The effects of gut microbiota on CNS function in humans

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The role of the gastrointestinal microbiota in human brain development and function is an area of increasing interest and research. Preclinical models suggest a role for the microbiota in broad aspects of human health, including mood, cognition, and chronic pain. Early human studies suggest that altering the microbiota with beneficial bacteria, or probiotics, can lead to changes in brain function, as well as subjective reports of mood. As the mechanisms of bidirectional communication between the brain and microbiota are better understood, it is expected that these pathways will be harnessed to provide novel methods to enhance health and treat disease.

Introduction

Humans have long considered the contents of the bowels as mere waste products, rather than a diverse and vital community whose intimate interactions with the body impact us on multiple levels. With increasing enthusiasm, however, both the lay public and the scientific community are turning attention to the gut's microbiome and its metabolic products to understand the mechanisms of obesity, systemic inflammation, and potentially even mood. Gastrointestinal pathogens have previously attracted more attention than the multitude of commensal organisms that also inhabit the gut. However, we know that many of these commensals are vital to our health, helping to digest carbohydrates and create energy rich short chain fatty acids, synthesizing vitamins, and metabolizing toxins. Now that identification of gastrointestinal microorganisms with metagenomic and metabolomic methods is becoming more accessible, there is an increased ability to examine more subtle interactions between the host and the microbiome. The gut provides the largest physical interface between the environment (including the microbiome) and self. Unlike the skin, our other contact interface with the world, the gut has a complex nervous system, which interfaces directly with the brain and allows bidirectional information flow between the microbiota and the brain. This close interaction suggests that aspects of brain development, function, mood, and cognition may be influenced by our gastrointestinal contents.

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The Microbiota and Brain Development

Microbiota-brain interactions likely begin early in development. While in utero, the intestines have been traditionally thought to be sterile. However, emerging evidence suggests that some maternal-fetal transmission of bacteria or bacterial products may occur via the amniotic fluid or umbilical cord blood.¹⁻⁴ Immediately upon delivery, the newborn is exposed to environmental flora and the gut begins true colonization. The mode of delivery, vaginal or cesarean section, as well as the mother's health status, will influence the bacteria that first take hold.⁵ During infancy, the factors influencing the developing gut microbiota include prematurity, breastfeeding, diet, antibiotic use, the presence of siblings, and hospitalizations.^{6,7} The microbiota matures over time and is thought to become relatively stable in childhood, while the brain continues to develop through adolescence into early adulthood.^{8,9} The composition of the microbiota in conjunction with other environmental factors likely influence DNA methylation and other epigenetic changes crucial to developing organs including the gut and brain.^{10,11} This important role of microbiota-brain interactions is supported by germ-free rodent studies in which it has been shown that the hypothalamic pituitary adrenal (HPA) axis develops abnormally in the absence of the normal gut microbes, leading to altered stress responsiveness and reduced hippocampal brain-derived neurotrophic factor (BDNF).¹² The observed abnormalities could then be ameliorated, at least in part, when the gastrointestinal tracts of the germ-free mice were reconstituted with stool from conventionally raised mice or with the specific organism *Bifidobacterium infantis*. In another study utilizing germ free mice, adult animal's anxiety-like behavior was found to be reduced in compared with mice with normal gut flora.¹³ The germ free mice also showed changes in the turnover of monoaminergic neurotransmitters in the striatum and reductions in neural growth factors in several brain regions associated with fear and anxiety. The abnormal behavior could be normalized when young germ free mice were colonized with normal gut bacteria, but could not be normalized when adult germ free mice were colonized with conventional bacteria, suggesting a developmental window for this specific microbe-brain interaction. Thus, it appears that multiple pathways within the brain are affected by the gut microbiota during development, but that some of these effects may be limited to childhood.

The Pathways of Microbiota-Brain Interaction

There is evidence for multiple pathways of communication from the intestinal microbiota to the CNS including vagal afferent nerves, immune and HPA axis modulation, and production of active metabolic products (Fig. 1).¹⁴⁻¹⁷ The vagus nerve, in addition to its many key effector functions in the gut, is a major sensory pathway and is made up of approximately 80% afferent fibers. Vagal afferents relay signals from peripheral organs (including a portion of the gut from the esophagus to the mid colon) to the central nervous system. It is well recognized that visceral afferent input can modulate cognition, emotion, and behavior via brain-stem nuclei as well as ascending cholinergic and noradrenergic projections to the cortex.¹⁸ An example of such behavioral changes can be seen in the anxiety response elicited by the gastrointestinal pathogen *Campylobacter jejuni*. *Campylobacter jejuni*, which has been correlated with post-infectious irritable bowel syndrome in humans, alters behavior in mice, presumably via vagally mediated pathways.^{19,20} When mice with subclinical *Campylobacter* infection were compared with uninfected mice they displayed anxiety-like behavioral changes. This change occurred in the absence of observed increases in peripheral inflammatory cytokines, suggesting that the behavior change was due to neural activation, rather than via circulating inflammatory mediators. Later studies have confirmed that *Campylobacter* does activate vagal afferent pathways.²¹ Further, studies of *Campylobacter* infected mice show vagally mediated rapid activation of visceral sensory and anxiety associated brain regions, including the amygdala, bed nucleus of the stria terminalis, parabrachial nucleus, nucleus of the solitary tract, and periaqueductal gray, which explain early infection related behavior change.^{22,23} The importance of the vagal connection was also noted in work by Bravo et al., in which the effects of *Lactobacillus rhamnosus* were observed on neurobiological and behavioral measures in mice only in the presence of an intact vagus nerve. In that study, gamma-aminobutyric acid (GABA) expression in the brain changed with chronic probiotic ingestion in conjunction with reduced stress-induced anxiety and depression-like behaviors compared with mice fed control diets. However, animals with vagotomy were comparable to the control group in both the behavioral and neurochemical parameters. Using a murine inflammatory model of chronic colitis with anxiety, Bercik et al. also showed that the anxiolytic effects of a probiotic were abolished by vagotomy.²⁴ On the other hand, in a study of specific pathogen free mice treated with antibiotics, altered hippocampal BDNF levels, and increased exploratory behavior occurred regardless of vagal integrity, suggesting that the intestinal microbiota can influence the CNS via other pathways as well.²⁵

One mode of vagally-independent communication from the microbiota to the CNS likely occurs via the immune system. Systemic immune modulation by gut microbes can stimulate circulating cytokines, which in turn can influence brain function.²⁶ The best described syndrome during which this occurs is the classic sickness behavior in which microbes trigger anorexia, anhedonia, lower pain thresholds, and slowed psychomotor function.²⁷ Microbe-triggered local immune activation in the gut may

be associated with altered barrier function, enteric nervous system activation, and changes in sensory-motor function.²⁸⁻³⁰ If such changes lead to increased gut sensation, then the amplified interoceptive signals could result in pain or anxiety. Conversely, decreased local and peripheral immune activation and improved intestinal barrier function have been shown to occur with the use of probiotics, leading to an improved sense of gastrointestinal wellbeing.³¹⁻³³

Microbe-gut-brain communication can also occur via modulation of the HPA axis. Exaggerated HPA responses to stress occur in germ-free animals exposed to stress, with increased cortisol levels.¹² The importance of microbial modulation of the HPA axis has been demonstrated by administration of beneficial bacteria to animals undergoing maternal separation and restraint stress, with amelioration of basal cortisol levels and intestinal permeability respectively.^{34,35} The effects of gut microbiota on the HPA axis has not been well studied in humans. A single study showed decreased urinary cortisol after probiotic consumption in healthy adult subjects whose mood symptoms also improved, suggesting this pathway may be relevant in the human microbe-gut-brain axis.^{36,37}

Several specific strains of gut bacteria have been shown to produce and secrete neurotransmitters locally, including biologically active compounds such as GABA, serotonin, catecholamines and histamine.³⁸⁻⁴⁰ These bacterially-derived neurotransmitters can relay signals to the CNS via enterochromaffin cells and/or enteric nerves.⁴¹ It is not clear whether they also act centrally via the systemic circulation. In hepatic encephalopathy, ammonia and other potential neurotoxins produced by microbes enter the portal system and due to poor liver function, gain access to the CNS, leading to impaired sleep, cognitive function, and emotional regulation.^{42,43} In health, it is presumed that other less dramatic interactions between the gut and brain occur via this route as metabolites such as short chain fatty acids, biogenic amines, neurotransmitters and neurotransmitter precursors produced by the microbiota gain access to the systemic circulation.^{15,44} Examples include the production of short chain fatty acids, which may affect satiety signals, or tryptophan production, which could influence circulating serotonin levels and mood.⁴⁵⁻⁴⁷ Anxiety-related metabolic profiles in blood and urine were demonstrated by Martin et al., to be consistent with altered microbial activity.⁴⁸ They found that daily intake of dark chocolate, often reported to have positive effects on mood, decreased two specific products of gut microbial activity, hippurate and p-creso sulfate, though changes in subjective mood states were not reported.^{49,50}

In humans, the interaction between gastrointestinal and psychological symptoms is complex with microbiota playing dual roles in the modulation of both peripheral and central processes. Studies in healthy individuals and patients with irritable bowel syndrome indicate that probiotics or antibiotics can improve unpleasant digestive symptoms and it is likely that these symptom changes are dependent on both direct and indirect effects of the microbiota.⁵¹⁻⁵⁴ In individuals with bothersome bloating, pain, or irregular bowel movements, there is the possibility that changes in the gut microbiota may lead to direct symptom improvements via local effects on gut function, such as improved motility or

Figure 1. Potential routes of communication between the microbiota and the brain are shown. Multiple inputs from the periphery can act centrally to modulate mood, pain sensitivity, cognition, and behavior.

secretion. An improved sense of digestive wellbeing, along with the central effects of altered immune or HPA function, may then modulate symptom related anxiety, depressive symptoms, or non-gastrointestinal discomfort. In the case of probiotics or other therapies, which may alter the gastrointestinal flora, non-specific central effects of ingesting a “healthy” food or starting a new treatment are also likely to occur. This has potential for decreased pain and discomfort that is similar to the CNS effects seen in the placebo response.⁵⁵

Effects of CNS on Bacterial Populations

Just as the gut microflora may impact the central nervous system and behavior, top down effects on local microbial populations appear to be important. Stress mediators may alter

intestinal permeability, which leads to local immune activation.⁵⁶ These mediators may also alter bacterial mucosal adherence and internalization into mucosal cells, which can change bacterial population profiles.⁵⁷⁻⁵⁹ Restraint and social stress in rodents and maternal separation in non-human primates lead to disrupted gut microbial composition.⁶⁰⁻⁶³ Clearly, the communication between the microbiota and the CNS is dynamic and bidirectional.

Gastrointestinal Microbiota and Central Nervous System Disease

Whether the changes in gut microbiota associated with early life events can influence cognitive or behavioral factors is unknown. A potential connection between gut microbiota,

gastrointestinal infection and autism had been raised given reports of symptom improvement with antibiotics, though these early reports have not been replicated.⁶⁴ Multiple reports have followed, however, suggesting over-representation of some gut bacterial species and altered metabolomic profiles in children with autism.⁶⁵⁻⁶⁸ Whether these changes are reproducible and reflect a consequence or potential cause of autism has not been determined. The cumulative data in humans has been supported by a mouse model of autism in which behavioral abnormalities, impaired gastrointestinal barrier function, and altered microbiota can be ameliorated with administration of a specific bacteria, *Bacteroides fragilis*.⁶⁹ At the other end of the age spectrum, hypotheses have been made suggesting that toxins from the intestinal flora can lead to brain changes such as Parkinson disease or dementia.⁷⁰ Early support for these hypotheses can be seen in the improvements in cognitive impairment after probiotic treatment observed in animal models of impaired cognition due to diabetes and inflammation.^{71,72}

Preclinical models, some of which are noted above, raise the possibility that mood symptoms may be influenced by intestinal microbiota. Taken further, suggestions have been made that prevention or treatment of psychiatric disease may one day be possible using manipulation of the microflora.⁷³ In an elegant example, Bravo and colleagues showed that treatment with *Lactobacillus rhamnosus* reduced stress-induced anxiety and depressive type behaviors in mice. In conjunction, the treatment altered gamma-aminobutyric acid (GABA) receptor expression in emotion related brain regions, such as the hippocampus, amygdala and locus coeruleus.⁷⁴ Similar anxiolytic benefits were found in another study of rats receiving a proprietary probiotic product containing lactic acid bacterial strains compared with diazepam, using a conditioned defensive burying test.³⁶ Symptoms suggestive of depression were also alleviated in a rat model of early life stress after treatment with *Bifidobacterium infantis*.⁷⁵ In that study, rats subjected to maternal separation underwent a forced swim test that measures motivational fortitude, peripheral cytokine levels, and brain monoamines. The probiotic treated rats had behavioral measures similar to non-maternally separated rats, and they also had normalization of brainstem noradrenaline and peripheral cytokines.

Human studies of brain-microbiota interactions have lagged behind the preclinical work, in part due to the difficulties of observing central nervous system changes in the human. One published study has successfully shown the effects of probiotics on brain function in healthy humans.⁷⁶ The response to an emotional faces attention task was measured with functional magnetic resonance imaging in healthy women before and after 4 weeks of chronic probiotic ingestion, ingestion of a control dairy product, or no treatment at all. Women who had taken the probiotic showed reductions in brain response to the task, particularly in sensory and interoceptive regions. While no associated group differences in mood were observed, the small sample size may have limited the ability to identify such changes. In contrast, improvements in several psychological parameters after 1-month treatment with probiotics were seen in a randomized, placebo-controlled study of healthy men and women, which also observed

reductions in urinary free cortisol.³⁶ In that study, global psychological distress, as measured by the global severity index of the Symptom Checklist-90, and anxiety symptoms, as measured by the Hospital Anxiety and Depression Scale, were improved in the group taking a *Lactobacillus* and *Bifidobacterium*-containing probiotic compared with those taking a matched control product. When viewed in the context of the preclinical work, these two studies support the concept that the gut microbial composition influences the CNS and that carefully designed trials will be able to further define this interaction.

Conclusions and Future Directions

The converging evidence suggests that gut microbiota is playing a role in multiple aspects of human health and disease. Understanding of the specific pathways linking microbiota to the central nervous system is increasing, both in terms of “bottom up” and “top down” effects. As a result, there is a great interest in pursuing clinical studies to assess the effects of microbiota manipulation in humans. Humans have been modulating their gut flora with increasing intensity in the past 100 years with the frequent use of antibiotics, probiotic pills, yogurts, and other fermented foods. However, despite these common interventions, few have identified clear associations between these factors and CNS symptoms or disease processes. This implies that in humans, as opposed to the rodent models, we should be prepared to look for more subtle effects. These effects may be variable, based on the host, baseline microbiota composition, and the mode of intervention. More dramatic effects may be expected in children (and perhaps the elderly) who have a less diverse microbiota and are more vulnerable to external influences in the developing (or declining) brain. Probiotics have generally been considered safe, but as more targeted or aggressive interventions are tested (i.e., fecal transplantation, antibiotic intervention for non-infectious disorders, etc.), our greater knowledge of the potentially wide-ranging effects of these treatments should generate added caution. Future studies evaluating manipulation of the microbiota in humans will require careful planning with controls in place both for safety and to avoid spurious interpretation. Ongoing studies of fecal transplantation for inflammatory bowel disease, irritable bowel syndrome, and *Clostridium difficile* infection will provide the opportunity to examine the secondary central consequences of making a dramatic change in the gut microbiota, albeit with clear confounds due to the treatment of the underlying diseases. Probiotic interventions as primary or adjunctive treatments for symptoms of anxiety, depression, or chronic pain, all presumed to be centrally mediated, should not only assess for symptom improvements, but should also address key factors such as whether the host’s baseline microbiota composition predicts response, if the vehicle in which the probiotic is administered is important, the role of dose response, and the durability of the treatment response. The long-term effects of microbial manipulation in humans will require careful examination beyond the time frames generally used in clinical trials to assess behavioral changes. While

it appears that our gastrointestinal microbiota are indeed communicating with the CNS, we are far from understanding the significance of this interaction in health and disease.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

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