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Gut-Brain Axis and Behavior

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Abstract

In the last 5 years, interest in the interactions among the gut microbiome, brain, and behavior has exploded. Preclinical evidence supports a role of the gut microbiome in behavioral responses associated with pain, emotion, social interactions, and food intake. Limited, but growing, clinical evidence comes primarily from associations of gut microbial composition and function to behavioral and clinical features and brain structure and function. Converging evidence suggests that the brain and the gut microbiota are in bidirectional communication. Observed dysbiotic states in depression, chronic stress, and autism may reflect altered brain signaling to the gut, while altered gut microbial signaling to the brain may play a role in reinforcing brain alterations. On the other hand, primary dysbiotic states due to Western diets may signal to the brain, altering ingestive behavior. While studies performed in patients with depression and rodent models generated by fecal microbial transfer from such patients suggest causation, evidence for an influence of acute gut microbial alterations on human behavioral and clinical parameters is lacking. Only recently has an open-label microbial transfer therapy in children with autism tentatively validated the gut microbiota as a therapeutic target. The translational potential of preclinical findings remains unclear without further clinical investigation.

Introduction

While alterations in bidirectional brain-gut microbiota interactions are believed to be involved in the pathogenesis of well-known gut disorders such as irritable bowel syndrome (IBS) and related functional gastrointestinal (GI) disorders [1], such alterations have also been suggested to play a role in the pathophysiology of several brain disorders, including disorders of mood and affect [2], autism spectrum disorders (ASD) [2], Parkinson disease (PD) [3], and chronic pain [4]. Despite the remarkable support for such associations based on behavioral studies in mouse models of these disorders, there is limited information regarding the translational relevance of these preclinical data for human diseases. Furthermore, there are considerable gaps in our understanding of the magnitude as well as the sites, pathways, and molecular mechanisms within the gut-brain axis that are responsible for these alterations, even though candidate molecules have recently been identified which may play a role in altered social behaviors [5] and in PD [6]. The intestinal microbiota and its metabolites have been shown to be involved in modulating GI functions, given their

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ability to affect intestinal permeability, mucosal immune function, intestinal motility and sensitivity, and release of GI hormones and neurotransmitters from enteroendocrine and enterochromaffin cells [2], as well as activity in the enteric nervous system [reviewed in 7]. Additionally, preclinical evidence suggests that the microbiota and its metabolites are likely to be involved in modulating behaviors and brain processes, including stress responsiveness [reviewed in 8], emotional behavior [reviewed in 9], pain modulation [reviewed in 2], ingestive behavior [reviewed in 10], and brain biochemistry [reviewed in 11].

To date, there is limited high-quality evidence regarding alterations in microbial ecology or production of microbial-derived metabolic products in human patients with brain or brain-gut disorders. For example, there is inconclusive evidence from human studies regarding the beneficial effects of manipulating the microbiota with prebiotics and antibiotics in patients with IBS, even though meta-analyses suggest a small therapeutic effect for probiotics [reviewed in 12]. Furthermore, it is not clear whether alterations observed in the microbiota of patients with these disorders arise from primary alterations at the gut microbial interface (bottom-up effects) and/or changes in brain-gut signaling (top-down effects).

Despite the limited clinical evidence, a large and growing number of review articles have appeared in the literature [2, 3], extrapolating the preclinical findings to human diseases. However, other than a series of case reports on the development of psychotic symptoms following broad-spectrum antibiotic intake [13], there is limited clinical evidence that acute alterations in the intestinal microbiota have an effect on clinical symptoms [reviewed in 3, 7].

This article will critically review the current preclinical literature about the role of the gut microbiota in behavior, explore the current evidence in humans consistent with the preclinical findings, and identify translational research areas required to identify a role of the gut microbiota in modulating the brain and the gut-brain axis.

Preclinical Studies

A number of experimental approaches have been employed to study the modulatory effects of gut microbiota on gut-brain interactions in experimental animals, including treatment with antibiotics [14] fecal microbial transplant [14–16], germ-free (GF) animal models [17], and treatment with probiotics. Considerable progress has been made since Sudo et al. [17] first observed that mice without normal gut microbiota exhibit marked differences in adult stress responsiveness and that these differences can be partially reversed by gut colonization. Microbiota-related effects have been reported in relation to anxiety-like behavior [5, 15, 18–23], depression-like behavior [15, 16, 22, 24], nociceptive responses [4, 25], stress responsiveness [22, 23], feeding behavior, taste preferences, and metabolic consequences [26–28].

These experimental approaches do have limitations which urge caution in translating findings to humans. GF models are born in aseptic conditions, often removed from the mother by cesarean section, and transferred immediately to an isolator in which air, food, and water are sterilized. There is a range of differences in brain and gut biochemistry [19];

blood-brain barrier permeability [29], hypothalamic-pituitary-adrenal (HPA) axis responses [17]; metabolic function [26–28]; and affective [5, 18–24], social [5, 24, 30], and ingestive [26–28] behaviors between GF animals and control animals that have normal or pathogen-free flora and who were reared by normally colonized mothers [19, 20]. The observed biochemical and behavioral changes could be mediated by a lack of gut microbiota directly or indirectly though one or several of the alterations not related to the brain.

Recent evidence suggests that the intrauterine environment is not sterile [31], and one may even speculate that the maternal gut microbial metabolites originating from the maternal gut microbiome may have an influence on fetal brain development. Furthermore, as GF pups are raised by GF mothers, the absence of fecal microbes may interfere with well-characterized maternal behaviors, such as arched-back nursing and anogenital licking. These behaviors have been associated with epigenetic changes at stress-related genes [32] that regulate the development of systems within the CNS [33]. However, in one study where maternal behavior was analyzed on days 2 and 3 postpartum, no effect of the GF status on such maternal behaviors was observed [17]. Altered signaling of the cecum to the brain, secondary to the massive cecal dilation associated with this model, could alter the development of brain regions processing such input. GF mice are leaner than control animals, despite consuming more calories [29]. Metabolic changes secondary to the loss of an important source of calories (gut microbiota-generated short-chain fatty acids) for the developing organism may affect brain development and alter the activity of brain circuits involved in feeding behavior and metabolism. Finally, the recently reported alterations in the permeability of the blood-brain barrier in GF mice is likely to result in significantly altered access of gut microbial metabolites to the brain [34]. Despite the extensive remodeling of biological systems in the GF animal, the fact that some observed behaviors and brain changes could be reversed by reconstitution of pathogen-free microbiota (conventionalization) validates some of the conclusions drawn. Nevertheless, as the GF animal has no counterpart in human brain development, premature conclusions about the translational relevance of these findings to humans should be avoided. Broad-spectrum antibiotics have well-documented transient effects on the composition and diversity of fecal microbiota [14] even though the effects on mucosa-associated microbial communities are not known.

In the studies published since 2010 using different strains of mice and rats, different strains of probiotics, and different experimental paradigms [11], a range of effects of gut microbial modulation was reported on emotional behavior [5, 15, 16, 18–24], learning and memory [22, 35, 36], social interactions [24, 30], and ingestive behaviors [27].

Emotional Behavior

When viewed together, reported findings demonstrate an increase in emotional behavior associated with infection/infestation with pathogens [18–20]; a reduction in basal or induced anxiety-like behavior in animals with normal gut microbiota, following the oral administration of probiotics [21–23, 25, 28]; and both reduced [18–20] and increased [36] anxiety in rodents raised in the absence of gut microbiota. A reduction in depression-like behaviors was observed in different rodent models with normal gut microbiota following

administration of a probiotic [22, 24]. Depression-like behavior in these models was induced by maternal separation [23] and experimental myocardial infarction [24].

Learning and Memory

While improvement in impaired memory function by probiotics was observed in a rodent model of diabetes [35], several studies showed a worsening with exposure to a pathogen [36], GF status [19], and administration of a probiotic [22].

Social and ASD-Like Behavior

Gut microbiota status was found to reduce social interactions in GF mice [30], and probiotics improved social interactions in a rat model after myocardial infarction [24, 30]. Gut microbiota-associated behavioral changes were reported in different ASD mouse models, including maternal immune activation where treatment with the probiotic *Bacteroides fragilis* had a beneficial effect on some of the behavioral abnormalities [5].

Ingestive Behavior

A limited number of studies suggest that gut microbial composition can influence ingestive behavior [26, 27]. Some of these effects are likely mediated by significant alterations in GF animals in intestinal taste receptors, fatty acid receptors, intestinal transport mechanisms, and changes in the release of satiety hormones.

In addition to specific behavioral domains, effects of gut microbial modulation on CNS elements with system-wide influence were reported, including the HPA axis and signaling systems.

HPA Axis Responsiveness

Increased basal or stimulated HPA axis activity (measured as blood corticosterone or ACTH levels) was reported in GF Swiss-Webster and BALB/c mice [18, 20, 36], while a probiotic-induced reduction in corticosterone levels was observed in normal mice [22]. The association between increased HPA axis responses and reduced anxiety-like behaviors observed in several of the studies performed in GF mice suggests that hypothalamic (HPA axis) and nonhypothalamic (anxiety-like behavior) components of central stress circuits may be affected differentially by the GF conditions, depending on species and mouse strain, a response pattern not seen in the majority of existing anxiety models in which these two components of the stress response are generally congruent. One may speculate that the increased HPA axis activity in GF animals represents a response of the organism to the loss of microbiota-related energy sources, but conflicting evidence does exist [reviewed in 7].

Brain Signaling Systems

Several studies have shown reduced expression of brain-derived neurotrophic factor in the brains of GF animals (primarily in hippocampus) and increased expression in infection models. Regional changes in the expression of GABA receptor A and B subunits, NMDA receptor subunits, serotonin 1A, tryptophan, and tryptophan metabolite levels have all been reported [reviewed in 7].

Clinical Studies

Ongoing recognition of the role of the gut microbiota in preclinical models of disease, especially neuropsychiatric and metabolic diseases, demands evaluation in clinical settings. While there is a significant and growing body of literature characterizing the differences between healthy controls and individuals suffering from particular diseases, these do not help to answer the question of causality better. Studies focused on ASD, major depressive disorder (MDD), and PD have made the most significant progress towards this objective.

Autism Spectrum Disorder

The wide-ranging and variable symptoms of ASD include the difficulty with social and communicative behavior, repetitive behavior, and restricted interests. Co-morbidities include intellectual disability, sleep disruption, feeding difficulty, and GI symptoms. The prevalence of GI symptoms is 9–90%, and children affected by ASD are nearly 8 times more likely to have at least one GI symptom. Moreover, GI symptom severity is strongly correlated with ASD symptom severity. These symptoms are also highly correlated with anxiety and sensory overresponsivity conditions modulated by gut microbiota in preclinical models [reviewed in 37].

Gut dysbiosis is an increasingly documented symptom of ASD, but causality remains limited to intriguing, albeit untested, hypotheses. The promising results from an uncontrolled study recently published by Kang et al. [38] showed that transfer of a standardized human gut microbiota led to reductions in GI and behavioral symptoms with a concordant maintenance of gut eubiosis, which remained 8 weeks after the intervention. Subsequent, randomized, double-blind clinical trials are essential to verify the gut microbiota as an effective therapeutic target for ASD maintenance symptoms.

Major Depressive Disorder

Preclinical studies have demonstrated the capacity of microbiota to influence parameters significant to depression pathogenesis and severity, including the levels of neurotransmitters and neuromodulators serotonin [reviewed in 39], brain-derived neurotrophic factor [17, 18, 20], and γ -amino butyric acid [22], synaptogenesis and synapse maturation [19]. Furthermore, MDD-associated gut dysbiosis is corroborated by the abnormal serum immunological parameters of depressed patients. An increased toll-like receptor 4 expression and enhanced immunoglobulin-mediated immune response to lipopolysaccharides of specific commensal bacteria implicates a “leaky gut” and increased bacterial translocation [reviewed in 39]. While studies characterizing the gut microbiome of MDD versus health have yielded marginally distinct assemblage correlations, 3 different types of studies suggest causality. Depressed human-to-rodent fecal microbial transplants have induced depressive behaviors in animal models [15, 16]; pre- and probiotic administration to healthy controls has improved anxiety and mood; and finally, incidences of *Escherichia coli* subtype outbreaks in Canada and Germany led to rises in depression and anxiety-related symptoms among the affected population [reviewed in 39].

Parkinson Disease

While the clinical hallmarks of PD remain motor deficits, there are numerous nonmotor symptoms present which contribute more detrimentally to patient quality of life. These nonmotor symptoms include psychiatric disorders, sensory alterations, and gastrointestinal problems related to dysfunctional autonomic and enteric nervous system activity. The risk of PD development increases with the infrequency of bowel movement and constipation severity. Moreover, constipation is among the earliest features, appearing as early as 15.3 years before motor dysfunction [reviewed in 40]. Early GI symptoms, thus, may be prodromal, making the gut microbiota a promising source of information for diagnosis, prognosis, and, potentially, pathogenesis. To date, clinical studies of PD and gut microbiota remain limited to characterizing the assemblage differences against healthy controls. However, Sampson et al. [6] have provided the first evidence suggesting causality by demonstrating that physical impairments in a PD rodent model are enhanced by microbiota from PD but not healthy controls.

Summary and Future Perspectives

Based on currently available evidence, there is no question that there is a relationship between the composition and function of the gut microbiota and brain function. While such a relationship has clearly been established in rodent models, the strongest evidence to date to support a significant role of such interactions in adult human subjects comes from a brain imaging study in healthy individuals and from several studies in MDD and PD patients and ingestive behavior in obesity. The majority of human studies have demonstrated associations rather than causality.

Plausible explanations for the apparent discrepancy between dramatic results in rodent models and the lack of conclusive evidence for the translatability of these findings into human disease populations include the limited homology of the human and the mouse brain in terms of brain networks relevant for human brain disorders, the limitations of the gnotobiotic mouse model, and the likelihood that brain-gut microbiome interactions in the adult are fairly stable and may have been established largely during the first 3 years of life. However, during this developmental period, there are many factors which have been shown to influence the assembly of the gut microbial architecture, including the diet and stress level of the pregnant mother, the mode of delivery, breast feeding, and early adverse life events. There is a need for large-scale, longitudinal human studies in well-phenotyped populations (including pediatric populations) as well as interventions targeted at the gut microbiome (pre- and probiotics) and the brain (mind-based therapies) to establish causality between brain and gut microbial influences.

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