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VIEWPOINT

The microbiome-gut-brain axis: from bowel to behavior

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Abstract

The ability of gut microbiota to communicate with the brain and thus modulate behavior is emerging as an exciting concept in health and disease. The enteric microbiota interacts with the host to form essential relationships that govern homeostasis. Despite the unique enteric bacterial fingerprint of each individual, there appears to be a certain balance that confers health benefits. It is, therefore, reasonable to note that a decrease in the desirable gastrointestinal bacteria will lead to deterioration in gastrointestinal, neuroendocrine or immune relationships and ultimately disease. Therefore, studies focusing on the impact of enteric microbiota on the host and in particular on the central nervous system are essential to our understanding of the influence of this system. Recent studies published in this Journal demonstrate that germ-free mice display alterations in stress-responsivity, central neurochemistry and behavior indicative of a reduction in anxiety in comparison to conventional mice. Such data offer the enticing proposition that specific modulation of the enteric microbiota may be a useful strategy for stress-related disorders and for modulating the co-morbid aspects of gastrointestinal disorders such as irritable bowel syndrome and inflammatory bowel disease.

Keywords *animal models, anxiety, brain-gut axis, microbiota, probiotics.*

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THE GUT-BRAIN AXIS

The bidirectional signaling between the gastrointestinal tract and the brain is vital for maintaining homeostasis and is regulated at the neural (both central and enteric nervous systems), hormonal and immunological levels. Perturbation of these systems results in alterations in the stress-response and overall behavior.[1] The high co-morbidity between stress-related psychiatric symptoms such as anxiety with gastrointestinal disorders including irritable bowel disorder (IBS) and inflammatory bowel disorder (IBD)[2,3] are further evidence of the importance of this axis. However, increasing evidence also suggests that the enteric microbiome greatly impacts on gut-brain communication leading to the coining of the phrase the brain-gut enteric microbiota axis[1] (illustrated in Fig. 1). The exact mechanisms governing such communication are unclear and most studies to date focus on the impact of altered signaling from the brain to the gut.[4,5] Recent emerging studies are investigating the impact of the guts' microbiota on brain and behavior. Approaches used to parse the role of gut microbiota on brain function include assessing the impact of probiotic agents, antibiotic-induced dysbiosis and pathogenic infections[6–9] each of which we will discuss below.

GERM-FREE/GNOTOBIOTIC ANIMALS

One approach that is being utilized to study the microbiome-gut-brain axis is the use of germ-free animals.[10] Germ-free mice, which are animals devoid of any bacterial contamination, offer the possibility to study the impact of the complete absence of a gastrointestinal microbiota on behavior. Germ-free mice also allow the study of the impact of a particular entity (e.g. probiotic) on the microbiome-gut-brain axis in isolation.[10] It should be noted that, while useful research tools in neurogastroenterology, the data from these

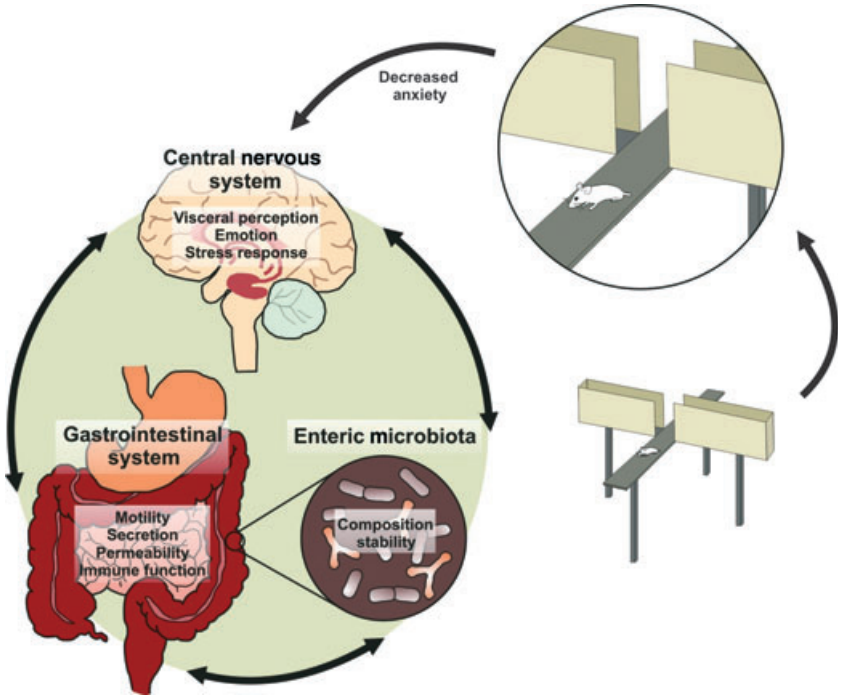

Figure 1 Representation of the enteric microbiota-gut-brain axis and how anxiety-like behavior can be measured using the elevated plus maze. Recent studies (refer to text) have shown that perturbations of the enteric microbiota can impact on this axis to alter behavioral responses in animal models.

animal studies are not translational to human disease as they do not represent any true situation in the human population. Despite this, their use has generated exciting data that is leading the way to directly answering the question: can enteric microbiota alter behavior?

BEHAVIORAL AND NEUROCHEMICAL CONSEQUENCES OF GROWING UP GERM-FREE

Neufeld and colleagues in this issue of this *Journal*[11] use female germ-free mice to demonstrate that the absence of a conventional microbiota results in a reduction in anxiety behavior in the elevated plus maze, a well validated model of anxiolytic action (see Fig. 1). These authors also show an upregulation in the expression of brain derived neurotrophic factor (BDNF) mRNA in the dentate gyrus of the hippocampus of these germ-free animals. Brain derived neurotrophic factor is crucial for supporting neuronal survival and encouraging the growth and differentiation of new neurons and synapses and thus is involved in the regulation of multiple aspects of cognitive and emotional behaviors.[12] Whilst there is a clear relationship between chronic stress states, major depression and BDNF,[13] the association between anxiety and BDNF appears to be more complex with the authors finding positive, negative and no correlation between hippocampal levels and anxiety.[14–16] Thus, it is unclear whether the changes in hippocampal BDNF observed in the study of Neufeld and colleagues actually relates to the behavioral changes observed.

Interestingly, and somewhat discordant with the behavioral data, an increase in the stress hormone corticosterone was noted in the plasma of the germ-free mice. Moreover, a decrease in the NR2B subunit of the NMDA receptor in the amygdala, but not hippocampus, of germ-free animals was observed which the authors speculate may contribute to the anxiolytic-like effect noted. In addition, a down-regulation of the 5-HT_{1A} auto receptor was also present in the dentate gyrus of the germ-free mice.[11]

These data together provide important and direct evidence that microbiota can influence brain and behavior, in this case anxiety. They build on previous studies from Sudo and colleagues[17] which demonstrate that male germ-free mice have an increased stress response (although no basal changes in hypothalamic pituitary adrenal axis function were noted in these studies) coupled with decreased hippocampal and cortical BDNF, and decreased NR1 (hippocampus) and

NR2A (hippocampus and cortex).[17] The reasons for the discrepancies between the molecular changes in these studies and that of Neufeld and colleagues are unclear at present. Gender may play a role in such effects. Indeed, recent data from our laboratory show that the neurochemical and endocrine but not immune effects of growing up in a germ-free environment is only evident in male animals.[18] Another important difference between the studies is the method of mRNA expression analysis (*in situ* hybridization *vs* quantitative real-time PCR). One significant caveat to the data generated by Neufeld and colleagues is that their studies were carried out on commercially sourced germ-free animals and were conducted just 48 h following arrival at their facility. The impact of transport stress and the lack of information regarding gnotobiotic status are two major confounds to the study.[19] Moreover, whereas Sudo and colleagues demonstrated that recolonization with *Bifidobacteria* species reversed the germ-free induced effects, no attempt at recolonization occurred in Neufeld *et al.*'s study. However, despite these limitations their work is among the first to show a direct link between anxiety-related behavior and the microbiota and thus is an exciting and important contribution to the literature.

PROBIOTICS AND BEHAVIOR/CENTRAL NEUROTRANSMITTERS

Probiotics are beneficial in the treatment of the gastrointestinal symptoms of disorders such as IBS.[20] Clinical evidence is mounting to support the role of probiotic intervention in reducing the anxiety and stress response as well as improving mood in IBS patients and those with chronic fatigue.[21–23] Recently, a study assessing the effect of a combination of *Lactobacillus helveticus* and *Bifidobacterium longum* on both human subjects and rats showed that these probiotics reduced anxiety in animals and had beneficial psychological effects with a decrease in serum cortisol in patients.[24] While the mechanism of action is not known, some probiotics do have the potential to lower inflammatory cytokines,[20,25] decrease oxidative stress and improve nutritional status.[21] The modulation of systemic inflammatory cytokines and oxidative stress could potentially lead to increased BDNF,[21] known to be involved in depression and anxiety.[13,14]

Lactobacillus reuteri, a potential probiotic known to modulate the immune system[26] decreases anxiety as measured on the elevated plus maze as well as reducing the stress-induced increase of corticosterone in mice.[27] This probiotic alters the mRNA expression of both $GABA_A$ and $GABA_B$ receptors in the central nervous system. Alterations in these receptors are associated with anxious and depressive-like behaviors in animal models. Vagotomy in these animals prevented the anxiolytic and antidepressant effects of this bacterium as well as the effects on the central GABA receptors. This suggests that parasympathetic innervation is necessary for *L. reuteri* to participate in the microbiota-brain interaction.

Previous studies[28] have shown that probiotic agents can modulate antidepressant-like behavior with *Bifidobacterium infantis* having antidepressant properties in the forced swim test, a well-established model in the evaluation of pharmacological antidepressant activity.[29] Chronic *B. infantis* administration also led to a suppression in stimulation-induced increases in peripheral pro-inflammatory cytokines and increases in plasma tryptophan,[28] both of which have been implicated in depression.[30,31] We have also investigated the impact of *B. infantis* on a preclinical model of IBS (maternal separation model)[32] and showed that this bacterium was able to reverse some of the early-life stress-induced changes.

Taken together, it is clear that certain probiotic strains can modulate various aspects of the microbiome-gut-brain axis.[33] However, these effects are bacterial strain dependent and care must be taken in extrapolating data obtained from one organism to another. Nonetheless, the accumulating data suggest a clear ability of probiotic and potential probiotic strains to modulate brain and behavior.

ANTIBIOTIC INTERVENTION AND BEHAVIOR

The administration of broad-spectrum antibiotics, frequently used in both adult and pediatric clinical practices, has been shown to reduce the biodiversity of the fecal microbiota and delay the colonization by some probiotic strains, e.g., lactobacilli.[34] Antibiotic disruption of the gut flora has also been linked to expression of functional gastrointestinal symptoms.[35]

The use of antimicrobial drugs is one of the most common artificial ways to induce intestinal dysbiosis in experimental animals. Preliminary data from Bercik and Collins[6] show that oral administration of neomycin and bacitracin along with the antifungal agent primaricin to perturb the microbiota for 7 days in adult BALB/c mice induced altered behavior when tested using the step-down and the light/dark box tests.[6] These data could be interpreted as a reduction in anxiety in antibiotic treated mice or alternatively an increase in locomotor behavior. Clearly, future studies examining the behavioral consequences of adult

antibiotic use and mechanisms underlying such changes are warranted.

Previously we have shown that stress in early life leads to altered behavior and fecal microbiota in adulthood in rats.[36] In line with this work we assessed the impact of perturbations in microbiota on adult behavior.[37] Therefore, we induced dysbiosis through the administration of the antibiotic vancomycin to neonatal rat pups. In this case behavior was measured in adulthood, 6 weeks after cessation of treatment. Our prediction was that an altered microbiota in neonatal life would lead to behavioral changes still present in adulthood. Intriguingly, although we did not see any alteration in anxiety or cognitive behavior, an increase in visceral pain behaviors was evident. Thus, there appears to be a temporal dissociation between the onset of microbiota disturbance and the alterations in anxiety-related behavior.

INFECTION, CENTRAL ACTIVATION AND BEHAVIOR

Studies observing behavior of animals following infection offer a better representation of the human condition in certain instances. Infections due to enteric bacterial pathogens causes acute mucosal inflammation[38] and is noted as a risk factor for the development of postinfectious IBS.[39,40] However, whether these short- and long-term intestinal changes affect behavior is not well defined.

In a recent series of studies, Bercik *et al.*,[41] sought to examine how chronic gut inflammation alters behavior; they infected mice with *Trichuris muris*, which is very closely related to the human parasite *T. trichiura*, and examined alterations in anxiety-like behavior and hippocampal BDNF. They demonstrated that treatment with the anti-inflammatory agents etanercept, and to a lesser degree of budesonide, normalized behavior, reduced cytokine and kynurenine levels, but did not influence BDNF expression. Moreover, the probiotic *B. longum* normalized behavior and BDNF mRNA but did not affect cytokine or kynurenine levels. Vagotomy did not prevent anxiety-like behavior in the infected mice. Clearly the mechanism of action of these interventions differ, nevertheless all three normalized the behavior induced by the infection indicating that the microbiota may signal to the brain through multiple routes.

There have been an increasing number of studies using *Citrobacter rodentium* as an infectious agent to investigate gut-brain axis function. *Citrobacter rodentium* does not affect baseline behavior when tested 14 and 30 days after infection in C57BL/6 mice.[10] Yet when CF-1 mice were infected and behavior tested at 7–8 h following infection, there was an increase in anxious-like behavior.[42] Psychological stress is known to affect intestinal barrier function[8] and host-microbe interactions.[43] With this in mind, the authors stressed the infected mice acutely which induced memory dysfunction following the resolution of the infection some 30 days later. This dysfunction was prevented by pretreatment with a commercially available combination of probiotics. This pretreatment also ameliorated serum corticosterone levels as well as preventing alterations in hippocampal BDNF and central c-*fos* expression. Germ-free mice were also included in this study and they displayed impaired memory both with and without the acute stress. Intriguingly, these germ-free mice were not less anxious when compared to controls which is at odds with the data presented by Neufield *et al.*[11] This maybe due to the fact that different tests were employed, the light-dark box test in the former as opposed to the elevated plus maze. This study highlights the joint impact of infection and stress on the central nervous system and also points to the fact that a commensal gut flora is necessary for both spatial and working memory. Since no overt systemic inflammation was observed and increased neuronal activation in vagal ganglia was seen, it is proposed that the gut to brain signaling in this instance was mediated through the vagus nerve.[42]

Another example of the impact of enteric microbiota affecting brain function was seen when *Camplobacter jejuni*, a food borne pathogen, led to activation of brain regions that are involved in the processing of gastrointestinal sensory information in mice.[44] c-*fos* was increased in visceral sensory nuclei in the brainstem (1 and 2 days after inoculation) and the paraventricular nucleus of the hypothalamus (2 days after inoculation). An interesting twist to this study is that the infection did not induce a system inflammatory response. Therefore, the vagal nerve may be implicated in the signaling of this pathogen and its effect on brain activation. These authors also noted that after 7 h, *C. jejuni* increased anxious-like behavior in the hole board test and the level of anxiety was proportional to neuronal activation as assessed by the number of c-*fos* expressing cells in the bed nucleus of the stria terminalis a key component of the extended amygdala fear system.[45] These and other findings by the same authors[9] allude to the amygdala and the bed nucleus stria of terminalis as interfaces between gastrointestinal pathogenic challenge and brain regions that are associated with the behavioral responses to stress. They also support the notion that these nuclei are anatomical substrates by which viscerosensory stimuli can influence behavior.

The vagus nerve is involved in the transmission of signals from the gastrointestinal tract to the central nervous system during a *Salmonella typhimurium* infection.[46] To establish this, rats were infected with the bacteria and vagotomy was performed in half of the animals 28 days prior to the infection. Salmonella increased inflammation in the ileum and the mesenteric lymph nodes while decreased aspects of the systemic immune response. c-*fos* expression was increased due to infection in the paraventricular nucleus and the supraoptic nucleus. Vagotomy prevented these infection associated changes. These results again imply that the vagus plays an important role in the transmission of immune information from gut to brain and also in homeostasis associated with the immune system.

CONCLUSIONS

All of the studies outlined above indicate that there is an increasing need to understand the molecular, cellular and physiological basis of enteric microbiome-gut-brain communication. The article by Neufeld and colleagues[11] illustrates that the complete absence of a conventional microbiota leads to decreased anxiety-like behaviors as well as alterations in central neurochemistry. Future studies will provide insight into the development of novel treatment strategies (probiotics or pharmacological), for gastrointestinal disorders that are associated with an altered signaling from the bowel to the brain.

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COMPETING INTERESTS

The authors have no competing interests.

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