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Man and the Microbiome: A New Theory of Everything?

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Abstract

The gut microbiome is implicated in the pathophysiology of a wide range of psychological disorders. Preclinical studies have provided us with key insights into the mechanisms by which the microbiome influences bidirectional gut–brain communication. There are many signaling pathways involved, including the hypothalamic–pituitary–adrenal axis, immune modulation, tryptophan and serotonin metabolism, bile acid transformation, microbial production of neuroactive compounds, and regulation of the endocannabinoid system. The complex and widespread influence of the microbiome on many physiological and psychological processes has generated a keen interest in its therapeutic potential for depression, anxiety, autism, and other psychiatric disorders. It has been shown that the microbiome composition of people suffering with such conditions differs significantly from that of healthy controls, and although the area is in its infancy, interventional studies that alter a person's microbiome through the use of probiotics, prebiotics, or dietary change can alleviate psychopathological symptoms.

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INTRODUCTION

Everybody is interested in the gut microbiome. From cardiology to oncology, from psychiatry to endocrinology, health-care professionals of all disciplines are beginning to realize the immense potential of the trillions of bacteria that reside in our gut. Microbiome research has exploded during the past two decades and presents a new paradigm from which to approach many of the common diseases that characterize the modern world. Our gut bacteria have been implicated in virtually all disease states, including obesity, diabetes, cancer, heart disease, asthma, allergies, depression, autism, Alzheimer's disease, and the list goes on. Many of these noncommunicable diseases share broad pathophysiological associations, such as immune dysregulation, a dysfunctional stress response, and lifestyle factors, including diet, exercise, and alcohol or tobacco use. The influence of the gut microbiome spans all of these domains. Physicists have long been seeking a theory of everything, a single, hypothetical, all-encompassing framework that explains and links together all physical aspects of the universe. Medicine may have found its own ultimate theory in the gut microbiome.

Microbiome: the collective genomes of the microorganisms found at a particular location

The relationship between psychological functioning and physical symptomatology has long been appreciated, although ideas regarding the nature of this relationship have changed dramatically over time. Freudian theory dominated psychiatric thinking in the early twentieth century and promoted the concept of conversion or hysteria. Freud hypothesized that unresolved emotional conflicts were converted into physical disorders and could account for many physical conditions [Breuer & Freud 2004 (1895)]. While psychiatry has moved beyond the case reports that

characterized Freudian psychoanalysis to a more evidence-based medical model of thinking, the concept of the mind–body link in the causation of disease is more relevant than ever. This is particularly true when it comes to the gastrointestinal tract (GIT). Irritable bowel syndrome (IBS) is the archetype of functional gastrointestinal disorders. While the etiology of this condition is unclear, it is well recognized that rates of psychiatric comorbidity, especially depression and anxiety, are extremely high (Whitehead et al. 2002), suggesting a significant etiological role for psychological factors. The same is true for many common, nonfunctional gastrointestinal disorders, including inflammatory bowel disease (IBD) (Graff et al. 2009), celiac disease (Zingone et al. 2015), and peptic ulceration (Lim et al. 2014). Although Freud was adept at dramatic theorizing, he was at least partially correct when suggesting that psychological stress or trauma could produce physical symptoms.

The bidirectional gut–brain axis allows for the top-down influence of our brain and emotional states on gastrointestinal homeostasis and function, as well as a bottom-up modulation of brain function and behavior via neural, endocrine, and immune systems. We have recently come to realize that our gut bacteria are a vital node in this signaling system. Approximately $1 \times 10^{13–14}$ bacteria reside in the human gut, a staggering number, especially when one considers that our gut bacteria far outnumber our own human cells. The presence of the human gut microbiota has long been known, but it was presumed that these bacteria were commensal organisms, that is, unharmful to but unhelpful for the human host. It is now recognized that this was a gross underappreciation of what is actually a complex symbiotic interaction that influences almost all human physiological, and many psychological, processes.

It is an exciting time in microbiome research. We are in great need of a new paradigm in our approach to mental health and psychological disorders. Depression and anxiety are the tuberculosis and cholera of the twenty-first century. The lifetime prevalence of major depressive disorder (MDD) is more than 10% (Lim et al. 2018), with lifetime anxiety disorders far in excess of this number, at estimated rates as high as 33.7% (Kessler et al. 2012). The World Health Organization predicts that by 2020 depression will rank second in global disease burdens, behind only ischemic heart disease. Could an etiological clue, and possibly a therapeutic answer, to this psychological epidemic reside in our gut? Could probiotics or prebiotics become a new class of antidepressant? Perhaps we are as guilty as Freud of wild hypothesizing, but the rapidly accumulating evidence would suggest that our interest and hope in the gut microbiome are merited.

THE MICROBIOME–GUT–BRAIN AXIS: MECHANISMS OF COMMUNICATION

During the past 15 years there has been a huge proliferation of preclinical studies attempting to elucidate the mechanisms by which the gut microbiome communicates with, and influences, the brain. Although we tend to view this work as a twenty-first century matter, an appreciation that gut microbes could alter brain chemistry was actually noted as far back as 1986. In a study designed to assess the influence of nephrectomy (removal of a kidney) on the brain, researchers found that germ-free (GF) mice had lower baseline levels of histamine in the hypothalamus than conventional controls (Hegstrand & Hine 1986). The implications of this finding were not fully appreciated at the time, and the paper exploring this remains relatively unknown. We can more accurately realize its importance today. The current widespread use of GF animals, along with animals whose microbiomes are altered by exposure to probiotics, prebiotics, pathogenic bacterial infections, antibiotics, and fecal microbiota transplantation, has allowed microbiome researchers to uncover the complex pathways that are involved in the microbiome–gut–brain axis. We provide an overview of these pathways in this section (**Figure 1**).

Functional gastrointestinal disorder: a disorder of the gastrointestinal tract characterized by the presence of clinical symptoms for which no underlying structural or biochemical cause can be found, for example, irritable bowel syndrome

Gut–brain axis: the bidirectional biochemical signaling that takes place between the gastrointestinal tract and the central nervous system

Microbiota: a collection of microorganisms found at a particular location, for example, in the human gut

Probiotic: a live organism that when ingested in adequate amounts can exert a health benefit

Prebiotic: a nondigestible carbohydrate selectively fermented by certain bacteria in the large intestine that can be used to target particular bacteria and enhance their growth

Germ-free (GF): animals that are delivered surgically and raised in a sterile environment with no microbial exposure

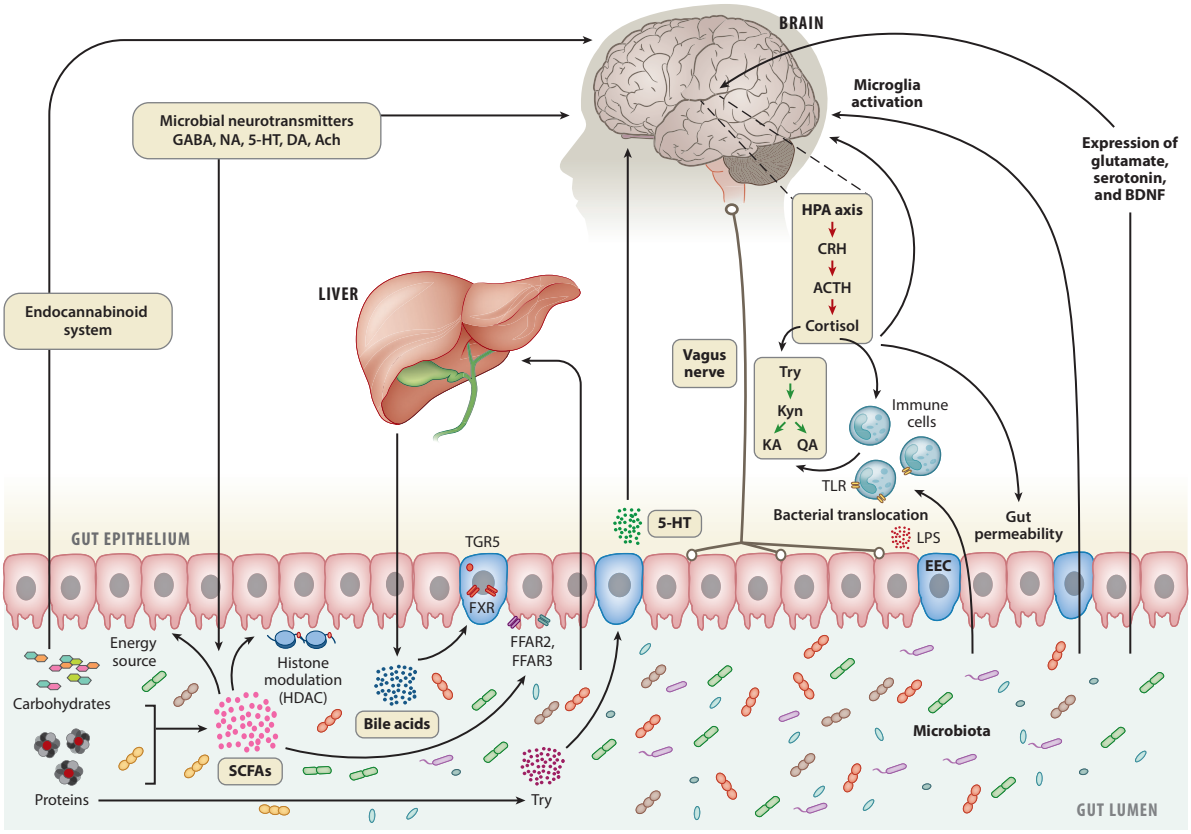

Figure 1

The varied and complex routes of communication between gut microbes and the brain. Abbreviations: Ach, acetylcholine; ACTH, adrenocorticotropic hormone; BDNF, brain-derived neurotrophic factor; CRH, corticotropin-releasing hormone; DA, dopamine; EEC, enteroendocrine cell; FFAR, free fatty acid receptor; FXR, farnesoid X receptor; GABA, gamma-aminobutyric acid; HDAC, histone deacetylase; HPA, hypothalamic–pituitary–adrenal; KA, kynurenic acid; Kyn, kynurenine; LPS, lipopolysaccharide; NA, noradrenaline; QA, quinolinic acid; SCFAs, short-chain fatty acids; TLR, Toll-like receptor; Try, tryptophan; 5-HT, serotonin.

The Microbiome and Stress Response

The hypothalamic–pituitary–adrenal (HPA) axis is best known for its role in mediating the stress response. Stress activates the axis by stimulating the production of corticotropin-releasing hormone (CRH) by the hypothalamus, resulting in subsequent downstream production of adrenocorticotropic hormone (ACTH) by the pituitary and, ultimately, cortisol by the adrenal glands. HPA axis dysfunction has been implicated in the pathophysiology of a variety of psychiatric disorders, in particular MDD (Holsboer 2000), and, in fact, appears to predict clinical symptom severity and cognitive impairment in depression (Keller et al. 2017).

Fecal microbiota transplantation: the process of transplanting fecal matter from one individual to another, thereby colonizing the recipient with the donor's microbiota

An individual's stress responsiveness is determined by a combination of gene–environment interactions. Early-life stress is a significant environmental variable that impairs HPA axis activity, contributing to future maladaptation of the stress response and increasing the risk of mental health difficulties in adulthood (Maniam et al. 2014). The gut microbiome has a profound effect on HPA axis development, as demonstrated by a milestone study in 2004 (Sudo et al. 2004). Researchers discovered that GF mice exhibited higher ACTH and corticosterone release following a mild

restraint stress in comparison to their control counterparts. This exaggerated stress response was partially reversed by colonization with feces from the control mice and completely reversed by giving the mice a single bacterial strain, *Bifidobacterium infantis*. However, the reversal was time dependent and occurred only if the bacterial reconstitution took place at an early stage, indicating that there is a critical time period in early life during which colonization of the GIT must occur for normal HPA axis development. Another study used maternal separation as an early life stressor, a paradigm that has proven to be of value as a model of depression in animals (O'Mahony et al. 2009). This study found increases in plasma corticosterone in the stressed rat pups along with an alteration of the fecal microbiota when compared with the control group. Thus, it appears that exposure to stress not only impacts the HPA axis but also modifies microbiome composition, a finding that has been replicated in several subsequent studies (Bailey et al. 2011, Bangsgaard Bendtsen et al. 2012).

Cytokines: signaling proteins produced by a broad range of cells, which have a key role in the immune response

One mechanism by which the microbiome may influence the HPA axis is via alteration of central gene expression in the amygdala, hippocampus, and prefrontal cortex, regions known to play a part in learning, memory, mood, and anxiety. Both glutamate and serotonin influence hypothalamic CRH release, and alterations in expression of these neurotransmitter receptors may thus alter HPA function in GF animals. Another protein of interest is brain-derived neurotrophic factor (BDNF), a key neurotrophin involved in neuronal plasticity, which is thought to have an important role in the etiology of depression (Castren et al. 2007). Microbiome-mediated changes in BDNF expression, along with changes in glutamate and serotonin receptor levels, have been confirmed in rodent studies, albeit inconsistencies are evident. Sudo et al. (2004) reported a decrease in BDNF along with decreased expression of the glutamate *N*-methyl-D-aspartate (NMDA) receptor in the hippocampus and cortex of male GF mice. However, Neufeld et al. (2011) reported an increase in hippocampal BDNF messenger RNA in female mice, while Clarke et al. (2013) reported decreases in male hippocampal BDNF messenger RNA levels and distinct changes in the hippocampal serotoninergic system. Such incongruities in animal studies obviously raise concerns in relation to applicability to the human system. Nevertheless, it is clear that the development and function of the HPA axis is affected by the compositional and functional status of our gut microbiome.

The Microbiome and Immunity

The immune system is a complex system extending its influence to virtually all disease processes. Although the effects of immune dysfunction are far from being fully understood in psychological illness, immune dysfunction is beginning to be appreciated as an important underlying pathophysiological factor in mental illness. An etiological role for immune dysfunction in depression was first considered in response to the study of cytokine-induced sickness behavior. Following peripheral infection, innate immune cells release signaling proteins called cytokines that act on the brain to cause symptoms such as loss of appetite, irritability, low mood, loss of motivation, social withdrawal, fatigue, and impaired attention. This constellation of symptoms is termed sickness behavior and is an adaptive response, encouraging the sick person to retreat, conserve energy, and fight off the infection. The symptoms of sickness behavior bear a striking resemblance to the clinical picture that characterizes depression. It has consistently been shown that depression is associated with a low-grade elevation in inflammatory markers (O'Brien et al. 2004), and, in fact, depression can actually be induced by the administration of cytokines (Udina et al. 2012). Bipolar disorder is also associated with chronic low-grade inflammation (Rosenblat & McIntyre 2017), and a recent meta-analysis confirmed through postmortem examination that the brains of people with schizophrenia demonstrate greater expression of proinflammatory genes and increased density of microglia (van Kesteren et al. 2017).

Toll-like receptors: pattern-recognition receptors present on the cell membrane of immune cells; they recognize characteristic molecular patterns on bacterial cells and initiate cytokine production in response

The gut microbiome is a long-overlooked modulator of immune function. In the first instance, the microbiome has a vital role in the early development of the immune system. Immune cells possess Toll-like receptors that when activated by the presence of bacteria, initiate a cascade of events, ultimately resulting in cytokine production. This process continues, and the immune system matures by constantly responding to different structural components of bacterial cells. A process that is initially located at the gut epithelium eventually extends throughout the body. The result is not only the production of a wide variety of cytokines but also the development of lymphocytes and guidance of antigen-specific acquired immunity (Akira & Takeda 2004). Without the microbiota, certain Toll-like receptors are not fully expressed in the gut (O'Hara & Shanahan 2006), and it appears that certain host-specific bacterial species need to be present for complete immune system development (Chung et al. 2012).

The source of the low-grade inflammation seen in depression and other related psychological conditions is unknown, and an interesting hypothesis involves the gut microbiome and the leaky gut. The intestinal epithelium plays a vital part in maintaining a selectively permeable barrier between the gut lumen and the rest of the body. The gut microbiome appears to regulate the homeostasis of the epithelial barrier, and it also influences colonic mucus secretion (Pearson & Brownlee 2010). The leaky gut theory supposes that increased permeability of the gut barrier—resulting in the translocation of gut bacteria or bacterial components such as lipopolysaccharides (LPS), which are normally safely confined to the gut lumen—can trigger a systemic inflammatory response. The hypothesis is certainly supported by the finding that raised LPS or corresponding immunoglobulin levels have been reported in depression (Maes et al. 2008), autism (Emanuele et al. 2010), and Alzheimer's disease (Zhang et al. 2009). Chronic stress is a significant risk factor for MDD, and it is well recognized that stress, through the production of cortisol and catecholamines, increases intestinal permeability, thus providing another plausible link in the leaky gut–inflammation–depression chain. It is noteworthy that probiotic bacteria such as *B. infantis* 35624, which reduce depressive behaviors in a rat model of depression, have also been shown to attenuate the proinflammatory state seen in these animals (Desbonnet et al. 2010). The same probiotic normalizes the proinflammatory state in patients with IBS as well as significantly reducing gastrointestinal symptomatology (O'Mahony et al. 2005).

While our discussion regarding the impact of the microbiome on the immune system has focused predominantly on the peripheral inflammatory response, it is worth noting an exciting study that revealed the microbiome can directly influence brain microglia. Erny et al. (2015) demonstrated that GF mice display widespread defects in the maturation and function of microglia, resulting in deficient innate immune responses. Furthermore, they demonstrated that a full repertoire of gut bacteria is needed for normal microglia development. The key signaling pathway mediating this influence was found to be the production of bacterial short-chain fatty acids (SCFAs), which are discussed in the following section.

The Microbiome and Short-Chain Fatty Acids

A major function of our gut bacteria is the digestion of carbohydrates and proteins that we consume in our diet. Principal metabolites of this digestive process are SCFAs, namely acetic acid, propionic acid, and *N*-butyric acid. At a local level, SCFAs serve many functions. They provide the major energy source for intestinal epithelial cells (Topping & Clifton 2001), regulate energy homeostasis (Ichimura et al. 2009), and directly influence the release of gastrointestinal signaling molecules, such as peptide YY (Holzer & Farzi 2014) and serotonin (Yano et al. 2015). Their role, however, is not just a local one, and we now realize that these molecules travel throughout the body and exert an effect at several distal organs (Clarke et al. 2014).

An important site of action appears to be the liver, given that a large proportion of circulating SCFAs are taken up here as they transit through the hepatic portal system (Cummings et al. 1987). Following hepatic uptake, peripheral blood concentrations are low, but the widespread presence of SCFA transporters and receptors suggests that these compounds must have a role outside the GIT. SCFAs are transported across cell membranes by monocarboxylate transporters, which are found not only at the gut but also at various other sites, including the kidney and brain (Ganapathy et al. 2008). Three specific SCFA receptors have been identified, free fatty acid receptors (FFARs) FFAR2 and FFAR3 (Bolognini et al. 2016) and hydroxycarboxylic acid receptor 2 (HCAR2) (Singh et al. 2014). FFAR2 and FFAR3 are widely expressed on leukocytes, thus supporting a role in immune regulation (Kim et al. 2014). Propionate has been shown to affect intracellular calcium concentrations in neutrophils, further endorsing an immune signaling role (Naccache et al. 1988). In addition, SCFA receptors are found in adipose tissue and are thought to be regulators of host adipocyte function and plasma lipid levels (Ge et al. 2008). There is no doubt that SCFAs could indirectly affect the brain by their influence on metabolism and immunity. However, the major question remains as to whether they can exert a direct influence on the central nervous system (CNS). The presence of specific SCFA receptors within the brain would support a direct role. FFAR3 (Bonini et al. 1997) and HCAR2 (Fu et al. 2015) are both expressed in rat brain tissue. However, human studies investigating the presence of SCFAs in the brain or cerebrospinal fluid are still awaited.

Once it gains access to a cell, butyrate demonstrates an exciting ability to influence gene transcription, no small feat for a small, single molecule produced by bacteria. It accomplishes this by inhibiting histone deacetylation (Boffa et al. 1978), an epigenetic process that allows it to influence the expression of a large number of genes and many different pathophysiological pathways. The potential of such a molecule cannot be overestimated, and this is reflected in the widespread research into butyrate across many disorders, neuropsychiatric and otherwise. Butyrate demonstrates antidepressant effects preclinically (Schroeder et al. 2007), and in an autism mouse model it has been shown to attenuate social deficits by transcriptional modification (Kratsman et al. 2016). It improves neurodegeneration and extends the life span in a mouse model of Huntington's disease (Ferrante et al. 2003), and it has been shown to be similarly beneficial in preclinical studies of Parkinson's (Sharma et al. 2015) and Alzheimer's diseases (Govindarajan et al. 2011). The therapeutic possibilities seem endless, but it is early days in butyrate and SCFA research.

The Microbiome and Neurotransmitter Production

Remarkably, our gut bacteria have the capability to directly produce neurotransmitters and neuromodulators that are an exact match to those produced by our human cells and widely used throughout the human body (Lyte 2011). *Lactobacillus* spp. and *Bifidobacterium* spp. produce gamma-aminobutyric acid (GABA); *Escherichia* spp., *Bacillus* spp., and *Saccharomyces* spp. produce noradrenaline; *Candida* spp., *Streptococcus* spp., *Escherichia* spp., and *Enterococcus* spp. produce serotonin; *Bacillus* spp. produce dopamine; and *Lactobacillus* spp. produce acetylcholine (Roshchina 2010). Of course, the quantities produced by bacteria are relatively small, and, therefore, their potential to exert a significant impact on human neurotransmission is questionable. However, these microbial neuroactive compounds do represent a potential mechanism whereby our microbes could directly interact with human cells, and even small amounts may impact important processes governing mood, anxiety, and cognition.

Let us take GABA as an example. GABA is the main inhibitory neurotransmitter in the brain (Roberts & Frankel 1950). It mediates its effects through two major classes of receptors: the ionotropic $GABA_A$ and the G protein–coupled $GABA_B$ receptors. The GABAergic system is implicated in the pathogenesis of depression and anxiety (Cryan & Kaupmann 2005), and these

receptors are important and effective pharmacological targets for antianxiety agents (e.g., benzodiazepines acting on $GABA_A$ receptors). Certain strains of *Lactobacillus* and *Bifidobacterium*, derived from the human intestine, can produce GABA from monosodium glutamate in vitro and from many foodstuffs containing probiotic bacteria; for example, Japanese funa sushi and Chinese traditional paocai contain levels of GABA in significant quantities. Thus, GABA, whether produced by intestinal bacteria from dietary glutamate or ingested in fermented foodstuffs, may be present in sufficient quantities intestinally to influence local receptor-mediated immune and neural systems. It is not just peripheral GABA concentrations that are impacted by gut bacteria. While the mechanism is unclear, gut bacteria can actually influence GABA concentrations in the brain. The probiotic *Lactobacillus rhamnosus*, which reduces anxiety and depressive behaviors in mice, also modulates GABA receptor expression in various brain regions, including the cortex, hippocampus, and amygdala (Bravo et al. 2011).

The Microbiome and the Vagus Nerve

The vagus nerve is the major parasympathetic nerve in the body and plays a key part in regulating several organ functions, including heart rate, bronchial constriction, and gut motility. Stimulation of this parasympathetic pathway has an anti-inflammatory effect, resulting in reduced proinflammatory cytokine production and attenuation of the systemic inflammatory response (Borovikova et al. 2000). Vagal stimulation has been to shown to produce antidepressant (Sackeim et al. 2001) and analgesic (Kirchner et al. 2000) effects.

The vagus nerve is an important signaling pathway in the gut–brain axis and appears to respond to, and relay information about, changes in gut bacteria. Vagal afferent nerve fibers are distributed throughout the intestinal wall, but are precluded from direct contact with the microbiota by the intestinal epithelial barrier (Bonaz et al. 2018). Thus, they respond to bacterial signals indirectly following exposure to bacterial neurometabolites, such as neurotransmitters or SCFAs, and through interaction with gut enteroendocrine cells (Raybould 2010). Key insights into the role of the vagus nerve in gut–brain signaling have been provided using animal models of GIT infection. A research team from the University of Virginia, in the United States (Gaykema et al. 2004, Goehler et al. 2008), sought to establish the anatomical basis for the anxiety-like behaviors observed in rodents infected with *Campylobacter jejuni*, a foodborne pathogen. Using Fos immunocytochemistry, they demonstrated that infection with *C. jejuni* resulted in increased activation in several brain areas that process visceral and autonomic information, including those that are typically activated following vagal stimulation. Another team highlighted the role of the vagus nerve by using rats who had undergone vagotomy, that is, surgical cutting of the vagus nerve. Rats infected with *Salmonella typhimurium* displayed functional activation of various brain regions and altered T cell counts. These responses were significantly reduced in rats who had previously been vagotomized (Wang et al. 2002). Probiotic studies have also used the vagotomy technique. A study mentioned previously, which demonstrated the positive effect of *Lactobacillus rhamnosus* strain JB-1 on anxiety behaviors and central GABA receptor levels, reported that neither the behavioral nor neurochemical effects of the probiotic were evident in vagotomized mice (Bravo et al. 2011).

The Microbiome and Serotonin

One of the earliest hypotheses relating to the pathophysiology of depression considered reduced serotonergic activity in the brain to be a major tenet in the etiology of the disorder (Maes & Melzer 1995). Selective serotonin reuptake inhibitors (SSRIs) are the most commonly used class of antidepressant and act to increase the availability of serotonin at the synapse. Interestingly,

SSRIs have also proven to be of benefit in gastrointestinal disorders such as IBS (Tack et al. 2006) and IBD (Macer et al. 2017). The overlap in serotonergic dysfunction between psychiatric and gastrointestinal disorders has prompted a keen interest in the role of the gut microbiome in the metabolism of tryptophan and serotonin.

We tend to think of serotonin as a predominantly CNS-based molecule, and, indeed, this is where most attention on the neurotransmitter has been focused. However, more than 90% of the body's serotonin is actually synthesized in the gut, predominantly by specialized endocrine cells called enterochromaffin cells. The metabolic pathway involves the conversion of tryptophan to 5-hydroxytryptophan by the rate-limiting enzyme, tryptophan hydroxylase (TPH). 5-Hydroxytryptophan is a short-lived intermediate product and quickly converted to serotonin (5-HT) (Berger et al. 2009). A forerunner in the investigation of this area was a metabolomics study in 2009, which revealed that conventionally colonized mice had 2.8 times greater plasma 5-HT levels than did GF mice (Wikoff et al. 2009). The mechanism behind this was subsequently revealed to be a microbiome-driven increase in transcription of the rate-limiting enzyme TPH1 (Yano et al. 2015). The elevated levels of TPH permitted increased 5-HT synthesis in enterochromaffin cells, thus resulting in increased peripheral 5-HT levels. Furthermore, this team demonstrated the specific microbes capable of promoting serotonin production to be spore-forming bacteria, primarily from the *Clostridium* genus.

Following absorption from the gut, tryptophan enters the circulation and travels to the brain where it is metabolized in several ways. A small amount is used for protein synthesis. Some is used for 5-HT production, and the synthetic cascade is identical to that in the gut, as detailed above. The remainder, accounting for the vast majority of tryptophan in the brain, is metabolized via an alternative route, the kynurenine pathway. We have been aware of this pathway for many decades, but it has only recently been appreciated that dysfunction may have important consequences for CNS function (Ruddick et al. 2006) and indeed play a part in disorders such as Alzheimer's disease (Bonda et al. 2010) and depression (Réus et al. 2015). The enzymes indoleamine-2,3-dioxygenase (IDO) and tryptophan-2,3-dioxygenase (TDO) catalyze the initial rate-limiting metabolic step of the kynurenine pathway and lead to the production of kynurenine. TDO is influenced significantly by glucocorticoid induction, whereas IDO levels are regulated by cytokines (Badawy 2017). Kynurenine can be metabolized via a number of different routes, and metabolism ultimately results in the production of neuroactive compounds, such as kynurenic acid and quinolinic acid. Kynurenic acid is thought to be a neuroprotective substance and acts as an NMDA antagonist (Albuquerque & Schwarcz 2013). Quinolinic acid, conversely, is an NMDA agonist and is neurotoxic (Schwarcz & Pellicciari 2002).

The kynurenine pathway offers a unifying theory in the pathogenesis of depression, integrating immune activation and HPA axis dysfunction with abnormal neurotransmission of 5-HT and glutamate (Maes et al. 2011). Immune activation, possibly mediated by a leaky gut as discussed previously, induces IDO. Chronic stress, HPA axis activation, and the ensuing glucocorticoid production increase TDO concentrations. The result is a dysfunctional kynurenine pathway with consequent abnormalities in 5-HT and glutamate neurotransmission. Although plasma tryptophan and kynurenine levels are not reduced in depressed patients, patients do have lower kynurenic acid concentrations, suggesting the metabolism of kynurenine is preferentially directed into the neurotoxic quinolinic pathway (Myint et al. 2007).

How does this relate to the microbiota? Interestingly, the gut microbiome appears to directly influence tryptophan breakdown at several points along the metabolic pathway. Although GF mice exhibit lower plasma 5-HT levels than do conventionally colonized mice, they have 40% greater plasma tryptophan levels (Wikoff et al. 2009). Clarke et al. (2013) expanded on this by demonstrating that GF mice exhibit a significant elevation in hippocampal concentrations of 5-HT.

Concentrations of tryptophan were also increased in the plasma of GF mice, and there was a reduced ratio of plasma kynurenine:tryptophan, suggesting that the microbiota alters central serotonergic neurotransmission via an influence on tryptophan metabolic pathways. Of note, these changes were present only in male mice, demonstrating a sex specificity not seen in studies exploring the immunological and neuroendocrine effects of the microbiome.

The Microbiome and Bile Acids

An exciting frontier in microbiome research is the exploration of cross talk between bile acids (BAs) and gut bacteria and the subsequent impact on host metabolism. BAs, which are metabolized by gut bacteria, have major regulatory and signaling roles. They are of increasing interest as potential etiological agents in a range of disease states, including obesity, metabolic syndrome, IBD, and GIT cancers (for a review, see Staley et al. 2017). This interest has extended in recent years to several neuropsychiatric conditions, including depression (Jia et al. 2016), autism (Golubeva et al. 2017), and Alzheimer's and Parkinson's diseases (Ackerman & Gerhard 2016). However, studies in the area of neuropsychiatry are sparse, and we are only beginning to appreciate a possible role for the gut microbiome's impact on the brain through BA transformation.

The liver synthesizes BAs as a product of cholesterol metabolism. Primary BAs are conjugated by liver cells and stored in the gallbladder prior to release into the duodenum following a meal, where they aid in the digestion and absorption of lipids. Gut bacteria deconjugate the BAs, which can subsequently be transformed into secondary BAs. Although deconjugation of BAs is an ability shared by most gut bacteria, the production of secondary BAs is a more exclusive function and is carried out by only a small number of anaerobic bacteria, predominantly of the *Clostridium* and *Eubacterium* genera (Ridlon et al. 2006). The interplay between BAs and the microbes that metabolize them is complex. While BAs depend on gut bacteria for transformation, BA pool size and composition appear to be major regulators of gut microbial structure. Secondary BAs have potent antimicrobial activity (Begley et al. 2005), and BA pool size is greatly reduced in conventional animals in comparison to their GF counterparts (Sayin et al. 2013).

While the major function of BAs is cholesterol clearance, they are now recognized as having a major role in glucose homeostasis, an action that obviously has far-reaching effects throughout the body. BAs regulate glucose metabolism through direct action on two receptors, a nuclear farnesoid X receptor (known as FXR) and a cytoplasmic G protein–coupled receptor (known as TGR5) (Gonzalez-Regueiro et al. 2017). They stimulate the release of fibroblast growth factor 19 (known as FGF19) from the ileum which, independent of insulin, plays an important part in mediating an appropriate postprandial response by the liver (Kir et al. 2011) and can regulate glucose metabolism via direct action on hypothalamic neurons, thus defining a new node in the gut–brain axis (Liu et al. 2018).

The gut microbiome may influence CNS disease states through BA alterations. A recent study using an animal model of autism demonstrated that a reduction in the abundance of particular bile-metabolizing bacteria was associated with marked gastrointestinal dysfunction and increased autistic-like behavioral scores (Golubeva et al. 2017). The authors described a significant reduction in the relative abundance of *Blautia* spp., a member of the *Clostridium* genus and one of a minority of bacteria capable of producing secondary BAs. Reduced numbers of secondary BAs, known for their potent antimicrobial activity, could lead to intestinal bacterial overgrowth and a disruption of the epithelial barrier, or leaky gut, which, as discussed previously, has been linked to the proinflammatory state seen in depression and autism spectrum disorder (ASD). Another study has linked depression and BA metabolism using a metabonomics approach (Jia et al. 2016). Researchers exposed rats to chronic unpredictable mild stress, a validated animal model of depression.

They subsequently measured a series of metabolic pathways in the liver and found primary BA biosynthesis to be one of the pathways playing a key part in the development of depression induced by chronic unpredictable mild stress. Our evolving understanding of the role of microbiome–BA interplay as an important component of the gut–brain axis will hopefully yield further insights into brain health.

The Microbiome and the Endocannabinoid System

Cannabis has long been used by humans seeking relief from a variety of symptoms, including anxiety, nausea, and chronic pain. The main psychoactive constituent of cannabis, Δ9-tetrahydrocannabinol, exerts the majority of its effects via activation of two cannabinoid receptors, CB_1 and CB_2. These receptors, along with their naturally occurring endogenous ligands, *N*-arachidonoylethanolamine (AEA) and 2-arachidonoylglycerol (2-AG), make up the endocannabinoid system (ECS). CB_1 is highly abundant in the CNS, while CB_2 is found predominantly in the gut, expressed on neurons and epithelial and immune cells. Our knowledge of this system has increased hugely in recent years, and we now appreciate it as an important component of the gut–brain axis, with emerging evidence that it interacts with the gut microbiome.

An important remit of the ECS is the regulation of energy metabolism, hunger signaling, and food intake, and it has been the focus of much interest in relation to the pathophysiology of obesity and type 2 diabetes (Gatta-Cherifi & Cota 2015). A Belgian group has published a series of papers exploring the interaction between the gut microbiome and the ECS. Using probiotics, antibiotics, and GF mice, they have demonstrated that the gut microbiota modulates ECS tone. This, in turn, regulates gut permeability and plasma LPS levels, which subsequently affect adipose tissue metabolism (Muccioli et al. 2010). A *Lactobacillus* probiotic demonstrated the ability to induce the expression of cannabinoid receptors in rodent intestine and mediate an analgesic effect (Rousseaux et al. 2007), while *Akkermansia muciniphila* also increased intestinal levels of endocannabinoids, reinforcing gut barrier function and improving the metabolic profile of obese mice (Everard et al. 2013).

It is not uncommon for individuals suffering with anxiety or mood issues to reportedly self-medicate with cannabis. While there is currently no sound evidence base for the therapeutic use of cannabis in psychiatry, the ECS does appear to be an important regulator of the stress response. Using a variety of stress models, rodent studies have demonstrated that stress results in reduced AEA and increased 2-AG levels. The low levels of AEA result in HPA axis activation and increased anxiety behaviors, while the increased 2-AG appears to modulate HPA adaptation responses as well as alter pain perception, memory, and synaptic plasticity (Morena et al. 2016). Concentrations of these endogenous cannabinoid ligands have been shown to be altered by stress exposure in healthy humans (Dlugos et al. 2012) and in patients with major depression (Hill et al. 2008). In addition, chronic stress causes the downregulation or loss of CB_1 receptors, and such impairment in receptor signaling is thought to increase an individual's susceptibility to stress-related pathology (Hill & Patel 2013).

A major challenge in harnessing the therapeutic potential of endocannabinoids is the widespread nature of their influence, and this was highlighted recently by the failure of a new pharmacological agent, rimonabant (Sanofi, Paris, France), which acted as a CB_1 receptor antagonist. This antiobesity drug was approved in Europe in 2006, but withdrawn from the market 2 years later due to serious concerns about psychiatric side effects, including depression, anxiety, and increased suicide risk (Sam et al. 2011). Without doubt, there is potential therapeutic value in the ECS for a variety of metabolic and psychiatric disorders, but safely channeling this capability requires a far greater understanding of its mechanisms.

THE MICROBIOME–GUT–BRAIN AXIS: HUMAN STUDIES

We can definitively conclude that the gut microbiome influences brain function, and the vast array of preclinical studies provides us with insights into the mechanisms by which this may be occurring. A major question is, of course, whether this preclinical evidence can actually be translated to the clinic. Unfortunately, there is a marked disparity between the swell of preclinical studies in recent years and the relative paucity of translational research. Human studies have followed one of two approaches. The first has been a case–control design, profiling the microbiome in patient populations and, by comparison with healthy controls, seeking to establish specific microbiome configurations associated with disease states. The major challenge with this method is that there is huge individual variability in microbiome composition, not to mention the many confounding factors to consider in patient populations, including diet and medication use. The second approach has been interventional, using various techniques to manipulate the microbiome of both healthy and clinical populations and assess the outcome on mood, anxiety, and other psychological functions. The most common intervention has been the use of probiotics, but prebiotics, antibiotics, and dietary change also feature in the literature. In this section, we review the current status of translational microbiome research as it pertains to various psychopathological states.

Mood Disorders: Microbiome Profile

Several case–control studies have reported altered gut microbiota composition in patients with depression. The first, published in 2014 (Naseribafrouei et al. 2014), found that the order Bacteroidales was overrepresented in patients with depression, while the family Lachnospiraceae was reduced. At lower taxonomic levels, the genus *Alistipes* was overrepresented in depressed patients, consistent with the findings of a similar study published the following year (Jiang et al. 2015). It is noteworthy that *Alistipes* has also been shown to be increased in chronic fatigue syndrome (Fremont et al. 2013) and IBS (Saulnier et al. 2011). A pronounced reduction in the Prevotellaceae family and subsequently in the *Prevotella* genus also appear to be associated with depression (Jiang et al. 2015, Kelly et al. 2016). Some inconsistencies have arisen, however. While one study (Naseribafrouei et al. 2014) reported no significant difference in species richness between depressed patients and controls, another study (Kelly et al. 2016) demonstrated that depression is associated with decreased gut microbiota richness and diversity.

Bipolar affective disorder (BPAD) has been the subject of one relatively large microbiome study involving 115 patients. Participants with BPAD displayed significantly decreased fractional representation of *Faecalibacterium* in comparison to healthy controls. Furthermore, this decrease correlated with better health outcomes across a range of parameters, including mood, anxiety, and sleep (Evans et al. 2017).

Mood Disorders: Interventional Studies

The majority of interventional studies exploring the effects of probiotics on mood have been performed in healthy participants and predominantly investigated various *Lactobacillus* and *Bifidobacterium* strains. The earliest was published in 2007 and assessed the effect of *Lactobacillus casei*. It was a large study involving 132 healthy adults, and although no overall effect of the probiotic was found, post hoc analysis revealed that those with the lowest baseline mood score showed significant improvements following probiotic supplementation (Benton et al. 2007). This suggests that probiotics may benefit those experiencing low mood while not offering any value to healthy adults with normal baseline mood states. Several subsequent probiotic studies revealed mixed results. A combination of *L. helveticus* and *B. longum* resulted in subtle improvements in mood (Messaoudi

et al. 2011). Another multispecies probiotic (*B. bifidum*, *B. lactis*, *Lactobacillus acidophilus*, *Lactobacillus brevis*, *Lactobacillus casei*, *Lactobacillus salivarius*, and *Lactococcus lactis*) demonstrated an ability to significantly reduce overall cognitive reactivity to sad mood, thought to represent a vulnerability to depression, in healthy adults (Steenbergen et al. 2015). An interesting imaging study involving healthy women found no benefit on mood scores following ingestion of a fermented milk product containing *B. animalis* subsp. *lactis*, *Streptococcus thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Lactococcus lactis* subsp. *lactis*. However, functional magnetic resonance imaging (fMRI) revealed alterations in the activity of brain regions controlling the central processing of emotion and sensation (Tillisch et al. 2013).

There have only been a handful of interventional studies in patients with a diagnosis of depression (**Table 1**). A polybiotic, containing *Lactobacillus acidophilus*, *Lactobacillus casei* and *B. bifidum*, significantly reduced depressive symptoms in patients with MDD (Akkasheh et al. 2016). Unfortunately, it was unclear whether the probiotic was a sole or adjunctive treatment, thus making it difficult to draw conclusions about its antidepressant potential. A more recent randomized controlled trial (RCT) specifically excluded patients who were taking psychotropic medication. Consumption of a probiotic preparation (*Lactobacillus helveticus* and *B. longum*) demonstrated no benefit in terms of improving mood or moderating inflammatory or other biomarkers (Romijn et al. 2017). A third trial reported significant benefits of *B. longum* strain NCC3001 in alleviating self-reported mild-to-moderate depressive symptoms. Although patients were not taking antidepressant medication, the study sample consisted of patients with IBS, an obvious confounder when looking at probiotic potential (Pinto-Sanchez et al. 2017).

There has been one probiotic interventional study in patients with BPAD. A team from the United States demonstrated that consumption of a probiotic (*Lactobacillus rhamnosus* strain GG and *B. animalis* subsp. *lactis* strain Bb12) significantly reduced the risk of relapse during a 6-month period following an episode of acute mania. Interestingly, the beneficial effect of the probiotic was increased in individuals with elevated levels of systemic inflammation at baseline, suggesting an immune basis for the observed effect (Dickerson et al. 2018).

Anxiety Disorders: Microbiome Profile

There is a striking absence of studies profiling the gut microbiome in patients with primary anxiety disorders, be it generalized anxiety disorder, social anxiety disorder, panic disorder, or agoraphobia. This gap is further highlighted by the fact that the most-studied condition in microbiome research is IBS, a disorder that, ironically, is conceptualized etiologically to be related to anxiety or stress. In addition, the most common psychological outcome in probiotic interventional studies is an anxiety or stress measure. Of course, these are dimensional concepts, and anxiety and stress, along with mood, are applicable to all human participants. Nonetheless, an obvious area for future research should involve profiling the microbiome in patients who cross that line between normal and pathological and have been diagnosed with a specific anxiety condition.

Posttraumatic stress disorder, while more correctly classified as trauma related as opposed to a primary anxiety disorder, is worth mentioning. It has been the subject of one small case–control study involving 18 patients in whom microbiome analysis revealed decreased total abundance of Actinobacteria, Lentisphaerae, and Verrucomicrobia, which was associated with greater severity of symptoms of posttraumatic stress disorder (Hemmings et al. 2017).

Anxiety Disorders: Interventional Studies

As with mood, most interventional trials exploring the effects of probiotics on anxiety and stress have been conducted in healthy populations and results are varied. A large Japanese study

Table 1 Probiotic interventional trials in psychiatric disorders

Psychiatric disorder (reference)	Number of participants and characteristics	Study design	Probiotic intervention	Outcomes	Points of note
Depression (Akkasheh et al. 2016)	40 adult patients with a diagnosis of MDD	8-week double-blind RCT	*Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum*	Probiotic group demonstrated significant improvement in depression scores on the BDI, as well as reduced serum insulin, reduced CRP, and increased plasma total glutathione levels	No information given on the use of psychotropic medications
Depression (Romijn et al. 2017)	79 adult patients recruited via an online screening resource; participants had self-reported depressive symptoms; psychotropic medications were an exclusion criterion	8-week double-blind RCT	*Lactobacillus helveticus* and *Bifidobacterium longum*	No effect of probiotic on any psychological outcome, including MADRS No effect of probiotic on serum inflammatory markers, BDNF, or vitamin D	No diagnostic interview performed to establish clinical diagnosis of depression
Depression (Pinto-Sanchez et al. 2017)	44 adult participants who had IBS and mild-to-moderate anxiety and/or depression scores based on self-report questionnaires; psychotropic medications were an exclusion criterion	6-week double-blind RCT	*Bifidobacterium longum* strain NCC3001	Probiotic group demonstrated significant reduction in depression scores on HADS; no effect on anxiety scores or IBS symptoms Probiotic reduced responses to negative emotional stimuli in multiple brain areas, including amygdala and frontolimbic regions No effect of probiotic on serum inflammatory markers, neurotransmitters (5-HT, substance P, and CGRP), or BDNF levels; no effect on fecal microbiome profile	No diagnostic interview performed to establish clinical diagnosis of depression IBS is a significant confounding factor
BPAD (Dickerson et al. 2018)	66 adult patients recruited during hospital admission for acute mania; patients were taking various psychotropic medications	24-week double-blind RCT	*Lactobacillus rhamnosus* strain GG and *Bifidobacterium animalis* subsp. *lactis* strain Bb12	Significant reduction in risk of rehospitalization in probiotic group Probiotic treatment also resulted in fewer days rehospitalized Effect of probiotic increased in those with elevated levels of systemic inflammation at baseline	No microbiome compositional analysis performed

(*Continued*)

Table 1 (*Continued*)

Psychiatric disorder (reference)	Number of participants and characteristics	Study design	Probiotic intervention	Outcomes	Points of note
Schizophrenia (Dickerson et al. 2014)	65 adult patients recruited from outpatient rehabilitation programs with at least moderately severe residual psychotic symptoms on PANSS; patients were taking various psychotropic medications	14-week double-blind RCT	*Lactobacillus rhamnosus* and *Bifidobacterium animalis*	No effect of probiotic on PANSS Probiotic group less likely to develop severe bowel difficulty during the course of the trial	No microbiome compositional analysis performed
ASD (Kaluzna-Czaplinska & Blaszczyk 2012)	22 children with diagnosis of ASD	8-week uncontrolled open-label trial	*Lactobacillus acidophilus*	Primary outcome was effect of probiotic on levels of D-arabinitol; probiotic reduced level of D-arabinitol Reported improvement in children's ability to concentrate and carry out orders	No information given on psychometric tools used Uncontrolled study
ASD (Parracho et al. 2010)	62 children with diagnosis of ASD	12-week double-blind crossover RCT	*Lactobacillus plantarum* strain WCSF1	Probiotic group demonstrated slight improvement in behavioral and emotional symptoms	Extremely high dropout rate; only 27% completed trial
ASD (Tomova et al. 2015)	29: 10 autistic children, their 9 nonautistic siblings, and 10 healthy controls	16-week open-label controlled trial	*Lactobacillus*, *Bifidobacterium*, and *Streptococcus*	Probiotic normalized dysbiotic microbiome of participants with ASD; no information on behavioral or neuropsychological effects	Autistic symptoms evaluated at baseline but not following probiotic intervention
ASD (Shaaban et al. 2017)	30 children with diagnosis of ASD	12-week uncontrolled open-label trial	*Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, and *Bifidobacterium longum*	Significant improvements in autistic and gastrointestinal symptoms	Uncontrolled study

Abbreviations: 5-HT, serotonin; ASD, autism spectrum disorder; BDI, Beck Depression Inventory; BDNF, brain-derived neurotrophic factor; BPAD, bipolar affective disorder; CGRP, calcitonin gene–related peptide; CRP, C-reactive protein; HADS, Hospital Anxiety and Depression Scale; IBS, irritable bowel syndrome; MADRS, Montgomery–Åsberg Depression Rating Scale; MDD, major depressive disorder; PANSS, Positive and Negative Syndrome Scale; RCT, randomized controlled trial.

involving more than 200 healthy participants reported a slight improvement in anxiety levels following 12-week consumption of *Lactobacillus gasseri* and *B. longum* (Nishihira et al. 2014). A similar probiotic combination (*Lactobacillus helveticus* and *B. longum*) also demonstrated a slight benefit on anxiety scores (Messaoudi et al. 2011). Another trial in healthy male participants revealed that 4-week consumption of *B. longum* resulted in a variety of psychological benefits, including reduced self-reported anxiety and reduced cortisol levels in response to an acute stressor, reduced daily perceived stress, and subtle improvements in visuospatial memory, with enhanced frontal midline mobility on electroencephalography (Allen et al. 2016). However, not all studies have been so positive. The probiotic *Lactobacillus casei* failed to impact anxiety measures in two separate studies (Benton et al. 2007, Reale et al. 2012), and *Lactobacillus rhamnosus*, a probiotic that had appeared promising in preclinical work, demonstrated no benefit over placebo in modifying a variety of biochemical and psychological stress-related measures in healthy participants (Kelly et al. 2017).

University students, subject to regular intermittent stress in the form of examinations, make for a useful healthy population in which to assess the effect of probiotics on anxiety and stress. Several interventional studies have used this population group. Takada et al. (2016) assessed the effects of *Lactobacillus casei* strain Shirota on gut–brain interactions in healthy Japanese medical students undergoing examination stress. As expected, academic stress resulted in increased salivary cortisol and an increase in physical symptomatology. Both of these stress-related responses were significantly suppressed in the group receiving *L. casei* strain Shirota. A similar study in Spain explored the effect of a fermented milk product containing *L. casei*, *L. delbrueckii* subsp. *bulgaricus*, and *Streptococcus salivarius* subsp. *thermophilus*. While the probiotic did modulate the immune response of the stressed students, there was no improvement in anxiety scores (Marcos et al. 2004).

It is worth mentioning those studies that have assessed anxiety outcomes in patients with various medical comorbidities. Several studies using multispecies probiotics, including various combinations of *Lactobacillus*, *Streptococcus*, and *Bifidobacterium* strains, reported no significant impact on anxiety symptoms in patients with IBS (Dapoigny et al. 2012, Han et al. 2017, Simren et al. 2010, Whorwell et al. 2006). However, a more positive finding was reported in patients with chronic fatigue syndrome, a disorder of unknown etiology that, like IBS, is commonly associated with anxiety and low mood. Authors described a significant improvement in anxiety scores in 39 patients with chronic fatigue syndrome following consumption of *L. casei* subsp. Shirota for 8 weeks (Rao et al. 2009). Yang et al. (2016) investigated the ability of *Clostridium butyricum* to reduce presurgical anxiety in patients prior to surgery for laryngeal cancer. Those in the probiotic group reported less anxiety and had reduced serum corticotropin-releasing factor levels, supporting a role for probiotics in ameliorating stress in presurgical cancer patients.

Prebiotics have also been investigated in relation to anxiety. A research group in Oxford, United Kingdom, found a significant impact of prebiotics on stress responses in healthy participants (Schmidt et al. 2015). Volunteers received one of two prebiotics [fructooligosaccharides or Bimuno galactooligosaccharides (Clasado, Reading, United Kingdom)] or a placebo (maltodextrin) daily for 3 weeks. Those in the Bimuno group showed significantly reduced waking cortisol responses as well as reduced attention and reactivity to negative emotions.

Psychotic Disorders: Microbiome Profile

Schizophrenia is a severe and typically chronic neuropsychiatric disorder characterized by symptoms of delusions and hallucinations, along with negative symptoms, including apathy, social withdrawal, and cognitive dysfunction. To date, there has been no analysis of the fecal microbiome in patients with schizophrenia, but the oropharyngeal microbiome, which offers greater ease of access, has been the focus of one study (Castro-Nallar et al. 2015). It was found that in patients

with schizophrenia, lactic acid bacteria were relatively more abundant, with functional metabolic pathways related to metabolite transport systems dominating, including siderophores, glutamate, and vitamin B12. This group went on to further investigate the bacteriophage component of the oropharyngeal microbiome in schizophrenia (Yolken et al. 2015). Bacteriophages are viruses that infect bacteria and alter their metabolism and replication. Investigators found one bacteriophage genome, *Lactobacillus* phage phiadh, to be significantly different in individuals with schizophrenia, the relevance of which is unknown at this time.

Psychotic Disorders: Interventional Studies

One probiotic interventional study has been undertaken in patients with schizophrenia. Given the range of immune abnormalities associated with schizophrenia, it was hoped that probiotics might, as a result of their immunomodulatory ability, have a beneficial effect. The 14-week trial involved the consumption of a probiotic product containing *L. rhamnosus* and *B. animalis*. While probiotic-treated participants were less likely to develop severe bowel difficulty during the course of the trial, the study did not find any effects of the probiotic on schizophrenia symptoms (Dickerson et al. 2014).

Neurodevelopmental Disorders: Microbiome Profile

Neurodevelopmental disorders are a heterogeneous group of conditions in which the development of the CNS is disturbed; they include ASD, attention-deficit/hyperactivity disorder (ADHD), and specific learning and language disorders.

ASD is characterized by the presence of stereotyped behaviors and interests, along with deficits in communication and social interaction. The mechanisms underlying its etiology remain poorly understood. Immune dysregulation and gastrointestinal disturbances are common features and have generated an interest in a potential role for the gut microbiome. Differences in microbiome composition between participants with ASD and healthy controls have been identified in several studies. Although findings have varied widely, a general trend has emerged that involves increased levels of Clostridiaceae, a family within the Firmicutes phylum (De Angelis et al. 2013, Finegold et al. 2002, Parracho et al. 2005, Song et al. 2004). Other elements of gut dysbiosis reported in ASD include increased *Lactobacillus* and *Desulfovibrio* spp. (Tomova et al. 2015), decreased *Prevotella* spp. (Kang et al. 2013), and elevated *Sutterella* spp. (Wang et al. 2013, Williams et al. 2012). However, apart from the increases in Clostridia, there is little consensus across studies in relation to alterations in specific bacterial species. Inconsistencies also exist when looking at microbial metabolites, with one study reporting elevated fecal SCFAs in children with ASD (Wang et al. 2012), while another demonstrated significantly lower SCFA levels in autistic participants (Adams et al. 2011).

One recent study has investigated the composition and functional status of the microbiome of children with ADHD (Aarts et al. 2017). ADHD is a common neuropsychiatric disorder, characterized by symptoms of inattention and/or impulsivity and hyperactivity, which are thought to be mediated by abnormalities in central dopamine and noradrenaline neurotransmission. This was a small study involving 19 adolescents and adults with ADHD. The authors reported an increase in several taxa in people with ADHD, including the *Bifidobacterium* genus. Using a hypothesis-driven approach, they sought to investigate whether the changes in microbiome composition were linked to functional changes involving dopamine precursor bacterial genes. They demonstrated that the increase in *Bifidobacterium* was linked to enhanced bacterial gene functionality related to the synthesis of phenylalanine, a precursor of dopamine. Furthermore, they demonstrated that

this increase in functionality was related to reduced reward anticipation in the ventral striatum on fMRI imaging, a finding characteristic of ADHD.

Neurodevelopmental Disorders: Interventional Studies

An early study in ASD, investigating the potential therapeutic benefit of the antibiotic vancomycin, was prompted by the observation that many parents reported significant antibiotic use in the months preceding the emergence of regressive-onset autism (Sandler et al. 2000). This small clinical trial found that vancomycin, an oral antibiotic effective against neurotoxin-producing Clostridia, resulted in improvement in ASD symptoms, which subsequently returned to baseline upon discontinuation of the treatment. This trial integrates well with later microbiome compositional studies, which have consistently revealed the predominant marker of gut dysbiosis in people with ASD to be elevated levels of Clostridia.

Human probiotic intervention studies in ASD are limited. Only one RCT has been undertaken (Parracho et al. 2010). Researchers employed a crossover design to investigate the effect of *Lactobacillus plantarum* strain WCSF1 and reported slight benefits of the probiotic in terms of behavioral and emotional symptoms. However, a major limitation was the extremely high dropout rate of 73%, highlighting the challenges of undertaking trials in this population. Several other open-label, primarily uncontrolled, trials have been published (**Table 1**), but although they suggest some benefit from probiotics, significant methodological difficulties make it difficult to draw any solid conclusions.

THE MICROBIOME IN PSYCHOLOGICAL MEDICINE: WHAT LIES AHEAD?

Research into the role of the gut microbiome in psychiatric disorders is in its infancy. The rapid expansion of preclinical microbiome–gut–brain research during the past two decades has generated cautious, but realistic, optimism about a potential new and effective paradigm for improving mental health. The dramatic advances in DNA sequencing technology and the use of animal models in which microbiome manipulation is a relatively easy task have afforded us a much greater understanding of the mechanisms by which microbiome–gut–brain communication occurs. We appear to have discovered the tip of an exciting iceberg that will hopefully offer new strategies for preventing and treating the epidemic of neuropsychiatric disorders that characterize modern life.

A major challenge lies in translating our laboratory findings to human studies. In the first instance, a healthy microbiome has not been defined. Likewise, the term dysbiosis, which refers to an altered or unbalanced microbiome, is an equally vague concept. Microbiome composition not only varies widely between individuals but also can be dramatically altered within individuals by many factors, including illness, diet, medications, and stress. Therefore, how does one characterize an optimal microbiome configuration? Large-scale population studies will certainly help. The American Gut and British Gut microbiome projects are joint crowd-funded initiatives whereby volunteers donate their biological samples and finance the subsequent microbiome analysis. These projects hope to collect information on the gut microbiota of thousands of citizens, which will give us a better idea of what constitutes a normal microbiome.

A further solution to this challenge may lie in moving our focus from microbiome composition to function. It is not enough to know "who" resides in our gut: We need to know what they do. Bacterial populations demonstrate properties of pleiotropy (the ability of a single bacterium to exert multiple actions) and redundancy (the ability of different bacteria to exert the same action), and this means that although microbiome composition may change, the overall functional

capacity may not. New bioinformatics software packages allow researchers to supplement compositional analysis with crucial information on different functional and metabolic pathways of the microbiome.

Psychobiotic: a live organism that, when ingested in appropriate quantities, has a positive mental health benefit

Another major concern for microbiome researchers is the lack of standardization in methodology across laboratories. The main source of information regarding human gut microbiota is the analysis of fecal samples. However, the fecal microbiome can be significantly affected by experimental design and procedures, including sample collection, storage protocols, and DNA extraction methods (Panek et al. 2018). Thus, the comparison of studies across laboratories is compromised until standard methodological practices have been agreed. The International Human Microbiome Standards project has been set up with the aim of developing standard operating procedures designed to optimize data quality and comparability in the human microbiome field.

A key challenge in the microbiome arena is trying to work out which of our trillions of gut bacteria may have mental health benefits. There are several difficulties inherent in investigating the probiotic or psychobiotic potential of individual bacterial species. First, even within specific bacteria genera, the probiotic potential of different species can vary considerably, and benefits are not necessarily generalizable between strains. Second, probiotics need to survive the hostile acidic environment of the stomach in order to reach the intestine, the site at which they can exert a therapeutic effect. Even if they do reach the intestine, many probiotics merely transit through and are unable to colonize the environment. Third, we lack any knowledge at this point about the optimal dose and duration of probiotic intervention, and a best-guess approach has been used in clinical trials. The alteration of our existing gut bacteria through the use of prebiotics or dietary change may be a more effective alternative to probiotic therapy. Prebiotics occur naturally in fruits, vegetables, oats, and wheat, foodstuffs that have increasingly been lacking in the heavily processed, high-fat, high-sugar diets that are common in developed countries. Dietary change is another highly effective way of rapidly and reproducibly altering microbiome composition (David et al. 2014). In societies that are becoming increasingly conscious of food choices, using diet as a therapeutic strategy for mental illness is not the pipe dream it might once have been. It is well recognized that there is a link between diet and depressive illness. The prophylactic benefits of a Mediterranean diet in protecting against depression and cognitive dysfunction have been confirmed in a meta-analysis (Psaltopoulou et al. 2013), and an innovative RCT has recently expanded the evidence base by demonstrating the efficacy of a Mediterranean-style diet as an effective adjunctive therapy in MDD (Jacka et al. 2017).

What is the future for microbiome research and the development of new microbiome-based therapies for psychiatric disorders? A move toward personalized medicine is an aspiration held by many health-care professionals and researchers alike, and a microbiome-based approach represents the perfect example of how this new paradigm of individualized health care might ultimately be realized. The Human Genome Project, which sequenced and mapped all the genes of the human genome, is undoubtedly one of the greatest achievements of our time. However, the expectation that it would completely revolutionize medicine and result in individualized treatments based on the genetic makeup of a patient has not been realized. This is primarily due to a gross underestimation of the importance of epigenetic phenomena, that is, the influence of the environment on gene expression. Nonetheless, the development of personalized medicine is an exciting aspiration, especially for psychiatric disorders that demonstrate extremely high rates of interindividual variability in terms of clinical presentation and treatment response.

The first step in precision medicine is the identification of biomarkers, that is, measurable indicators of a disease state. Despite decades of research, we still do not have a reliable biomarker for depression, although it appears that using a panel of multiple biomarkers may be of some diagnostic use (Schmidt et al. 2011). It is certainly plausible that an individual's microbiome

fingerprint could be a valuable component of an MDD biomarker panel and, more importantly, indicate whether microbiome-based treatment may represent a useful part of the therapeutic arsenal. The microbiome represents the perfect target for precision medicine, given the significant contribution it makes to interindividual variability in all aspects of health and disease. In addition, the huge amount of genetic material carried by our gut bacteria is readily and easily modifiable, unlike our human genes, thus making it of potentially immense therapeutic value for a wide range of multifactorial disease states, including depression. Moreover, it is not only the genetic signature of our gut bacteria that is available. The rapid development of new technologies affords a multiomics approach. Metatranscriptomics provides information about which genes are expressed under different conditions. Metabolomics and metaproteomics help identify metabolites and proteins and represent the end products of metabolic interactions between the microbiome and the human. Therefore, in addition to the genetic code, we can easily access a huge amount of information about the downstream consequences of specific genes and increase our capacity for identifying potential biomarkers. The future of psychological medicine will likely include microbiome analysis, with resultant targeted therapeutic interventions incorporating evidence-based microbiome manipulation through the use of probiotics, prebiotics, or specific dietary recommendations. Although such an approach may appear to some to be a distant dream, the prevailing mood of enthusiasm and excitement among the microbiome research community and the rapid pace of discovery mean that patients may not have so long to wait for a personalized microbiome mental health plan.

SUMMARY POINTS

1. The gut microbiome is a key component of the gut–brain axis.
2. Gut bacteria communicate with the brain through a variety of pathways, including the hypothalamic–pituitary–adrenal axis, immune modulation, tryptophan metabolism, and the production of various neuroactive compounds.
3. Patients with a wide variety of psychological conditions—including depression, bipolar affective disorder, posttraumatic stress disorder, and autism spectrum disorder—show distinct compositional changes in their gut microbiome.
4. Probiotics containing *Lactobacillus* and *Bifidobacterium* spp. have demonstrated their ability to improve mood, anxiety, and cognitive function both in healthy populations and in patient groups.

FUTURE ISSUES

1. Further elucidation of the mechanisms by which gut bacteria influence brain function is needed and this may be clarified through ongoing animal and human studies.
2. Particular emphasis needs to be placed on furthering human research to build on current preclinical work. This will include analyzing the compositional structure of the microbiome in specific patient groups as well as conducting interventional trials using probiotics, prebiotics, fecal microbiota transplantation, and specific dietary modifications.
3. More accurate characterization of a normal microbiome, in terms of both composition and function, is required, and this can be achieved through large-scale population studies.

4. A coordinated effort throughout the microbiome research community must occur to promote the standardization of techniques and methodologies across laboratories to ensure greater consistency and generalizability of microbiome data.

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