Review

Gut–brain axis: how the microbiome influences anxiety and depression

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Within the first few days of life, humans are colonized by commensal intestinal microbiota. Here, we review recent findings showing that microbiota are important in normal healthy brain function. We also discuss the relation between stress and microbiota, and how alterations in microbiota influence stress-related behaviors. New studies show that bacteria, including commensal, probiotic, and pathogenic bacteria, in the gastrointestinal (GI) tract can activate neural pathways and central nervous system (CNS) signaling systems. Ongoing and future animal and clinical studies aimed at understanding the microbiota–gut–brain axis may provide novel approaches for prevention and treatment of mental illness, including anxiety and depression.

Introduction

The human intestine harbors nearly 100 trillion bacteria that are essential for health [1]. These organisms make critical contributions to metabolism by helping to break down complex polysaccharides that are ingested as part of the diet and they are critical to the normal development of the immune system. Recent studies reveal the importance of gut microbiota to the function of the CNS [2–6]. Bidirectional communication between the brain and the gut has long been recognized. Established pathways of communication include the autonomic nervous system (ANS), the enteric nervous system (ENS), the neuroendocrine system, and the immune system. Recently, there has been a rethinking of how the CNS and periphery communicate, largely due to a growing body of experimental data from animal studies focused on the microbiome (see Glossary). Neuroscientists are now taking notice of these novel reports that highlight the 'bottom-up' influence of microbes themselves, with several studies showing that commensal bacteria are important to CNS function.

In this review, we discuss current experimental data on how gut microbiota influence the brain. Based on recent discoveries, we suggest that gut microbiota are an important player in how the body influences the brain, contribute to normal healthy homeostasis, and influence risk of disease, including anxiety and mood disorders (Figure 1). Although much of this work is preclinical, we also review the limited work in the clinical arena to date.

Overview of the microbiome

Early postnatal life in mammals represents a period of bacterial colonization. Resident or commensal microbiota colonize the mammalian gut shortly after birth and remain there throughout life. In humans, the lower intestine contains 10^{14}–10^{15} bacteria, that is, there are 10–100 times more bacteria in the gut than eukaryotic cells in the human body (10^{13}) [1,7,8]. The presence of commensal microbiota is critical to immune function, nutrient processing, and other aspects of host physiology [9–13]. As we discuss here, microbiota are also important in the function of the CNS.

To understand effectively the role of commensal microbiota in health and disease, we must be able to describe the complex ecology of the microbiome. Recently developed molecular and metagenomic tools have allowed researchers to better understand the structure and function of the microbial gut community. Several bacterial phyla are represented in the gut, and commensals exhibit considerable diversity, with as many as 1000 distinct bacterial species involved [14–16]. The two most prominent phyla are Firmicutes and Bacteroides, accounting for at least 70–75% of the microbiome [15–17]. Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia are also present but in reduced numbers [15]. The dynamic nature and the diversity of the microbiome determined to date extends far beyond what researchers expected. We are only beginning to understand how the diversity and distribution of these

Glossary

Bacterial colonization: naturally occurring bacterial colonization of infants (human) or pups (rodents) begins at birth and continues through postnatal life. Experimentally, mice lacking microbiota (GF mice) can be colonized by removal from the gnotobiotic rearing conditions, followed by exposure to microbiota (often exposure to mouse feces); these mice are referred to as 'conventionalized' mice.

Bacterial phyla: several bacteria phyla are represented in the intestinal microbiome, including Firmicutes, Bacteroides, Proteobacteria, Actinobacteria, Fusobacteria, and Verrucomicrobia. Recent metagenomic population studies have attempted to classify different profiles of bacterial phyla across groups of humans that are referred to as 'enterotypes'.

Commensal intestinal microbiota: the human intestine is home to nearly 100 trillion microbes. The relation between these microbes and their host begins at birth and continues throughout life as a mutually beneficial relation. These naturally occurring, ever-present microbes are referred to as commensal intestinal microbiota or commensals.

Microbiome: refers to the collection of microbes and their genetic material in a particular site, for example the human GI tract.

Probiotics: live microorganisms that are administered as dietary supplements or as food products, such as yogurt. Experimentally, several probiotic bacteria have been tested for health benefits, including *Lactobacillus* sp. (Firmicutes) and *Bifidobacterium* sp. (Actinobacteria), which are both gram-positive anaerobic bacteria.

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Keywords: microbiota; behavior; anxiety; gut–brain axis; germ-free; stress.

 http://dx.doi.org/10.1016/j.tins.2013.01.005

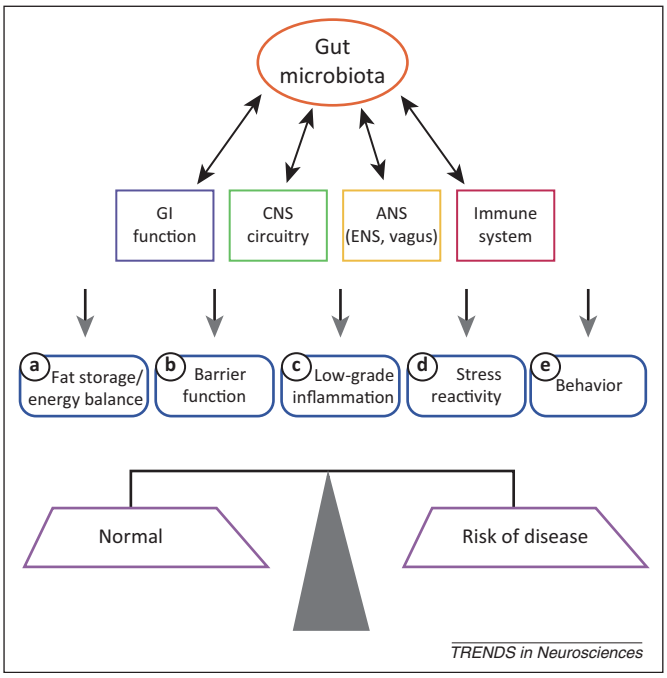

Figure 1. Bidirectional communication between gut microbiota and components of the gut–brain axis influence normal homeostasis and may contribute to risk of disease. Alterations in gastrointestinal (GI), central nervous system (CNS), autonomic nervous system (ANS), and immune systems by microbiota may lead to alterations in **(a)** fat storage and energy balance; **(b)** GI barrier function; **(c)** general low-grade inflammation (GI and systemic); **(d)** increased stress reactivity; and **(e)** increased anxiety and depressive-like behaviors. Each of these mechanisms is implicated in the pathophysiology of mood and anxiety disorders. Abbreviation: ENS, enteric nervous system.

prominent phyla contribute to health and disease. To this end, metagenomic population approaches have shown that certain bacterial populations, identified as enterotypes, are shared among groups of humans [18]. Beyond this phyla-level characterization, detailed analyses demonstrate considerable individual variability in bacterial content between related and unrelated individuals [1,19]. The microbiome is a dynamic entity, influenced by several factors, including genetics, diet, metabolism, age, geography, antibiotic treatment, and stress [20–27]. As such, the microbiota profile may be a good representation of the environmental history of the individual and could contribute to individual differences in risk of illness, disease course, and treatment response. These tools are now being used in both human and animal studies, and it will be important to determine how the microbiome in humans differs and/or is similar to that in mice.

Stress and microbiota

Alterations in HPA function

Clinically, depressive episodes are associated with dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis [28] and resolution of depressive systems with normalization of the HPA axis [29,30]. A direct link between microbiota and HPA reactivity was established with the 2004 report that showed an exaggerated corticosterone (CORT) and adrenocorticotrophin (ACTH) response to restraint stress in germ-free (GF) mice when compared with conventionally house-specific pathogen-free (SPF) mice [5]. GF mice have no commensal microbiota and exhibit an undeveloped immune system [10,31–33]. The use of mice raised in a GF environment allows investigators to assess directly the contribution of the microbiota to the development of brain and body systems. This landmark study showing increased stress reactivity in GF mice [5] was the catalyst for neuroscientists to consider the importance of microbiota in CNS function. Recent work has reproduced these findings, showing enhanced stress reactivity in both male and female mice to a novel environmental stressor [6].

Over the past few years, it has become clear that gut microbiota play a role in both the programming of the HPA axis early in life and stress reactivity over the lifespan. The stress response system is functionally immature at birth and continues to develop throughout the postnatal period, a developmental period coinciding with intestinal bacterial colonization. Studies using maternal separation in rats show that neonatal stress leads to long-term changes in the diversity and composition of gut microbiota [34,35], which may contribute to long-term alterations in stress reactivity and stress-related behavior observed in these rats. In support of this, concurrent treatment with probiotics (*Lactobacillus* sp.) during the early stress period has been shown to normalize basal CORT levels, which are elevated following maternal separation [36]. An indirect role for microbiota in the stress response was recently demonstrated in an animal model of stress-induced social disruption, where it was shown that microbiota are necessary for some of the stress-induced changes in inflammation [37]. Stress is known to increase intestinal permeability, thus affording bacteria an opportunity to translocate across the intestinal mucosa and directly access both immune cells and neuronal cells of the ENS [38,39]. This is therefore a potential pathway whereby the microbiota can influence the CNS via the immune system and ENS in the presence of stress. Intriguingly, a recent study has shown that pretreating rats with probiotic *Lactobacillus farciminis* reduced the intestinal permeability that typically results from restraint stress and also prevented associated HPA hyper-reactivity [40].

Direct influences on stress circuits

In addition to modulating HPA axis function, microbiota may influence CNS function directly through neuronal activation of stress circuits. Studies using oral administration of food-borne pathogens, *Citrobacter rodentium* and *Campylobacter jejuni*, provide evidence that bacteria residing in the GI tract can activate stress circuits through activation of vagal pathways [41,42]. During the acute phase of infection with *C. jejuni*, induction of the neuronal activation marker cFOS was evident in vagal sensory neurons in the absence of a systemic immune response [42]. Central brain regions also showed cFOS activation following oral administration of *C. rodentium* [41]. cFOS activation of neurons in the paraventricular nucleus of the hypothalamus (PVN) has been shown in GF mice following oral feeding with probiotic *Bifidobacterium infantis*, enteropathogenic *Escherichia coli*, or a mutated noninfectious strain of *E. coli* (ΔTir) [5]. The cFOS response to *E. coli* was stronger and accompanied by a robust peripheral cytokine response, suggesting that both neural and immune routes contributed to HPA activation in response to

infection. By contrast, HPA activation in response to probiotic *B. infantis* and mutated *E. coli* was not only shorter in duration, but also showed activation of central circuitry in the absence of a systemic immune response [5]. Together these reports provide clear evidence of bottom-up signaling between both pathogenic and commensal bacteria in the GI tract and neurons in central stress circuits.

When considering direct neural routes whereby the microbiota may be influencing the CNS, the ENS must also be included. Sensory neurons of the myenteric plexus in the ENS are the first point of contact for the intestinal microbiota residing in the gut lumen. These sensory neurons synapse on enteric motor neurons controlling gut motility. In addition, there is anatomical evidence of close, synaptic-like connections with vagal nerve endings in the gut [43]. Recent work has demonstrated via intracellular recordings that these sensory neurons are less excitable in GF mice than in control SPF mice, an effect that normalized after conventionalizing adult GF mice with SPF microbiota [44]. These same neurons have also been shown to become more excitable after feeding rats the probiotic *Lactobacillus rhamnosus* [45]. These findings are intriguing because they demonstrate altered electrophysiological properties in ENS neurons due to changes in commensal microbiota, providing a potential mechanism whereby the brain is informed of changes to the bacterial status of the intestinal lumen.

Gut–brain axis and behavior

Evidence gathered from experiments carried out in animals with altered commensal intestinal microbiota, whether GF mice, or conventionally housed animals either treated with probiotics and/or antibiotics or infected with pathogenic bacteria, all indicate that rodent behavioral responses are impacted when the bacterial status of the gut is manipulated. Genetic differences across strains influence behavior and, therefore, it is important to note that work studying the role of microbiota in behavior has been conducted in several strains, including inbred Balb/C, outbred Swiss Webster, NMR1 (a Swiss-type), outbred CF-1 (not Swiss), and AKR mice. Balb/C mice are readily used by neuroscientists in studies of neuroimmunology and immune–brain communication, including many behavioral studies. Swiss Webster and NMR1 mice are less often used by neuroscientists in behavioral studies; however, CD1 mice derived from Swiss Webster mice are commonly used. Table 1 provides a detailed summary of the behavioral data generated by experiments in which the microbiota profile of mice or rats has been manipulated. To date, several findings related to microbiota alterations have

Table 1. Summary of the impact of altered microbiota on anxiety-like and depressive-like behaviors[a]

Strain	Sex	Test	Main findings	Refs
GF versus SPF mice				
Swiss Webster (outbred)	F	EPM	GF mice showed reduced anxiety-like behavior: increased time spent in the open arm by GF mice and increased number of open-arm entries by GF mice	[4]
NMRI (Swiss-type, outbred)	M	OF, L/D and EPM	GF mice showed reduced anxiety-like behavior: increased center distance travelled by GF mice in OF; increased time spent in the light box by GF mice and increased time spent in the open arm by GF mice	[2]
Swiss Webster	M	L/D	GF mice showed reduced anxiety-like behavior: increased transitions between chambers by GF mice	[6]
Swiss Webster	F	L/D	GF mice showed no difference in anxiety-like behavior: no difference in transitions or time spent in light chamber by GF mice compared with SPF mice	[46]
Reconstitution of microbiota in GF mice				
NMRI	M	EPM and L/D	Colonization of GF mice early in life reversed EPM phenotype but not L/D phenotype: increased time spent in the light box by GF mice and no difference in open-arm time in conventionalized GF mice compared with SPF mice	[2]
Swiss Webster	M	L/D	Colonization at 3 weeks of age reversed L/D transitions	[6]
Swiss Webster	F	EPM	Colonization of GF mice at 10 weeks of age; reduced anxiety-like phenotype persisted	[3]
Swiss Webster Balb/C	M	Step Down	Colonization of GF Balb/C mice with NIH Swiss microbiota reduced anxiety-like behavior; latency to step down reduced in GF-Balb/C + Swiss microbiota compared with SPF Balb/C mice; colonization of GF NIH Swiss mice with Balb/C microbiota increased anxiety-like behavior; and latency to step down increased in GF-Swiss + Balb/C microbiota compared with SPF-Swiss mice	[52]
Effects of infection and gut inflammation on anxiety-like behavior				
CF-1	M	EPM and holeboard	Low levels of pathogenic bacteria administered orally increased anxiety-like behavior	[41,42,55]
Balb/C AKR	M	L/D	Infection with the parasite *Trichuris muris* increased anxiety-like behavior	[57]
AKR	M	Step down	Dextran sodium sulfate-induced gut inflammation increased anxiety-like behavior	[56]
Influence of probiotics on anxiety-like and depressive-like behaviors				
Balb/C	M	EPM and FST	Probiotic treatment reduced anxiety-like and depressive-like behavior in adult Balb/C mice in EPM and FST	[53]
Sprague-Dawley	M	FST	Probiotic treatment reversed the impact of maternal separation on depressive-like behavior in rats in FST	[54]
AKR	M	Step down	Probiotic treatment reversed inflammatory-induced increase in anxiety-like behavior	[56]
Balb/C AKR	M	L/D	Probiotic treatment reversed parasite-induced increase in anxiety-like behavior	[57]

[a]Abbreviations: F, female; M, male.

been replicated in more than one strain and, in particular, the impact of probiotics on behavior has been effective in several strains (Table 1).

GF housing and antibiotic treatment reduced anxiety-like behavior

Several independent laboratories have demonstrated that adult GF mice have reduced anxiety-like behavior [2–4,6] in the elevated plus maze (EPM), the light/dark test (L/D), and the open field (OF), with the results showing increased exploration of typically aversive zones (open arms in EPM, light chamber in L/D box, and center of the OF); however, one report did not observe changes in transitions or light time in the L/D in GF female mice [46]. Surprisingly, increased basal levels of plasma CORT were observed in GF mice compared with SPF mice [4]. Although it may be unexpected that GF mice show elevated CORT and reduced anxiety-like behavior, these observations are in line with previous findings showing that anxiety-like behaviors in the EPM are not related to CORT levels [47]. Interestingly, reconstitution of microbiota to GF mice early in life was able to normalize EPM behavior and some aspects of L/D behavior [2,6]. By contrast, in GF mice conventionalized with SPF microbiota in adulthood, the reduced anxiety-like phenotype observed in the EPM persisted [2,3]. These data suggest that there is a critical window during development where microbiota influence the CNS wiring related to stress-related behaviors (Figure 2).

The use of broad-spectrum antibiotics in drinking water has been reported to reduce significantly the microbial number and diversity in healthy adult C57Bl/6 [48]. In models of diet-induced obesity and in genetically modified obese (ob/ob) mice, administration of a broad-spectrum antibiotic improved glucose tolerance [49,50], reduced weight gain and fat mass [49] and lowered adipose inflammatory markers [49]. It has been suggested that the benefit of altering the profile of microbiota in these models results from reducing intestinal permeability and thereby decreasing inflammatory tone [49,51]. Male adult mice exposed to a mixture of antibiotics (neomycin 5 mg/ml and bacitracin 5 mg/ml) together with the antifungal agent, pimaricin, for 7 days showed reduced anxiety-like and increased exploratory behavior in the step-down and L/D tests [52]. The microbiota profile following 1 week of antibiotic treatment was significantly different from baseline; however, after a 2-week wash-out period, the microbiota profile normalized, as did the behavior [52]. Antibiotic treatment in GF mice had no effect on behavior, supporting the conclusion that the behavioral changes were mediated by the alterations in microbiota. Interestingly, when GF male Swiss Webster mice were colonized with microbiota from SPF Balb/C mice, an increased anxiety-like behavior was observed, reflecting the behavioral phenotype that is readily observed in SPF Balb/C mice [52]. In the reverse experiment, GF Balb/C mice that received microbiota from SPF Swiss Webster mice showed a reduction in anxiety-like behavior, similar to that seen in SPF Swiss Webster mice. The behavioral differences observed in these reconstitution experiments were associated with distinct strain-specific microbiota profiles [52].

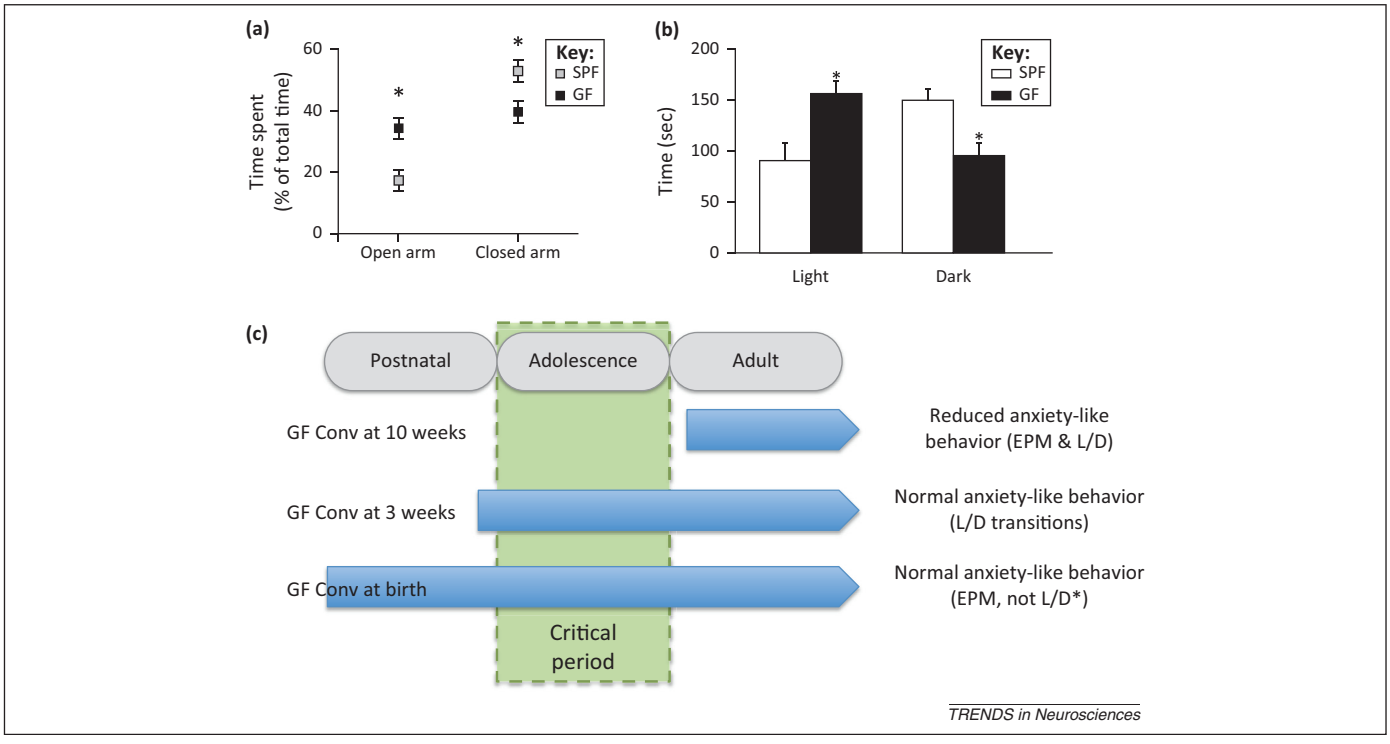

Figure 2. Several groups have demonstrated that germ-free (GF) mice, raised without exposure to microbes, show reduced anxiety-like behavior. **(a)** Testing in the elevated plus maze (EPM) revealed reduced anxiety-like behavior in GF mice compared with specific pathogen free (SPF) mice. (Values are means +/– S.E.M., $*P<0.05$.) **(b)** GF mice also spent more time in the light side of the light/dark (L/D) box and significantly less time in the dark side (values are means +/– S.E.M., $*P<0.05$). **(c)** Conventionalization of GF mice early in life normalizes anxiety-like behavior. GF mice conventionalized with SPF feces at birth (EPM not L/D) or at 3 weeks of age showed normal anxiety-like behavior, whereas GF mice conventionalized at 10 weeks of age showed reduced anxiety-like behavior similar to that of adult GF mice [2,3,6]. These data suggest that adolescence is a critical period where the gut–brain axis influences adult anxiety-like behavior. Reproduced, with permission, from [4] (a) and [2] (b).

Probiotics influence anxiety-like and depressive-like behaviors

A recent study has demonstrated that feeding healthy male Balb/C mice *L. rhamnosus* decreased anxiety-like and depressive-like behaviors in the EPM, forced swim test (FST), and OF [53]. The probiotic-treated group showed increased entries into the open arms of the EPM, spent less time immobile in the FST, and increased entries and time spent in the center of the OF. In a similar study, adult rats that had undergone maternal separation in the neonatal period showed a reduction in depressive-like symptoms after treatment with probiotic *B. infantism*, a behavioral effect that was also observed following antidepressant (citalopram) treatment [54].

Infection and gut inflammation increase anxiety-like behavior

Exposure to a subpathogenic infection of *C. jejuni* increased anxiety-like behavior measured in the EPM 2 days after infection, which was notable given the absence of an immune response in the periphery [55]. Two additional studies with *C. rodentium* and *C. jejuni* showed increased anxiety-like behavior 8 h post-infection, again with no difference in plasma cytokine levels or intestinal inflammation compared with control mice [41,42]. These studies show that the presence of pathogenic bacteria in the GI tract, in the absence of a systemic immune response, can increase anxiety-like behavior.

In experiments that result in increased GI inflammation, there are notable increases in anxiety-like behavior [56,57]. Mice with *Trichuris muris* showed GI inflammation and related increased anxiety-like behavior when were tested in both the L/D test and step-down test [57]. Treatment with the probiotic *Bifidobacterium longum* was able to normalize anxiety-like behavior in infected mice [57]. In a well-established mouse model of colitis (GI inflammatory disease), animals treated with dextran sodium sulfate (DSS) show GI inflammation and increased anxiety-like behavior; however, mice pretreated with DSS showed a reduction of anxiety-like symptoms after treatment with probiotic *B. longum* [56].

Behavioral studies suggest that inflammatory state influences behavior

The studies described above suggest that increased inflammation is associated with increased anxiety-like behavior. This relation observed across many studies is summarized in Figure 3. Of note, animal studies show that probiotic treatment can reverse inflammation-related increased anxiety-like behavior [56,57]. Additional animal studies with a neuroscience focus and clinical studies in psychiatric populations are needed in the area of probiotic treatment. Importantly, recent progress has resulted in the availability of tools to study microbiota in clinical populations [58], and we expect that this area of research will continue to expand in the immediate future.

Clinical evidence of probiotic use for mood and anxiety symptoms to date

Although the use of probiotics in animal studies has consistently shown an impact on anxiety- and depressive-like behaviors, there is little published work concerning the effects of probiotics on depression or anxiety symptoms in humans. In the limited work that does exist, however, there is evidence that probiotics have similar antidepressive and anxiolytic effects as those observed in preclinical studies. In a double-blind, placebo-controlled, randomized parallel group clinical trial, healthy subjects were given a mixture of probiotics containing *Lactobacillus helveticus* R0052 and *B. longum* R0175 or placebo for 30 days and then evaluated. Using various questionnaires designed to assess anxiety, depression, stress, and coping mechanisms, the probiotic treatment group demonstrated significantly less psychological distress than did matched controls [59]. Similarly, in another double-blind, placebo-controlled trial, healthy subjects were fed either a probiotic-containing milk drink or placebo control for 3 weeks, with mood and cognition assessed before treatment and after 10 and 20 days of consumption. Subjects who initially scored in the lowest third for depressed mood showed significant improvement in symptoms after probiotic treatment [60]. Chronic fatigue syndrome (CFS) is a functional somatic disorder that is frequently comorbid with anxiety and GI disturbance, and previous work suggested that these patients also demonstrate an altered microbial profile in the gut [61]. In a pilot study, patients with CFS receiving *Lactobacillus casei* daily for 2 months showed significantly fewer anxiety symptoms than did the placebo group in the Beck Depression & Anxiety Inventories [62]. Although these clinical studies examining the impact of probiotics on mood and anxiety are in the early stages and, to date, are limited to studies in nonpsychiatric patients, the results point us in a promising direction whereby intestinal bacteria could be targeted for their therapeutic potential in mood and anxiety disorders.

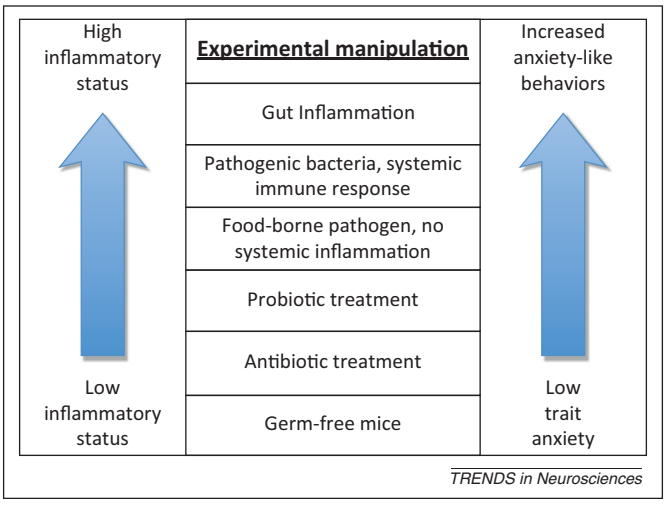

Figure 3. Microbiota may play a role in the relation between inflammation and anxiety-like behaviors. Several reports show that experimental manipulations that alter intestinal microbiota impact anxiety-like behavior. In relation to this, the observed behavioral changes relate to inflammatory status and are associated with differences in the microbiota profile in the gastrointestinal tract. This figure is based on data across many animal studies and represents generalized trends in these studies [2–4,6,41,42,52–57,80].

Gut–brain axis and neurochemistry

Bidirectional communication between gut microbiota and components of the gut–brain axis influence normal

homeostasis and may contribute to risk of disease through alterations in GI, CNS, ANS, and immune systems (Figure 1). A critical question facing neuroscientists is whether changes in behavior mediated by microbiota are a result of long-term changes in central signaling systems. To date, investigators have provided evidence that both neuroplasticity-related systems and neurotransmitter systems are influenced by the gut–brain axis.

Brain-derived neurotrophic factor

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophin family, influences many processes, such as the survival and differentiation of neurons, formation of functional synapses, and neuroplasticity during development and in adulthood [63–65]. Changes in hippocampal BDNF mRNA and protein have been noted in relation to the gut–brain axis. In infection models known to lead to alterations in the microbiota profile, reduced expression of hippocampal BDNF mRNA or protein was associated with increased anxiety-like behaviors [52,57]. Reversal of behavioral changes by probiotic treatment in these studies was associated with a return to control levels of BDNF expression [52,57]. This work is consistent with previous work linking stress to reduced hippocampal BDNF expression and restoration of normal levels following administration of antidepressants [66,67].

In the case of low levels of anxiety, as observed in GF mice, the reports related to hippocampal BDNF are varied. BDNF protein levels, measured by ELISA, were reduced in the hippocampus and cortex of male GF mice compared with SPF. By contrast, an increase in BDNF mRNA specifically in the dentate gyrus of the hippocampus of female GF mice has been reported [4]. A recently released report showed that a decrease in hippocampal BDNF mRNA expression was observed only in male GF mice. In female GF mice, a qualitative increase in BDNF mRNA expression was present, suggesting that BDNF expression differences are related to sex. A limitation to a broader interpretation of these results is the mismatch between sex differences in this molecular readout and the reduced anxiety-like behavior that is observed in both male and female GF mice. Although the importance of sexual dimorphism to CNS function and behavior is evident, determining the precise roles for various sex-specific factors will require additional study.

GABAergic signaling

GABA is a major inhibitory neurotransmitter in the CNS, and dysfunctions in GABA signaling are linked to anxiety and depression [68]. Interestingly, *Lactobacillus* and *Bifidobacterium* bacteria are capable of metabolizing glutamate to produce GABA in culture [69,70]. *In vivo* feeding of *L. rhamnosus* to mice, noted above to influence anxiety- and depressive-like behaviors, also altered central expression of GABA receptors in key CNS stress-related brain regions. Importantly, in these healthy mice, CNS effects on gene expression and behavioral effects may be mediated by the vagus nerve, because vagotomized mice did not show behavioral or CNS changes [53].

Serotonergic signaling

The serotonergic system is recognized as a major biological substrate in the pathogenesis of mood disorders [71,72], and pharmacological and genetic studies also provide evidence for the role of serotonergic signaling molecules in the neurobiology of anxiety [73–79]. Increased serotonin turnover and altered levels of related metabolites in the striatum of GF mice [2] and hippocampus [6] have been reported. At the level of gene expression, increased hippocampal expression of 5-hydroxytryptamine 1A (5HT1A) receptor mRNA [3] and 5HT2C receptor mRNA [2] has been observed. Together, these initial studies show an association between microbiota and serotonin signaling; however, studies are needed to provide a better understanding of how changes in serotonergic signaling, peripheral [6] and central, might influence neural function. In particular, given that microarray profiling revealed altered gene expression in a cluster of genes functionally related to synaptic long-term potentiation [2], there is a clear need for physiology experiments to determine the impact of microbiota on neurotransmission.

Box 1. Outstanding questions

- How do sex differences influence microbiota–brain communication?
  To date, alterations in microbiota have resulted in sex-dependent changes in molecular signaling in the CNS [6,53]; however, associated changes in behavior have not been identified. Sex differences are of particular importance because women are twice as likely as men to suffer from anxiety and depression [81–83]. The challenge going forward is to link sex differences in behavior to related neurobiological substrates.
- Do microbiota influence learning and memory?
  A few studies have shown an association between microbiota, learning, and memory [46,84]. It will be important to expand this area of research, particularly related to the role of microbiota in normal healthy CNS development of cognition and in childhood learning disorders.
- What is the impact of gut microbiota on CNS development?
  The use of antibiotics in children influences the profile of microbiota present [20], and yet the impact of early life antibiotic treatment on CNS development is not known. Importantly, childhood and adolescence may represent the periods when microbiota structure and function are the most dynamic and, therefore, it is timely and necessary to study how gut–brain interactions influence healthy brain development and risk of mental illness.
- Does the gut–brain axis play a role in childhood neurodevelopmental disorders, such as autism spectrum disorder (ASD)?
  Several studies have now reported changes in microbiota profile in patients with ASD [85–91]. Although this area of research is new and consensus across studies has not yet been established, this is clearly an emerging area of interest. Studies considering possible mechanisms for gut–brain communication in autism suggest that an altered metabolic phenotype in association with microbiota dysbiosis contributes to ASD [90,92], pointing to the importance of metabolomics in the study of how microbiota may influence the brain.
- How important are microbiota to CNS function in patient populations?
  Future work is needed to determine whether behavioral changes in animal studies related to microbiota translate to the clinic, specifically in psychiatric patient populations. This work may also consider how microbiota influence personality in humans. Do pharmacotherapies influence the microbiome and are adverse effects from these treatments, such as weight gain, related to gut microbiota dysbiosis?

Concluding remarks

Significant progress has been made over the past decade in recognizing the importance of gut microbiota to brain function. Key findings show that stress influences the composition of the gut microbiota and that bidirectional communication between microbiota and the CNS influences stress reactivity. Several studies have shown that microbiota influence behavior and that immune challenges that influence anxiety- and depressive-like behaviors are associated with alterations in microbiota. Emerging work notes that alterations in microbiota modulate plasticity-related, serotonergic, and GABAergic signaling systems in the CNS. Going forward, there is a significant opportunity to consider how the gut–brain axis and, in particular, new tools will allow researchers to understand how dysbiosis of the microbiome influences mental illness. Neuroscientists, armed with the results to date in this area, are well positioned to tackle outstanding questions (Box 1) and develop innovative approaches to prevent and treat stress-related disorders, including anxiety and depression.

Acknowledgments

Operating funds from the National Science and Engineering Research Council of Canada (NSERC, to J.A.F.), and equipment funds from Canadian Foundation for Innovation (to J.A.F.) contributed to this project. Graduate stipend support (to K.A.N.) was provided by Ontario Graduate Scholarship and Ontario Graduate Scholarship in Science and Technology.

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