumption of a fermented milk containing the probiotic *L. casei* Shirota (109). This strain was previously shown to reduce anxiety symptoms in patients with chronic fatigue syndrome (110). Increases in salivary cortisol observed during an exam stress period were also diminished in a healthy student population consuming an *L. plantarum* 299v probiotic strain (111). Furthermore, improvements in depression and anxiety scores were observed in a probiotic intervention study in arms examining probiotic-supplemented yoghurt and an arm using capsules containing probiotic, versus standard yoghurt alone, and the improvements were found to be unrelated to changes in kynurenine signaling (112). Positive changes in stress measures and salivary cortisol and improvements in immune response have also been observed in a 12-week intervention of *Lactobacillus gasseri* and *B. longum* in healthy controls (113). A recent study further described multiple benefits of *L. plantarum* in stressed but otherwise healthy adults, including a reduction of stress and anxiety measures and improvements in multiple aspects of memory and cognition, enhanced serotonergic signaling, and reductions in cortisol and proinflammatory cytokines (114).

Interestingly, probiotics have also been studied as a potential adjunct therapy in combination with antidepressant drugs in a cohort of treatment-resistant depressions, showing some promise in an open-label study (115). These findings are supported by systematic reviews of the use of

the therapeutic potential of probiotics in MDD (116–118). On the other hand, some studies have failed to show any benefit of probiotic supplementation to MDD patients in terms of ameliorating depression scores, reinforcing the strain-dependent nature of putative psychobiotics (119, 120), as highlighted in one recent systematic review (95).

Neurology-related studies. There has been a limited number of studies using probiotic interventions to attempt to ameliorate some of the cognitive deficits resulting from AD. One such RCT, using a multistrain probiotic in a milk drink (*L. acidophilus*, *L. casei*, *B. bifidum*, and *Lactobacillus fermentum* species) resulted in improvements in Mini–Mental State Exam scores (121). However, no improvements in cognition were observed in a severe AD cohort (122) in another RCT by the same group, using other *Lactobacilli* and *Bifidobacterium* strains, again highlighting the differential effects of the strain level in formulating effective probiotics as well as emphasizing the need for clarity with regard to the best time to therapeutically intervene in progressive disease. Cognitive performance, as measured in tests of attention and verbal learning, was improved in a recent study of an MDD patient cohort due to supplementation with *L. plantarum* (123). Cognition was also improved following a 6-month intervention with a multistrain probiotic in a cohort of HIV patients (124). Furthermore, emotional memory performance was improved in a cohort of healthy volunteers following a 4-week intervention with a multistrain probiotic (125).

Certain probiotic strains have shown promise in ameliorating behavioral and neurochemical perturbations in preclinical models of ASD (126, 127). Human research on the use of probiotics in ASD is in its infancy and is focused on improvement in gut-related comorbidities (128), though there is also some preliminary evidence for behavioral change (129). Adjunctive *L. rhamnosus* and *Bifidobacterium animalis* treatment of schizophrenic patients improved gut health with no beneficial effects on psychiatric symptom severity (130). Improvements to peripheral inflammatory profiles have also been observed due to a 14-week intervention with *L. rhamnosus* plus *B. animalis* as an adjunctive treatment in chronic schizophrenia patients (131). A recent open-label study on *Bifidobacterium breve* supplementation in schizophrenic patients reported a beneficial effect on anxiety and depression scores, although there was no placebo arm to this study (132).

Prebiotics

Much work has been done on attempting to directly modulate the gut microbiota by the introduction of living microorganisms, probiotics, to the system. However, there are many technical and pragmatic difficulties with this approach. These include ensuring the survival of the probiotic transiting through the hostile environment of altered pH levels, from the acidity of the stomach to the alkali bile salt environment, to the location in the intestines where it can exert its potential therapeutic effect; the ease of fermentation and issues in continuity and consistency in batch production; the inability of probiotics to colonize the environment in the long term; and the selection of appropriate strains. Alternatively, targeting the beneficial host microbiota and enabling these bacteria to thrive is now presenting an exciting possibility. Prebiotics are defined as a "substrate that is selectively utilized by host microorganisms conferring a health benefit" (133, p. 493). For the most part, prebiotics consist of fibers such as inulin, fructo-oligosaccharides, galacto-oligosaccharides (GOSs), and resistant starch, which fail to be absorbed in the small intestine and are selectively fermented by gut microbes. Prebiotics are found in a wide variety of fruits, vegetables, and grains, entities that are increasingly diminished in the Western-style diet. Prebiotics are also found in human milk (human milk oligosaccharides) (134). Interventions with prebiotics have the advantage of potentially being able to improve gut microbial status more globally, as opposed to the use of single- or multistrain probiotics.

Regarding the microbiota-gut-brain axis, there are now multiple studies using prebiotics that resulted in beneficial outcomes. Administration of a 3-week course of B-GOS resulted in a decreased cortisol awakening response in a healthy volunteer cohort (135), while another study determined that GOS supplementation caused a significant decrease in anxiety scores in an IBS cohort (136). However, other studies have failed to find evidence that prebiotic intervention improved mood scores in an MDD cohort (101). A B-GOS intervention resulted in behavioral improvements in a cohort of children with ASD when included with a restrictive diet intervention (137). Administration of resistant starch in a cohort of schizophrenia or bipolar disorder patients on stable atypical antipsychotic medication showed variable alterations in gut microbiome profiles in a recent study that aimed to examine the tolerability of the prebiotic in this population (138).

Synbiotics

Synbiotics are a combination of probiotics and prebiotics, whereby the prebiotics are in place to improve the viability of the probiotic, providing a source of fermentable fiber as well as acting as a general prebiotic. A synbiotic comprising GOS and a dual-strain probiotic (*L. helveticus* and *B. longum*) caused a reduction in depression scores and positively impacted tryptophan signaling in mild to moderate MDD (101). Functional GI symptoms were improved in a PD cohort in an RCT on a synbiotic containing multistrain probiotics plus a prebiotic (139). There is limited evidence for ameliorations in microbiota-gut-brain axis signaling in PTSD, with one study showing positive results of a fermented soy drink in a PTSD cohort (140). A recent pilot study has shown some promise for the use of a synbiotic (*Bifidobacterium infantis* plus oligosaccharides) in ameliorating some of the gut-related comorbidities related to ASD (141).

Postbiotics

Postbiotics are nonviable entities that are metabolites of bacterial fermentation and include bioactives such as SCFAs. We also consider the consequences of bacterial interaction with the host and the resultant bioactives such as gut peptides as being postbiotics. There are limited studies in humans regarding the administration of bioactives and their effect on brain health; however, the general impacts of the gut microbiome and SCFAs on metabolic traits and disease have recently been described (142). Regarding brain health, preclinical studies have described anxiolytic effects of an SCFA combination (a mixture of acetate, propionate, and butyrate) administered to mice undergoing psychosocial stress (143). Propionic acid administration to rats resulted in an autism-like phenotype (144). In one human interventional study of an inulin-propionate ester that increases propionate production in the colon, brain imaging (via functional MRI) done to determine responses to a food picture evaluation task showed a reduction in anticipatory reward responses in the striatum due to the supplement. It is therefore plausible that behaviors can be targeted through supplementation with such bioactives.

Gut peptides have a well-established role in influencing behavior and also a role in stress, anxiety, and depression (for a review, see 145). While technical issues may hinder the use of gut peptides directly as an intervention in gut-brain axis signaling, targeting specific microbiota in order to modulate specific gut peptides may be a useful psychobiotic therapy.

Paraprobiotics, or nonviable probiotics, e.g., heat-killed probiotics, can also be included in the category of postbiotics (146) in that they contain structural components that may exert biological activity in the host, and there are now a number of human studies on the microbiota-gut-brain axis regarding supplementation with paraprobiotics. Stress experienced due to university exams in medical students was alleviated by a 12-week intervention with a heat-killed, washed, paraprobiotic CP2305, with some improvements in state anxiety scores in female students in addition to

improved basal cortisol output and improvements in sleep measures (147). In preclinical studies, several heat-killed probiotics have described antidepressant and anxiolytic effects, with heat-killed *Lactobacillus paracasei* PS23 having benefits in a corticosterone-induced depressive phenotype, reversing hippocampal and prefrontal cortex reductions in dopamine levels (148). Heat-killed paraprobiotics would have a distinct marketing advantage over probiotics (live biotherapeutics) by having an increased shelf life and possibly in terms of an increased safety profile. However, not all probiotic strains will be effective as heat-killed preparations, as observed in an intervention study of an *L. plantarum* strain to improve anxiety-like behaviors in mice (149).

Fermented Foods and Diet

Fermented foods have long been staple parts of traditional cultures' diets; indeed, this is how probiotics were originally described by Elie Metchnikoff in a Bulgarian population. Fermented foods contain probiotics, prebiotics, and bacterially derived bioactives (for a recent review, see 150). Two of the most common strains used in the fermentation process include *Lactobacillus delbrueckii* subsp. *bulgaricus* and *S. thermophilus*. In dairy products, lactic acid–producing *Bifidobacterium* and *Lactobacillus* are commonly used. Studies using fermented food interventions in humans are limited; however, there is some evidence showing ameliorations in anxiety and mood scores. Fermented milk drinks have been found to result in positive benefits in emotional processing (151) and stress (109, 152), while others have failed to find gut-brain axis benefits (153).

Mediterranean diets have described mental health benefits (154). One large-cohort, cross-sectional study in women found healthier dietary patterns to be associated with better general health scores and decreased incidence of anxiety and depression outcomes (155). A meta-analysis of 22 studies also showed decreased risk of depression and cognitive impairment, including risk of having AD, with increased adherence to a Mediterranean diet (156). Further meta-analyses have corroborated these findings and described the benefits of Mediterranean diet consumption in addition to the detrimental effects of more Western-style diets (154, 157, 158). A recently published meta-analysis of 14 observational studies described no significant association between the risk of depression and adherence to the Mediterranean diet, but it did find an association with the odds of depression in the cohorts (159). There is a need for more prospective dietary studies on the benefits of diets rich in potential psychobiotics to fully elucidate the potential benefits regarding stress, anxiety, and depressive outcomes. The first such study, the SMILES trial, described a clear benefit of a dietary improvement intervention in a cohort with moderate to severe depression (160), with an economic evaluation indicating this could potentially be a cost-effective option for wider implementation (161). Dietary improvements also resulted in improved anxiety and depression scores in a cohort of patients with diagnosed depression and/or anxiety following a 12-week dietary advice intervention encompassing such positive changes as increasing fruit and vegetable servings and reducing portion sizes (162). A further study also found improvements to depression scores and quality of life scores in a cohort with diagnosed or self-reported depressive symptoms following an intervention of a fish oil–supplemented Mediterranean diet (163). A recent meta-analysis of dietary improvements on depression and anxiety found evidence of a benefit to ameliorate depression measures but not anxiety measures (164).

There is clearly an increasing interest in harnessing the benefits of healthy diets in psychiatric populations (165). The Mediterranean diet encompasses many elements that are rich in psychobiotics, particularly prebiotics. The combination of such a diet with items such as fermented foods and postbiotics could lead to a diet with particularly beneficial microbiota-gut-brain axis signaling. Such a psychobiotic diet is worthy of investigation for its potential benefits in mitigating stress, anxiety, and depressive symptoms.

MEDICATIONS AND THE MICROBIOME

While antibiotic treatment clearly has profound effects on the gut microbiome (19), a number of recent studies highlighted the potential confounding effects of other medications in research on the gut microbiome. In particular, a study on T2DM determined that metformin, a commonly used antidiabetic medication, was responsible for much of the alterations in the gut microbiota profiles of T2DM patients, which had previously been reported to be due to the disease. The authors determined there was a specific T2DM gut microbiome signature when the confounding effects of medication were taken into account and that the beneficial effects of metformin could actually be mediated via the microbiome (166). There have since been several studies that have described a plethora of drugs that may alter the gut microbiota profile (20, 21). Proton pump inhibitors (PPIs) have a well-described effect on gut microbiota (167–170). Furthermore, multiple nonantibiotic drugs have since been determined to have antimicrobial properties (171), including several psychotropic medications, which may exert their beneficial or adverse effects, at least in part, via altering microbial profiles or by activating microbiota-gut-brain axis signals (92, 172–176). Indeed, a small, recently published study described increases in microbial alpha diversity in a cohort of depressed patients who were hospitalized and treated with the selective serotonin reuptake inhibitor antidepressant escitalopram for 6 weeks, compared with baseline samples that were taken following a 1-week washout (177). However, it was noted that there was no placebo group in this study on depressed patients due to ethical considerations, and the impact of the hospital diet could be a confounding factor (177). Regarding PD, gut microbes have also been determined to be responsible for a portion of the breakdown of levodopa (178). Overall, it is becoming more apparent that the gut microbiota needs to be considered in any study on the mechanisms of drug action in the body and in relation to either beneficial or adverse effects. The gut microbiota could be harnessed to provide new therapeutic options in many cases or to limit the metabolic side effects in others, such as with antipsychotics (92, 179), and the gut microbiota may even be responsible for decreased efficacy of certain drugs in others.

SCREENING THE MICROBIOME FOR PSYCHOBIOTICS

Screening the microbiome to identify putative psychobiotics involves a very different process than traditional drug screening (for reviews, see 97, 98) (**Figure 3**). Fresh fecal sampling, in conjunction with fresh plating of resultant bacteria in a specific agar medium, and picking resultant colonies are the first step. These fecal samples can be taken from a cross-sectional cohort, but targeting individuals who are more resilient to stress-related measures could be of particular benefit, in addition to targeting strains present in low abundance in those with neurological and psychiatric disorders. The strains are then identified using genome sequencing. While strains of certain genera, e.g., *Lactobacilli* and *Bifidobacteria*, have recognized safety status in a number of jurisdictions, other new probiotics require extensive profiling before being approved for use (for a review of regulatory guidelines across a number of jurisdictions, see 180, 181). In the European Union, in order to meet the European Food Safety Authority's Qualified Presumption of Safety, the group of microorganisms must meet certain criteria, including the identification of their taxonomy, establishment of their safety, exclusion of pathogenic properties, and clear definition of their intended usage (182).

Once the strains have been chosen, they can be used in a battery of assays, such as enzyme assays, in addition to in vitro cell testing, whereby cells of interest are incubated with varying concentrations of the probiotic candidates. Depending on the cell type of interest, the cells can be monitored for growth; morphology; and the production of anti-inflammatory cytokines and other bioactives such as SCFAs, neurotransmitters, and hormones. G protein–coupled receptors (GPCRs) within

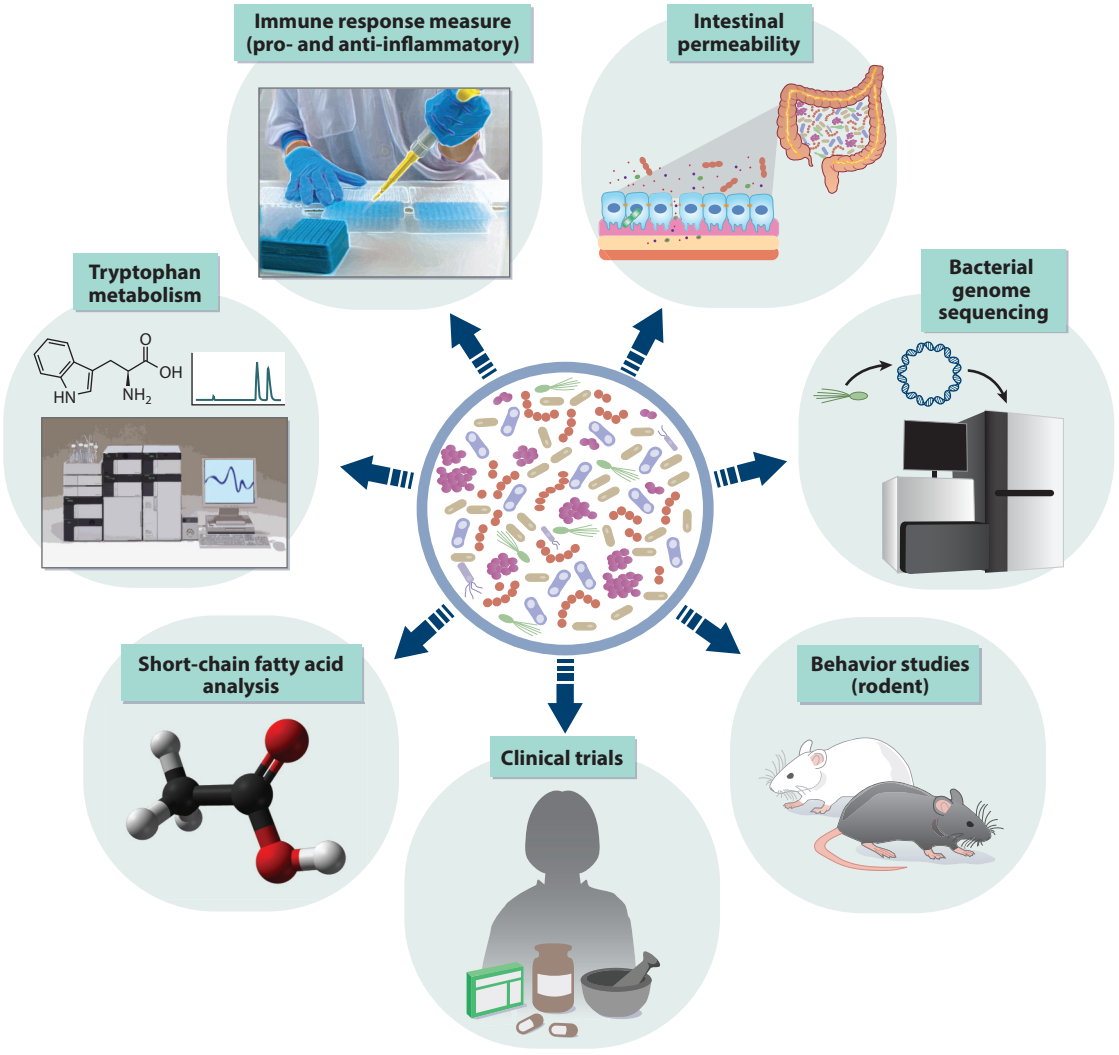

Figure 3

Screening for potential psychobiotic therapies. The search for psychobiotics is multimodal and commences with the identification of strains of interest, followed by a battery of testing to determine if there are positive effects on microbiota-gut-brain axis signaling.

the gut can be directly targeted by microbially produced bioactives (183), and classical pharmacological approaches can be used to determine microbial bioactive effects on GPCRs. If a candidate probiotic shows particular benefits with regard to any of these factors, the probiotic can then be used in preclinical in vivo testing, and further safety analysis can be performed. Decisions on when to provide the supplement, whether in early life, adolescence, or adulthood and prior to or during a stressor, need to be carefully considered. A further battery of behavioral testing is usually done to determine whether any anxiolytic or antidepressant phenotypes can be elucidated.

PERSPECTIVES AND CONCLUSION

Rumpling the jerkin or the lining, the mind or the body, requires much reflection in the age of the microbiome for those working in the areas of neuropharmacology and neurotoxicology. We

have moved from a focus on single bacterial strains as pathogens to an emphasis on nurturing an entire community of microbes, lest they become a pathological entity. There are many challenges to conventional wisdom at play with the possibility that the alterations in the gut microbiota noted in many CNS disorders may have a causal role in symptom expression and that many of the drugs used to treat those disorders may be toxic to or support the diversity of our gut microbes. Understanding the fundamental rules of engagement, and their implications for pharmacokinetics and pharmacodynamics, is a big project and one in need of support with greater mechanistic and translational insights. It is, however, difficult to see how future drug development efforts cannot incorporate this knowledge into discovery pipelines, and screening of human-directed drugs for antimicrobial properties may soon become routine.

Direct targeting of the gut microbiome is, of course, a great opportunity to produce a new generation of therapeutic options with superior safety profiles for CNS disorders by containing the site of initial action within the gut. Supporting good mental health and increasing resilience can easily be reconciled with a move to a more psychobiotic lifestyle that also stands to benefit our general health on many fronts. However, it is also becoming more apparent that targeted alterations in microbiota-gut-brain axis signaling represent a very real and exciting therapeutic opportunity for adjunctive therapy for a number of psychological and psychiatric disorders. Mining the gut microbiome for potential psychobiotics may pave the way for developing these new interventions.

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LITERATURE CITED

1. Sender R, Fuchs S, Milo R. 2016. Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell* 164:337–40
2. Hum. Microbiome Proj. Consort. 2012. Structure, function and diversity of the healthy human microbiome. *Nature* 486:207–14
3. Shkoporov AN, Hill C. 2019. Bacteriophages of the human gut: the "known unknown" of the microbiome. *Cell Host Microbe* 25:195–209
4. Forbes JD, Bernstein CN, Tremlett H, Van Domselaar G, Knox NC. 2018. A fungal world: Could the gut mycobiome be involved in neurological disease? *Front. Microbiol.* 9:3249
5. Konturek SJ, Konturek JW, Pawlik T, Brzozowski T. 2004. Brain-gut axis and its role in the control of food intake. *J. Physiol. Pharmacol.* 55:137–54
6. Dinan TG, Cryan JF, Stanton C. 2018. Gut microbes and brain development have black box connectivity. *Biol. Psychiatry* 83:97–99

7. Dominguez-Bello MG, Costello EK, Contreras M, Magris M, Hidalgo G, et al. 2010. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *PNAS* 107:11971–75
8. Ng PC. 2000. The fetal and neonatal hypothalamic-pituitary-adrenal axis. *Arch. Dis. Child Fetal Neonatal Ed.* 82:F250–54
9. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, et al. 2014. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* 11:506–14
10. Tamburini S, Shen N, Wu HC, Clemente JC. 2016. The microbiome in early life: implications for health outcomes. *Nat. Med.* 22:713–22
11. Mueller NT, Whyatt R, Hoepner L, Oberfield S, Dominguez-Bello MG, et al. 2015. Prenatal exposure to antibiotics, cesarean section and risk of childhood obesity. *Int. J. Obes.* 39:665–70
12. Metsala J, Lundqvist A, Virta LJ, Kaila M, Gissler M, Virtanen SM. 2015. Prenatal and post-natal exposure to antibiotics and risk of asthma in childhood. *Clin. Exp. Allergy* 45:137–45
13. Vatanen T, Plichta DR, Somani J, Munch PC, Arthur TD, et al. 2019. Genomic variation and strain-specific functional adaptation in the human gut microbiome during early life. *Nat. Microbiol.* 4:470–79
14. Sandhu KV, Sherwin E, Schellekens H, Stanton C, Dinan TG, Cryan JF. 2017. Feeding the microbiota-gut-brain axis: diet, microbiome, and neuropsychiatry. *Transl. Res.* 179:223–44
15. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, et al. 2010. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *PNAS* 107:14691–96
16. McFadzean R. 2014. *Exercise can help modulate human gut microbiota*. PhD Thesis, Univ. Colorado, Boulder, CO
17. Clarke SF, Murphy EF, O'Sullivan O, Lucey AJ, Humphreys M, et al. 2014. Exercise and associated dietary extremes impact on gut microbial diversity. *Gut* 63:1913–20
18. O'Sullivan O, Cronin O, Clarke SF, Murphy EF, Molloy MG, et al. 2015. Exercise and the microbiota. *Gut Microbes* 6:131–36
19. Antunes LC, Han J, Ferreira RB, Lolic P, Borchers CH, Finlay BB. 2011. Effect of antibiotic treatment on the intestinal metabolome. *Antimicrob. Agents Chemother.* 55:1494–503
20. Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, et al. 2016. Population-level analysis of gut microbiome variation. *Science* 352:560–64
21. Zhernakova A, Kurilshikov A, Bonder MJ, Tigchelaar EF, Schirmer M, et al. 2016. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science* 352:565–69
22. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, et al. 2014. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505:559–63
23. Mitsou EK, Kakali A, Antonopoulou S, Mountzouris KC, Yannakoulia M, et al. 2017. Adherence to the Mediterranean diet is associated with the gut microbiota pattern and gastrointestinal characteristics in an adult population. *Br. J. Nutr.* 117:1645–55
24. Sonnenburg JL, Backhed F. 2016. Diet-microbiota interactions as moderators of human metabolism. *Nature* 535:56–64
25. Sarkar A, Lehto SM, Harty S, Dinan TG, Cryan JF, Burnet PWJ. 2016. Psychobiotics and the manipulation of bacteria-gut-brain signals. *Trends Neurosci.* 39:763–81
26. Kennedy PJ, Cryan JF, Dinan TG, Clarke G. 2017. Kynurenine pathway metabolism and the microbiota-gut-brain axis. *Neuropharmacology* 112:399–412
27. Clarke G, Stilling RM, Kennedy PJ, Stanton C, Cryan JF, Dinan TG. 2014. Minireview: gut microbiota: the neglected endocrine organ. *Mol. Endocrinol.* 28:1221–38
28. Rea K, Dinan TG, Cryan JF. 2016. The microbiome: a key regulator of stress and neuroinflammation. *Neurobiol. Stress* 4:23–33
29. Vanuytsel T, van Wanrooy S, Vanheel H, Vanormelingen C, Verschueren S, et al. 2014. Psychological stress and corticotropin-releasing hormone increase intestinal permeability in humans by a mast cell–dependent mechanism. *Gut* 63:1293–99

30. Alonso C, Guilarte M, Vicario M, Ramos L, Rezzi S, et al. 2012. Acute experimental stress evokes a differential gender-determined increase in human intestinal macromolecular permeability. *Neurogastroenterol. Motil.* 24:740–46
31. Fulling C, Dinan TG, Cryan JF. 2019. Gut microbe to brain signaling: what happens in vagus…. *Neuroview* 101:998–1002
32. Svensson E, Horvath-Puho E, Thomsen RW, Djurhuus JC, Pedersen L, et al. 2015. Vagotomy and subsequent risk of Parkinson's disease. *Ann. Neurol.* 78:522–29
33. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM, et al. 2011. Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. *PNAS* 108:16050–55
34. Breit S, Kupferberg A, Rogler G, Hasler G. 2018. Vagus nerve as modulator of the brain-gut axis in psychiatric and inflammatory disorders. *Front. Psychiatry* 9:44
35. Daban C, Martinez-Aran A, Cruz N, Vieta E. 2008. Safety and efficacy of vagus nerve stimulation in treatment-resistant depression: a systematic review. *J. Affect. Disord.* 110:1–15
36. Cristancho P, Cristancho MA, Baltuch GH, Thase ME, O'Reardon JP. 2011. Effectiveness and safety of Vagus Nerve Stimulation for severe treatment-resistant major depression in clinical practice after FDA approval: outcomes at 1 year. *J. Clin. Psychiatry* 72:1376–82
37. Han W, Tellez LA, Perkins MH, Perez IO, Qu T, et al. 2018. A neural circuit for gut-induced reward. *Cell* 175:665–78.e23
38. Kaelberer MM, Buchanan KL, Klein ME, Barth BB, Montoya MM, et al. 2018. A gut-brain neural circuit for nutrient sensory transduction. *Science* 361:eaat5236
39. Bellono NW, Bayrer JR, Leitch DB, Castro J, Zhang C, et al. 2017. Enterochromaffin cells are gut chemosensors that couple to sensory neural pathways. *Cell* 170:185–98.e16
40. Kelly JR, Borre Y, O'Brien C, Patterson E, El Aidy S, et al. 2016. Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. *J. Psychiatr. Res.* 82:109–18
41. Allen AP, Dinan TG, Clarke G, Cryan JF. 2017. A psychology of the human brain-gut-microbiome axis. *Soc. Personal. Psychol. Compass* 11:e12309
42. Bharwani A, Mian MF, Foster JA, Surette MG, Bienenstock J, Forsythe P. 2016. Structural & functional consequences of chronic psychosocial stress on the microbiome & host. *Psychoneuroendocrinology* 63:217–27
43. Gareau MG, Jury J, MacQueen G, Sherman PM, Perdue MH. 2007. Probiotic treatment of rat pups normalises corticosterone release and ameliorates colonic dysfunction induced by maternal separation. *Gut* 56:1522–28
44. Golubeva AV, Crampton S, Desbonnet L, Edge D, O'Sullivan O, et al. 2015. Prenatal stress-induced alterations in major physiological systems correlate with gut microbiota composition in adulthood. *Psychoneuroendocrinology* 60:58–74
45. O'Mahony SM, Hyland NP, Dinan TG, Cryan JF. 2011. Maternal separation as a model of brain-gut axis dysfunction. *Psychopharmacology* 214:71–88
46. Partrick KA, Chassaing B, Beach LQ, McCann KE, Gewirtz AT, Huhman KL. 2018. Acute and repeated exposure to social stress reduces gut microbiota diversity in Syrian hamsters. *Behav. Brain Res.* 345:39–48
47. Bailey MT, Coe CL. 1999. Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. *Dev. Psychobiol.* 35:146–55
48. Bailey MT, Lubach GR, Coe CL. 2004. Prenatal stress alters bacterial colonization of the gut in infant monkeys. *J. Pediatr. Gastroenterol. Nutr.* 38:414–21
49. Foster JA, Rinaman L, Cryan JF. 2017. Stress & the gut-brain axis: regulation by the microbiome. *Neurobiol. Stress* 7:124–36
50. Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N, et al. 2004. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. *J. Physiol.* 558:263–75
51. Clarke G, Grenham S, Scully P, Fitzgerald P, Moloney RD, et al. 2013. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol. Psychiatry* 18:666–73

52. Kennedy PJ, Cryan JF, Dinan TG, Clarke G. 2014. Irritable bowel syndrome: a microbiome-gut-brain axis disorder? *World J. Gastroenterol.* 20:14105–25
53. Labus JS, Hollister EB, Jacobs J, Kirbach K, Oezguen N, et al. 2017. Differences in gut microbial composition correlate with regional brain volumes in irritable bowel syndrome. *Microbiome* 5:49
54. Zijlmans MA, Korpela K, Riksen-Walraven JM, de Vos WM, de Weerth C. 2015. Maternal prenatal stress is associated with the infant intestinal microbiota. *Psychoneuroendocrinology* 53:233–45
55. Naseribafrouei A, Hestad K, Avershina E, Sekelja M, Linlokken A, et al. 2014. Correlation between the human fecal microbiota and depression. *Neurogastroenterol. Motil.* 26:1155–62
56. Zheng P, Zeng B, Zhou C, Liu M, Fang Z, et al. 2016. Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol. Psychiatry* 21:786–96
57. Aizawa E, Tsuji H, Asahara T, Takahashi T, Teraishi T, et al. 2016. Possible association of *Bifidobacterium* and *Lactobacillus* in the gut microbiota of patients with major depressive disorder. *J. Affect. Disord.* 202:254–57
58. Jiang H, Ling Z, Zhang Y, Mao H, Ma Z, et al. 2015. Altered fecal microbiota composition in patients with major depressive disorder. *Brain Behav. Immun.* 48:186–94
59. Huang Y, Shi X, Li Z, Shen Y, Shi X, et al. 2018. Possible association of Firmicutes in the gut microbiota of patients with major depressive disorder. *Neuropsychiatr. Dis. Treat.* 14:3329–37
60. Valles-Colomer M, Falony G, Darzi Y, Tigchelaar EF, Wang J, et al. 2019. The neuroactive potential of the human gut microbiota in quality of life and depression. *Nat. Microbiol.* 4:623–32
61. Dinan TG, Cryan JF. 2019. Gut microbes and depression: still waiting for Godot. *Brain Behav. Immun.* 79:1–2
62. Unger MM, Spiegel J, Dillmann KU, Grundmann D, Philippeit H, et al. 2016. Short chain fatty acids and gut microbiota differ between patients with Parkinson's disease and age-matched controls. *Parkinsonism Relat. Disord.* 32:66–72
63. Bedarf JR, Hildebrand F, Coelho LP, Sunagawa S, Bahram M, et al. 2017. Functional implications of microbial and viral gut metagenome changes in early stage L-DOPA-naive Parkinson's disease patients. *Genome Med.* 9:39
64. Hasegawa S, Goto S, Tsuji H, Okuno T, Asahara T, et al. 2015. Intestinal dysbiosis and lowered serum lipopolysaccharide-binding protein in Parkinson's disease. *PLOS ONE* 10:e0142164
65. Hill-Burns EM, Debelius JW, Morton JT, Wissemann WT, Lewis MR, et al. 2017. Parkinson's disease and Parkinson's disease medications have distinct signatures of the gut microbiome. *Mov. Disord.* 32:739–49
66. Keshavarzian A, Green SJ, Engen PA, Voigt RM, Naqib A, et al. 2015. Colonic bacterial composition in Parkinson's disease. *Mov. Disord.* 30:1351–60
67. Scheperjans F, Aho V, Pereira PA, Koskinen K, Paulin L, et al. 2015. Gut microbiota are related to Parkinson's disease and clinical phenotype. *Mov. Disord.* 30:350–58
68. Sampson TR, Debelius JW, Thron T, Janssen S, Shastri GG, et al. 2016. Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson's disease. *Cell* 167:1469–80.e12
69. Braak H, de Vos RA, Bohl J, Del Tredici K. 2006. Gastric α-synuclein immunoreactive inclusions in Meissner's and Auerbach's plexuses in cases staged for Parkinson's disease-related brain pathology. *Neurosci. Lett.* 396:67–72
70. Liu B, Fang F, Pedersen NL, Tillander A, Ludvigsson JF, et al. 2017. Vagotomy and Parkinson disease: a Swedish register–based matched-cohort study. *Neurology* 88:1996–2002
71. Dobbs SM, Dobbs RJ, Weller C, Charlett A, Bjarnason IT, et al. 2010. Differential effect of *Helicobacter pylori* eradication on time-trends in brady/hypokinesia and rigidity in idiopathic parkinsonism. *Helicobacter* 15:279–94
72. Shen X, Yang H, Wu Y, Zhang D, Jiang H. 2017. Meta-analysis: association of *Helicobacter pylori* infection with Parkinson's diseases. *Helicobacter* 22:e12398
73. Dardiotis E, Tsouris Z, Mentis AA, Siokas V, Michalopoulou A, et al. 2018. *H. pylori* and Parkinson's disease: meta-analyses including clinical severity. *Clin. Neurol. Neurosurg.* 175:16–24
74. Vogt NM, Kerby RL, Dill-McFarland KA, Harding SJ, Merluzzi AP, et al. 2017. Gut microbiome alterations in Alzheimer's disease. *Sci. Rep.* 7:13537

75. Zhuang ZQ, Shen LL, Li WW, Fu X, Zeng F, et al. 2018. Gut microbiota is altered in patients with Alzheimer's disease. *J. Alzheimers Dis.* 63:1337–46
76. Arnold SE, Arvanitakis Z, Macauley-Rambach SL, Koenig AM, Wang HY, et al. 2018. Brain insulin resistance in type 2 diabetes and Alzheimer disease: concepts and conundrums. *Nat. Rev. Neurol.* 14:168–81
77. Nho K, Kueider-Paisley A, MahmoudianDehkordi S, Arnold M, Risacher SL, et al. 2019. Altered bile acid profile in mild cognitive impairment and Alzheimer's disease: relationship to neuroimaging and CSF biomarkers. *Alzheimers Dement*. 15:232–44
78. Manderino L, Carroll I, Azcarate-Peril MA, Rochette A, Heinberg L, et al. 2017. Preliminary evidence for an association between the composition of the gut microbiome and cognitive function in neurologically healthy older adults. *J. Int. Neuropsychol. Soc.* 23:700–5
79. Cattaneo A, Cattane N, Galluzzi S, Provasi S, Lopizzo N, et al. 2017. Association of brain amyloidosis with pro-inflammatory gut bacterial taxa and peripheral inflammation markers in cognitively impaired elderly. *Neurobiol. Aging* 49:60–68
80. Severance EG, Prandovszky E, Castiglione J, Yolken RH. 2015. Gastroenterology issues in schizophrenia: why the gut matters. *Curr. Psychiatry Rep.* 17:27
81. Severance EG, Gressitt KL, Stallings CR, Katsafanas E, Schweinfurth LA, et al. 2017. Probiotic normalization of *Candida albicans* in schizophrenia: a randomized, placebo-controlled, longitudinal pilot study. *Brain Behav. Immun.* 62:41–45
82. Zheng P, Zeng B, Liu M, Chen J, Pan J, et al. 2019. The gut microbiome from patients with schizophrenia modulates the glutamate-glutamine-GABA cycle and schizophrenia-relevant behaviors in mice. *Sci. Adv.* 5:eaau8317
83. Coury DL, Ashwood P, Fasano A, Fuchs G, Geraghty M, et al. 2012. Gastrointestinal conditions in children with autism spectrum disorder: developing a research agenda. *Pediatrics* 130(Suppl. 2):S160–68
84. Strati F, Cavalieri D, Albanese D, De Felice C, Donati C, et al. 2017. New evidences on the altered gut microbiota in autism spectrum disorders. *Microbiome* 5:24
85. Mayer EA, Padua D, Tillisch K. 2014. Altered brain-gut axis in autism: comorbidity or causative mechanisms? *Bioessays* 36:933–39
86. Coretti L, Paparo L, Riccio MP, Amato F, Cuomo M, et al. 2018. Gut microbiota features in young children with autism spectrum disorders. *Front. Microbiol.* 9:3146
87. Sandler RH, Finegold SM, Bolte ER, Buchanan CP, Maxwell AP, et al. 2000. Short-term benefit from oral vancomycin treatment of regressive-onset autism. *J. Child Neurol.* 15:429–35
88. Kang DW, Adams JB, Gregory AC, Borody T, Chittick L, et al. 2017. Microbiota transfer therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. *Microbiome* 5:10
89. Aizawa E, Tsuji H, Asahara T, Takahashi T, Teraishi T, et al. 2018. *Bifidobacterium* and *Lactobacillus* counts in the gut microbiota of patients with bipolar disorder and healthy controls. *Front. Psychiatry* 9:730
90. Hemmings SMJ, Malan-Muller S, van den Heuvel LL, Demmitt BA, Stanislawski MA, et al. 2017. The microbiome in posttraumatic stress disorder and trauma-exposed controls: an exploratory study. *Psychosom. Med.* 79:936–46
91. Dinan TG, Stanton C, Cryan JF. 2013. Psychobiotics: a novel class of psychotropic. *Biol. Psychiatry* 74:720–26
92. Cussotto S, Clarke G, Dinan TG, Cryan JF. 2019. Psychotropics and the microbiome: a chamber of secrets. *Psychopharmacology* 236:1411–32
93. Butler MI, Sandhu K, Cryan JF, Dinan TG. 2019. From isoniazid to psychobiotics: the gut microbiome as a new antidepressant target. *Br. J. Hosp. Med.* 80:139–45
94. Kelly JR, Allen AP, Temko A, Hutch W, Kennedy PJ, et al. 2017. Lost in translation? The potential psychobiotic *Lactobacillus rhamnosus* (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. *Brain Behav. Immun.* 61:50–59
95. Ng QX, Peters C, Ho CYX, Lim DY, Yeo WS. 2018. A meta-analysis of the use of probiotics to alleviate depressive symptoms. *J. Affect. Disord.* 228:13–19

96. Ostlund-Lagerstrom L, Kihlgren A, Repsilber D, Bjorksten B, Brummer RJ, Schoultz I. 2016. Probiotic administration among free-living older adults: a double blinded, randomized, placebo-controlled clinical trial. *Nutr. J.* 15:80
97. Bambury A, Sandhu K, Cryan JF, Dinan TG. 2018. Finding the needle in the haystack: systematic identification of psychobiotics. *Br. J. Pharmacol.* 175:4430–38
98. O'Toole PW, Marchesi JR, Hill C. 2017. Next-generation probiotics: the spectrum from probiotics to live biotherapeutics. *Nat. Microbiol.* 2:17057
99. Benton D, Williams C, Brown A. 2007. Impact of consuming a milk drink containing a probiotic on mood and cognition. *Eur. J. Clin. Nutr.* 61:355–61
100. Akkasheh G, Kashani-Poor Z, Tajabadi-Ebrahimi M, Jafari P, Akbari H, et al. 2016. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: a randomized, double-blind, placebo-controlled trial. *Nutrition* 32:315–20
101. Kazemi A, Noorbala AA, Azam K, Eskandari MH, Djafarian K. 2018. Effect of probiotic and prebiotic versus placebo on psychological outcomes in patients with major depressive disorder: a randomized clinical trial. *Clin. Nutr.* 38:522–28
102. Pinto-Sanchez MI, Hall GB, Ghajar K, Nardelli A, Bolino C, et al. 2017. Probiotic *Bifidobacterium longum* NCC3001 reduces depression scores and alters brain activity: a pilot study in patients with irritable bowel syndrome. *Gastroenterology* 153:448–59.e8
103. Messaoudi M, Violle N, Bisson JF, Desor D, Javelot H, Rougeot C. 2011. Beneficial psychological effects of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in healthy human volunteers. *Gut Microbes* 2:256–61
104. Diop L, Guillou S, Durand H. 2008. Probiotic food supplement reduces stress-induced gastrointestinal symptoms in volunteers: a double-blind, placebo-controlled, randomized trial. *Nutr. Res.* 28:1–5
105. Colica C, Avolio E, Bollero P, Costa de Miranda R, Ferraro S, et al. 2017. Evidences of a new psychobiotic formulation on body composition and anxiety. *Mediators Inflamm.* 2017:5650627
106. Steenbergen L, Sellaro R, van Hemert S, Bosch JA, Colzato LS. 2015. A randomized controlled trial to test the effect of multispecies probiotics on cognitive reactivity to sad mood. *Brain Behav. Immun.* 48:258–64
107. Slykerman RF, Hood F, Wickens K, Thompson JMD, Barthow C, et al. 2017. Effect of *Lactobacillus rhamnosus* HN001 in pregnancy on postpartum symptoms of depression and anxiety: a randomised double-blind placebo-controlled trial. *EBioMedicine* 24:159–65
108. Allen AP, Hutch W, Borre YE, Kennedy PJ, Temko A, et al. 2016. *Bifidobacterium longum* 1714 as a translational psychobiotic: modulation of stress, electrophysiology and neurocognition in healthy volunteers. *Transl. Psychiatry* 6:e939
109. Takada M, Nishida K, Kataoka-Kato A, Gondo Y, Ishikawa H, et al. 2016. Probiotic *Lactobacillus casei* strain Shirota relieves stress-associated symptoms by modulating the gut-brain interaction in human and animal models. *Neurogastroenterol. Motil.* 28:1027–36
110. Rao AV, Bested AC, Beaulne TM, Katzman MA, Iorio C, et al. 2009. A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. *Gut Pathog.* 1:6
111. Andersson H, Tullberg C, Ahrne S, Hamberg K, Lazou Ahren I, et al. 2016. Oral administration of *Lactobacillus plantarum* 299v reduces cortisol levels in human saliva during examination induced stress: a randomized, double-blind controlled trial. *Int. J. Microbiol.* 2016:8469018
112. Mohammadi AA, Jazayeri S, Khosravi-Darani K, Solati Z, Mohammadpour N, et al. 2016. The effects of probiotics on mental health and hypothalamic-pituitary-adrenal axis: a randomized, double-blind, placebo-controlled trial in petrochemical workers. *Nutr. Neurosci.* 19:387–95
113. Nishihira J, Kagami-Katsuyama H, Tanaka A, Nishimura M, Kobayashi T, Kawasaki Y. 2014. Elevation of natural killer cell activity and alleviation of mental stress by the consumption of yogurt containing *Lactobacillus gasseri* SBT2055 and *Bifidobacterium longum* SBT2928 in a double-blind, placebo-controlled clinical trial. *J. Funct. Foods* 11:261–68
114. Chong HX, Yusoff NAA, Hor YY, Lew LC, Jaafar MH, et al. 2019. *Lactobacillus plantarum* DR7 alleviates stress and anxiety in adults: a randomised, double-blind, placebo-controlled study. *Benef. Microbes* 10:355–73

115. Miyaoka T, Kanayama M, Wake R, Hashioka S, Hayashida M, et al. 2018. *Clostridium butyricum* MIYAIRI 588 as adjunctive therapy for treatment-resistant major depressive disorder: a prospective open-label trial. *Clin. Neuropharmacol.* 41:151–55
116. Wallace CJK, Milev R. 2017. The effects of probiotics on depressive symptoms in humans: a systematic review. *Ann. Gen. Psychiatry* 16:14
117. Pirbaglou M, Katz J, de Souza RJ, Stearns JC, Motamed M, Ritvo P. 2016. Probiotic supplementation can positively affect anxiety and depressive symptoms: a systematic review of randomized controlled trials. *Nutr. Res.* 36:889–98
118. Huang R, Wang K, Hu J. 2016. Effect of probiotics on depression: a systematic review and meta-analysis of randomized controlled trials. *Nutrients* 8:483
119. Chung YC, Jin HM, Cui Y, Kim DS, Jung JM, et al. 2014. Fermented milk of *Lactobacillus helveticus* IDCC3801 improves cognitive functioning during cognitive fatigue tests in healthy older adults. *J. Funct. Foods* 10:465–74
120. Romijn AR, Rucklidge JJ, Kuijer RG, Frampton C. 2017. A double-blind, randomized, placebo-controlled trial of *Lactobacillus helveticus* and *Bifidobacterium longum* for the symptoms of depression. *Aust. N. Z. J. Psychiatry* 51:810–21
121. Akbari E, Asemi Z, Daneshvar Kakhaki R, Bahmani F, Kouchaki E, et al. 2016. Effect of probiotic supplementation on cognitive function and metabolic status in Alzheimer's disease: a randomized, double-blind and controlled trial. *Front. Aging Neurosci.* 8:256
122. Agahi A, Hamidi GA, Daneshvar R, Hamdieh M, Soheili M, et al. 2018. Does severity of Alzheimer's disease contribute to its responsiveness to modifying gut microbiota? A double blind clinical trial. *Front. Neurol.* 9:662
123. Rudzki L, Ostrowska L, Pawlak D, Malus A, Pawlak K, et al. 2018. Probiotic *Lactobacillus plantarum* 299v decreases kynurenine concentration and improves cognitive functions in patients with major depression: a double-blind, randomized, placebo controlled study. *Psychoneuroendocrinology* 100:213–22
124. Ceccarelli G, Brenchley JM, Cavallari EN, Scheri GC, Fratino M, et al. 2017. Impact of high-dose multi-strain probiotic supplementation on neurocognitive performance and central nervous system immune activation of HIV-1 infected individuals. *Nutrients* 9:1269
125. Bagga D, Reichert JL, Koschutnig K, Aigner CS, Holzer P, et al. 2018. Probiotics drive gut microbiome triggering emotional brain signatures. *Gut Microbes* 9:486–96
126. Wang X, Yang J, Zhang H, Yu J, Yao Z. 2019. Oral probiotic administration during pregnancy prevents autism-related behaviors in offspring induced by maternal immune activation via anti-inflammation in mice. *Autism Res.* 12:576–88
127. El-Ansary A, Bacha AB, Bjorklund G, Al-Orf N, Bhat RS, et al. 2018. Probiotic treatment reduces the autistic-like excitation/inhibition imbalance in juvenile hamsters induced by orally administered propionic acid and clindamycin. *Metab. Brain Dis.* 33:1155–64
128. Patusco R, Ziegler J. 2018. Role of probiotics in managing gastrointestinal dysfunction in children with autism spectrum disorder: an update for practitioners. *Adv. Nutr.* 9:637–50
129. Shaaban SY, El Gendy YG, Mehanna NS, El-Senousy WM, El-Feki HSA, et al. 2018. The role of probiotics in children with autism spectrum disorder: a prospective, open-label study. *Nutr. Neurosci.* 21:676–81
130. Dickerson FB, Stallings C, Origoni A, Katsafanas E, Savage CL, et al. 2014. Effect of probiotic supplementation on schizophrenia symptoms and association with gastrointestinal functioning: a randomized, placebo-controlled trial. *Prim. Care Companion CNS Disord.* 16:PCC.13m01579
131. Tomasik J, Yolken RH, Bahn S, Dickerson FB. 2015. Immunomodulatory effects of probiotic supplementation in schizophrenia patients: a randomized, placebo-controlled trial. *Biomark Insights* 10:47–54
132. Okubo R, Koga M, Katsumata N, Odamaki T, Matsuyama S, et al. 2019. Effect of *Bifidobacterium breve* A-1 on anxiety and depressive symptoms in schizophrenia: a proof-of-concept study. *J. Affect. Disord.* 245:377–85
133. Gibson GR, Hutkins R, Sanders ME, Prescott SL, Reimer RA, et al. 2017. Expert consensus document: the International Scientific Association for Probiotics and Prebiotics (ISAPP) consensus statement on the definition and scope of prebiotics. *Nat. Rev. Gastroenterol. Hepatol.* 14:491–502

134. Verkhnyatskaya S, Ferrari M, de Vos P, Walvoort MTC. 2019. Shaping the infant microbiome with non-digestible carbohydrates. *Front. Microbiol.* 10:343
135. Schmidt K, Cowen PJ, Harmer CJ, Tzortzis G, Errington S, Burnet PW. 2015. Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. *Psychopharmacology* 232:1793–801
136. Silk DB, Davis A, Vulevic J, Tzortzis G, Gibson GR. 2009. Clinical trial: the effects of a trans-galactooligosaccharide prebiotic on faecal microbiota and symptoms in irritable bowel syndrome. *Aliment Pharmacol. Ther.* 29:508–18
137. Grimaldi R, Gibson GR, Vulevic J, Giallourou N, Castro-Mejia JL, et al. 2018. A prebiotic intervention study in children with autism spectrum disorders (ASDs). *Microbiome* 6:133
138. Flowers SA, Baxter NT, Ward KM, Kraal AZ, McInnis MG, et al. 2019. Effects of atypical antipsychotic treatment and resistant starch supplementation on gut microbiome composition in a cohort of patients with bipolar disorder or schizophrenia. *Pharmacotherapy* 39:161–70
139. Barichella M, Pacchetti C, Bolliri C, Cassani E, Iorio L, et al. 2016. Probiotics and prebiotic fiber for constipation associated with Parkinson disease: an RCT. *Neurology* 87:1274–80
140. Gocan AG, Bachg D, Schindler AE, Rohr UD. 2012. Balancing steroidal hormone cascade in treatment-resistant veteran soldiers with PTSD using a fermented soy product (FSWW08): a pilot study. *Horm. Mol. Biol. Clin. Investig.* 10:301–14
141. Sanctuary MR, Kain JN, Chen SY, Kalanetra K, Lemay DG, et al. 2019. Pilot study of probiotic/colostrum supplementation on gut function in children with autism and gastrointestinal symptoms. *PLOS ONE* 14:e0210064
142. Sanna S, van Zuydam NR, Mahajan A, Kurilshikov A, Vich Vila A, et al. 2019. Causal relationships among the gut microbiome, short-chain fatty acids and metabolic diseases. *Nat. Genet.* 51:600–5
143. van de Wouw M, Boehme M, Lyte JM, Wiley N, Strain C, et al. 2018. Short-chain fatty acids: microbial metabolites that alleviate stress-induced brain-gut axis alterations. *J. Physiol.* 596:4923–44
144. Shultz SR, MacFabe DF, Ossenkopp KP, Scratch S, Whelan J, et al. 2008. Intracerebroventricular injection of propionic acid, an enteric bacterial metabolic end-product, impairs social behavior in the rat: implications for an animal model of autism. *Neuropharmacology* 54:901–11
145. Lach G, Schellekens H, Dinan TG, Cryan JF. 2018. Anxiety, depression, and the microbiome: a role for gut peptides. *Neurotherapeutics* 15:36–59
146. de Almada CN, Almada CN, Martinez RCR, Sant'Ana AS. 2016. Paraprobiotics: evidences on their ability to modify biological responses, inactivation methods and perspectives on their application in foods. *Trends Food Sci. Technol.* 58:96–114
147. Nishida K, Sawada D, Kuwano Y, Tanaka H, Sugawara T, et al. 2017. Daily administration of paraprobiotic *Lactobacillus gasseri* CP2305 ameliorates chronic stress-associated symptoms in Japanese medical students. *J. Funct. Foods* 36:112–21
148. Wei CL, Wang S, Yen JT, Cheng YF, Liao CL, et al. 2019. Antidepressant-like activities of live and heat-killed *Lactobacillus paracasei* PS23 in chronic corticosterone-treated mice and possible mechanisms. *Brain Res.* 1711:202–13
149. Liu WH, Chuang HL, Huang YT, Wu CC, Chou GT, et al. 2016. Alteration of behavior and monoamine levels attributable to *Lactobacillus plantarum* PS128 in germ-free mice. *Behav. Brain Res.* 298:202–9
150. Aslam H, Green J, Jacka FN, Collier F, Berk M, et al. 2018. Fermented foods, the gut and mental health: a mechanistic overview with implications for depression and anxiety. *Nutr. Neurosci.* In press. **https://doi.org/10.1080/1028415X.2018.1544332**
151. Tillisch K, Labus J, Kilpatrick L, Jiang Z, Stains J, et al. 2013. Consumption of fermented milk product with probiotic modulates brain activity. *Gastroenterology* 144:1394–401.e4
152. Kato-Kataoka A, Nishida K, Takada M, Suda K, Kawai M, et al. 2016. Fermented milk containing *Lactobacillus casei* strain Shirota prevents the onset of physical symptoms in medical students under academic examination stress. *Benef. Microbes* 7:153–56
153. Simren M, Ohman L, Olsson J, Svensson U, Ohlson K, et al. 2010. Clinical trial: the effects of a fermented milk containing three probiotic bacteria in patients with irritable bowel syndrome—a randomized, double-blind, controlled study. *Aliment Pharmacol. Ther.* 31:218–27

154. Lassale C, Batty GD, Baghdadli A, Jacka F, Sanchez-Villegas A, et al. 2018. Healthy dietary indices and risk of depressive outcomes: a systematic review and meta-analysis of observational studies. *Mol. Psychiatry* 24:965–86
155. Jacka FN, Pasco JA, Mykletun A, Williams LJ, Hodge AM, et al. 2010. Association of Western and traditional diets with depression and anxiety in women. *Am. J. Psychiatry* 167:305–11
156. Psaltopoulou T, Sergentanis TN, Panagiotakos DB, Sergentanis IN, Kosti R, Scarmeas N. 2013. Mediterranean diet, stroke, cognitive impairment, and depression: a meta-analysis. *Ann. Neurol.* 74:580–91
157. Li Y, Lv MR, Wei YJ, Sun L, Zhang JX, et al. 2017. Dietary patterns and depression risk: a meta-analysis. *Psychiatry Res.* 253:373–82
158. Molendijk M, Molero P, Sanchez-Pedreno FO, Van der Does W, Martinez-Gonzalez MA. 2018. Diet quality and depression risk: a systematic review and dose-response meta-analysis of prospective studies. *J. Affect. Disord.* 226:346–54
159. Shafiei F, Salari-Moghaddam A, Larijani B, Esmaillzadeh A. 2019. Adherence to the Mediterranean diet and risk of depression: a systematic review and updated meta-analysis of observational studies. *Nutr. Rev.* 77:230–39
160. Jacka FN, O'Neil A, Opie R, Itsiopoulos C, Cotton S, et al. 2017. A randomised controlled trial of dietary improvement for adults with major depression (the 'SMILES' trial). *BMC Med.* 15:23
161. Chatterton ML, Mihalopoulos C, O'Neil A, Itsiopoulos C, Opie R, et al. 2018. Economic evaluation of a dietary intervention for adults with major depression (the "SMILES" trial). *BMC Public Health* 18:599
162. Forsyth A, Deane FP, Williams P. 2015. A lifestyle intervention for primary care patients with depression and anxiety: a randomised controlled trial. *Psychiatry Res.* 230:537–44
163. Parletta N, Zarnowiecki D, Cho J, Wilson A, Bogomolova S, et al. 2019. A Mediterranean-style dietary intervention supplemented with fish oil improves diet quality and mental health in people with depression: a randomized controlled trial (HELFIMED). *Nutr. Neurosci.* 22:474–87
164. Firth J, Marx W, Dash S, Carney R, Teasdale SB, et al. 2019. The effects of dietary improvement on symptoms of depression and anxiety: a meta-analysis of randomized controlled trials. *Psychosom. Med.* 81:265–80
165. Morkl S, Wagner-Skacel J, Lahousen T, Lackner S, Holasek SJ, et al. 2018. The role of nutrition and the gut-brain axis in psychiatry: a review of the literature. *Neuropsychobiology*. In press. **https://doi.org/10.1159/000492834**
166. Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, et al. 2015. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* 528:262–66
167. Imhann F, Bonder MJ, Vich Vila A, Fu J, Mujagic Z, et al. 2016. Proton pump inhibitors affect the gut microbiome. *Gut* 65:740–48
168. Freedberg DE, Toussaint NC, Chen SP, Ratner AJ, Whittier S, et al. 2015. Proton pump inhibitors alter specific taxa in the human gastrointestinal microbiome: a crossover trial. *Gastroenterology* 149:883–85.e9
169. Jackson MA, Goodrich JK, Maxan ME, Freedberg DE, Abrams JA, et al. 2016. Proton pump inhibitors alter the composition of the gut microbiota. *Gut* 65:749–56
170. Takagi T, Naito Y, Inoue R, Kashiwagi S, Uchiyama K, et al. 2018. The influence of long-term use of proton pump inhibitors on the gut microbiota: an age-sex-matched case-control study. *J. Clin. Biochem. Nutr.* 62:100–5
171. Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, et al. 2018. Extensive impact of non-antibiotic drugs on human gut bacteria. *Nature* 555:623–28
172. Cussotto S, Strain CR, Fouhy F, Strain RG, Peterson VL, et al. 2018. Differential effects of psychotropic drugs on microbiome composition and gastrointestinal function. *Psychopharmacology* 236:1671–85
173. Dinan TG, Cryan JF. 2018. Schizophrenia and the microbiome: time to focus on the impact of antipsychotic treatment on the gut microbiota. *World J. Biol. Psychiatry* 19:568–70
174. Davey KJ, Cotter PD, O'Sullivan O, Crispie F, Dinan TG, et al. 2013. Antipsychotics and the gut microbiome: olanzapine-induced metabolic dysfunction is attenuated by antibiotic administration in the rat. *Transl. Psychiatry* 3:e309

175. Davey KJ, O'Mahony SM, Schellekens H, O'Sullivan O, Bienenstock J, et al. 2012. Gender-dependent consequences of chronic olanzapine in the rat: effects on body weight, inflammatory, metabolic and microbiota parameters. *Psychopharmacology* 221:155–69
176. Lyte M, Daniels KM, Schmitz-Esser S. 2019. Fluoxetine-induced alteration of murine gut microbial community structure: evidence for a microbial endocrinology-based mechanism of action responsible for fluoxetine-induced side effects. *PeerJ* 7:e6199
177. Liskiewicz P, Pelka-Wysiecka J, Kaczmarczyk M, Loniewski I, Wronski M, et al. 2019. Fecal microbiota analysis in patients going through a depressive episode during treatment in a psychiatric hospital setting. *J. Clin. Med.* 8:164
178. van Kessel SP, Frye AK, El-Gendy AO, Castejon M, Keshavarzian A, et al. 2019. Gut bacterial tyrosine decarboxylases restrict levels of levodopa in the treatment of Parkinson's disease. *Nat. Commun.* 10:310
179. Kao AC, Spitzer S, Anthony DC, Lennox B, Burnet PWJ. 2018. Prebiotic attenuation of olanzapine-induced weight gain in rats: analysis of central and peripheral biomarkers and gut microbiota. *Transl. Psychiatry* 8:66
180. Kumar H, Salminen S, Verhagen H, Rowland I, Heimbach J, et al. 2015. Novel probiotics and prebiotics: road to the market. *Curr. Opin. Biotechnol.* 32:99–103
181. Sanders ME, Akkermans LM, Haller D, Hammerman C, Heimbach J, et al. 2010. Safety assessment of probiotics for human use. *Gut Microbes* 1:164–85
182. Herman L, Chemaly M, Cocconcelli PS, Fernandez P, Klein G, et al. 2019. The qualified presumption of safety assessment and its role in EFSA risk evaluations: 15 years past. *FEMS Microbiol. Lett.* 366:fny260
183. Cryan JF, Clarke G, Dinan TG, Schellekens H. 2018. A microbial drugstore for motility. *Cell Host Microbe* 23:691–92