The Microbiota-Gut Brain Axis in Health and Disease

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Abstract

The "gut-brain axis" is a bidirectional communication system between the CNS and the gastrointestinal system that is comprised of neural and humoral pathways. There is accumulating evidence, mainly from animal studies using perturbation of the microbiota by antimicrobials and gnotobiotic models, that intestinal bacteria play an important role as modulators and signalling components of the gut-brain axis.

Glossary of Abbreviations
BDNF: Brain-Derived Neurotrophic Factor
CNS: Central Nervous System
ENS: Enteric Nervous System
IBS: Inflammatory Bowel Syndrome
ME: Median Eminence
NGF: Nerve Growth Factor
POMC: Pro-Opio-Melanocortin
SPF: Specific Pathogen-Free Flora

Introduction

Clinicians and researchers have long recognized the link between gastrointestinal function and the central nervous system (CNS). The "gut-brain axis" is a bidirectional communication system comprised of neural pathways, such as the enteric nervous system (ENS), vagus, sympathetic and spinal nerves, and humoral pathways, which include cytokines, hormones and neuropeptides as signalling molecules. Recently, results in animal models have generated great interest into the role of intestinal microbes as key players in gut-brain communication (Figure 1).

The intestinal microbiota involves a wide diversity of microbial species[1] and can be considered a postnatal acquired organ that performs different functions for the host. Intestinal microbes have developed a mutual relationship with their host, and they play a crucial role in the development of innate and adaptive immune responses[2,3] and influence physiological systems throughout life by modulating gut motility, intestinal barrier homeostasis,[4,5] absorption of nutrients, and the distribution of somatic and visceral fat.[6,7]

Until recently, composition of this microbial community was considered unique for each individual and relatively stable over time.[8,9] However, using deep sequencing of stool samples from several hundred individuals, the European MetaHit consortium study has shown that human microbiota profiles can be grouped in three major bacterial enterotypes dominated by *Bacteroides, Prevotella* and *Ruminococcus*, respectively.[10] Distinct enterotypes strongly associated with long-term diets has been confirmed by Wu et al., linking protein and animal fat with *Bacteroides* and consumption of carbohydrates with *Prevotella*.[11] This indicates that despite large numbers of bacterial strains in the human intestine, there is a limited number of well-balanced host-microbial symbiotic states that might respond differently to diet and drug intake.

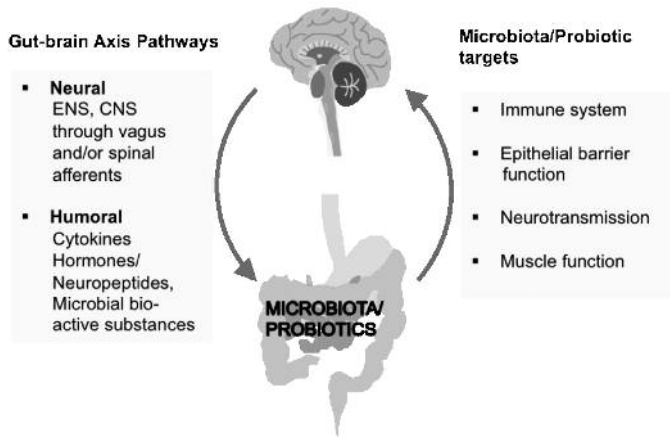

Figure 1: The gut-brain axis. Pathways of communication and probiotic targets.

Microbiota-Gut-Brain Axis

The concept that gut bacteria are a driving force for immune maturation and gut function in the host is well accepted. The notion that bacteria could also influence brain function and behavior is seemingly implausible, but clinicians routinely use laxatives and oral antibiotics to treat patients with altered mental status due to hepatic encephalopathy.[12] Several clinical studies have also described altered composition of gut microbiota in patients with autism[13] and suggested at least a short-term beneficial effect of antibiotic treatment,[14,15] though no randomized clinical trial currently is available. There also are multiple reports of patients developing psychoses after administration of different antibiotics.[16] No current studies have characterized the gut microbiota associated with depression or anxiety, but earlier studies demonstrated that depression in females is associated with increased fermentation of carbohydrates, indirectly implicating changes in the composition or metabolic activity of the gut microbiota.[17]

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Lessons from Animal Models: Effects of Bacteria on the CNS

At this point, the brunt of evidence linking microbes with behavior and brain biochemistry comes from animal studies. Pivotal experiments performed by Lyte et al. have shown that mice display altered, anxiety-like behavior during the early phase of acute infection with *Campylobacter jejuni*.[18] This abnormal behavior occurred within several hours after introduction of the intestinal pathogen into the GI tract, before any significant immune response was mounted, suggesting that this was not a consequence of cytokine-induced sickness behavior. Subsequent studies showed that presence of *C. jejuni* triggers activity of vagal ascending pathways and a specific activation pattern in multiple brain regions previously implicated in anxiety-like behavior[19,20] This clearly illustrates that the neural system can detect an acute change in the gut and can selectively identify a pathogen in the gut lumen.

Studies using chronic *H. pylori* infection in mice have shown that this pathogen alters gastric physiology, namely delayed gastric emptying and visceral sensitivity, with upregulation of SP and CGRP-containing nerves in the stomach and the spinal cord.[21,22] Furthermore, chronic *H. pylori* infection leads to abnormal feeding behavior characterized by frequent feeding bouts with less food consumed per feeding than controls, which is reminiscent of early satiety observed in patients with functional dyspepsia.[22] The abnormal feeding pattern was accompanied by downregulation of regulatory peptide pro-opio-melanocortin (POMC) in the arcuate nucleus and upregulation of the proinflammatory cytokine TNF-α in the median eminence (ME) of the hypothalamus. The ME is part of the circumventricular organ, an area of the brain where the blood-brain barrier is relatively leaky, enabling metabolites/molecules from the systemic circulation to enter the CNS. Interestingly, altered behavior and biochemical abnormalities persisted for at least two months post-bacterial eradication, suggesting that changes induced by chronic infection in the CNS may be long-lasting or permanent.

To establish a link between commensal bacteria and the CNS, several experimental approaches can be undertaken. One is to compare germ-free animals with animals colonized with specific pathogen-free flora (SPF). Sudo et al. demonstrated an abnormal HPA axis with elevated ACTH and corticosterone levels in response to restraint stress in germ-free mice, which normalized after colonization with commensal bacteria.[23] Furthermore, germ-free mice had lower brain-derived neurotrophic factor (BDNF) levels in the cortex and hippocampus. Several recent studies have compared behavior and brain biochemistry in germ-free and SPF mice.

Overall, using standard behavioral tests, such as elevated plus maze, open field and light/dark preference tests, germ-free mice displayed higher exploratory and lower anxiety-like behavior than SPF mice.[24,25] Heijtz et al. showed that compared to germ-free mice, SPF mice had higher central expression of neurotrophins, such as nerve growth factor (NGF) and BDNF.[24] Furthermore, there was differential expression of multiple genes involved in the secondary messenger pathways and synaptic long-term potentiation in the hippocampus, frontal cortex and striatum. Similarly, Neufeld et al. demonstrated increased expression of NMDA receptor subunit NR2B in the central amygdala and serotonin receptor 1A (5-HT 1A) expression in the hippocampus in SPF mice compared to germ-free mice.[25] The pronounced differences between germ-free mice and mice colonized with complex microbiota may relate to the ability of gut bacteria to affect multiple aspects of host metabolism, immunity and physiology. Colonization with a single commensal bacterium, *B. thetaiotomicron*, was shown to change expression of a vast array of genes in the intestine encoding for metabolism, intestinal permeability and angiogenesis as well as for glutamate uptake, GABA production and neurotransmitter release.[26]

A different approach to investigate the role of microbiota in gut-brain axis is to perturb a previously "stable" microbiota in healthy adult mice by oral administration of nonabsorbable antimicrobials. Combination of neomycin, bacitracin and pimaricin induced changes in colonic microbiota composition (gut dysbiosis) in SPF mice, with a marked increase in *Firmicutes*, mainly *Lactobacilli* spp, and decrease in γ-proteobacteria. This was accompanied by an increase in mouse exploratory behavior and altered BDNF levels in hippocampus and amygdala[27] (Figure 2A).

The same antimicrobial treatment failed to induce behavior abnormalities in germ-free mice or in mice treated intraperitoneally with antimicrobials. The antimicrobial regime used in this study did not induce measurable changes in gut inflammation or change levels of intestinal serotonin (5-HT), noradrenalin (NA) or dopamine. Interestingly, studies using subdiaphragmatic vagotomy or chemical sympathectomy before antimicrobials suggest that vagal and sympathetic pathways are not involved in gut-brain communication in this experimentally induced dysbiosis model of altered behavior.

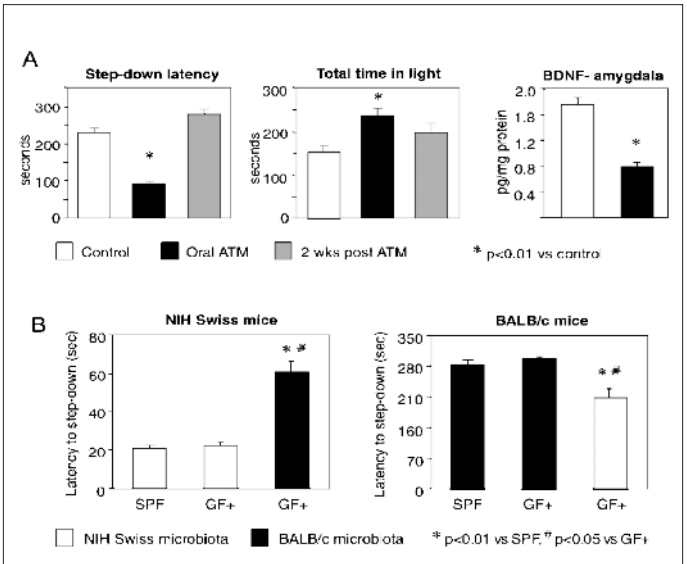

Figure 2: Effects of commensal bacteria on behavior.

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Behavior has a genetic component, and it is known that mouse strains differ in their behavioral phenotype. There also is a difference in microbiota composition among mouse strains, and the "SPF" status does not indicate uniformity of the microbiota, only that mice have been screened for the most common murine pathogens. BALB/c and NIH Swiss mice are on opposite ends of the behavior phenotype: BALB/c mice are timid and less exploratory, while NIH Swiss mice display a high exploratory drive. BALB/c and NIH Swiss mice were reared under germ-free conditions and then colonized with SPF microbiota from either NIH Swiss or BALB/c mice. Germ-free mice colonized with microbiota from the same strain exhibited similar behavior as the SPF mice. However, mice colonized with microbiota from the other strain exhibited a behavior profile similar to the donor[27] (Figure 2B). This was not accompanied by measurable changes in systemic or gut immune activation or levels of intestinal 5-HT, NA or dopamine. A change in central neurotrophins was observed one week post-colonization. We can therefore speculate that host behavioral phenotype is also influenced by microbial factors.

Probiotics and the CNS Function

Psychiatric comorbidities, such as anxiety and depression, are common in patients with chronic bowel disorders, including inflammatory bowel syndrome (IBS) and inflammatory bowel disease.[28,29] Both disorders also are associated with abnormal intestinal microbiota profiles. In this respect, chronic infection with a noninvasive parasite or mild chemically induced colitis was shown to be associated with anxiety/depression-like behavior and decreased levels of hippocampal BDNF expression.[30,31] Interestingly, both abnormalities were normalized with treatment of the probiotic *B. longum* NC3001 but not with *L. rhamnosus* NCC4007. *B. longum* did not improve gut inflammation or circulating cytokines, however, its anxiolytic effect was absent in mice with previous vagotomy, suggesting that its action was neurally mediated. This was further confirmed by *ex vivo* studies, in which electroresponsiveness of enteric neurons was assessed after perfusion with *B. longum* supernatant. Compared to controls, *B. Longum*-treated neurons fired less action potentials in response to suprathreshold depolarizing current.[31]

The beneficial effect of probiotic bacteria may extend to healthy individuals. A study by Desbonnet et al. showed that administration of *Bifidobacterium infantis* to healthy Sprague-Dawley rats reduced concentrations of serotonin and dopamine metabolites in the frontal and the amygdaloid cortex, respectively.[32] The authors suggested that this bacterium may have an anxiolytic potential, although no difference in behavior was found in that study. Subsequent experiments with the same bacterium using maternal separation models demonstrated beneficial effects on altered behavior together with normalization of noradrenaline concentrations in the brainstem.[33]

Bravo et al. have recently demonstrated that administration of the probiotic *L. rhamnosus* JB1 promoted exploratory behavior and attenuated despair-like behavior, as assessed by an elevated plus maze and forced swim test, respectively, in healthy BALB/c mice. This was accompanied by region-dependent alterations in GABA(B1b) and GABA(Aα2) mRNA in the brain,[34] which was vagally dependent, as subdiaphragmatic vagotomy abolished both changes in brain biochemistry and behavior. Thus, animal studies support the notion that commensal bacteria and specific probiotics can influence brain chemistry and the function of the central nervous system.

Conclusion

While clinical observation and psychiatric comorbidity in various chronic intestinal disorders support a role of the intestinal microbiota in gut-brain axis communication, the strongest evidence for a role of microbes as signalling components in the gut-brain axis comes from animal studies using perturbation of the microbiota by antimicrobials and gnotobiotic models. Mechanisms of communication are likely to be multiple and involve neural, humoral and inflammatory pathways, depending on the host and environmental factors.

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