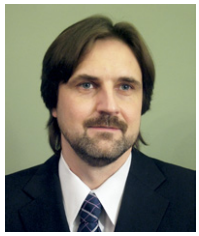
The Relationship Between Intestinal Microbiota and the Central Nervous System in Normal Gastrointestinal Function and Disease



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Although many people are aware of the communication that occurs between the gastrointestinal (GI) tract and the central nervous system, fewer know about the ability of the central nervous system to influence the microbiota or of the microbiota's influence on the brain and behavior. Within the GI tract, the microbiota have a mutually beneficial relationship with their host that maintains normal mucosal immune function, epithelial barrier integrity, motility, and nutrient absorption. Disruption of this relationship alters GI function and disease susceptibility. Animal studies suggest that perturbations of behavior, such as stress, can change the composition of the microbiota; these changes are associated with increased vulnerability to inflammatory stimuli in the GI tract. The mechanisms that underlie these alterations are likely to involve stress-induced changes in GI physiology that alter the habitat of enteric bacteria. Furthermore, experimental perturbation of the microbiota can alter behavior, and the behavior of germ-free mice differs from that of colonized mice. Gaining a better understanding of the relationship between behavior and the microbiota could provide insight into the pathogenesis of functional and inflammatory bowel disorders.

The gut–brain axis (GBA) is a bidirectional neurohumoral communication system that integrates brain and gastrointestinal (GI) functions. The GBA has been implicated in the pathophysiology of functional GI disorders, and evidence is emerging for its role in the pathogenesis of inflammatory disorders of the gut such as inflammatory bowel disease (IBD). It would be a relatively straightforward matter to integrate information about the intestinal microbiota with that of the GBA by simply reviewing literature on interactions between flora and the GI tract. However, the brain is the most influential organ within the axis, and communication is bidirectional. Thus, it is important to consider the influence of the brain on the microbial content of the gut and, conversely, to examine the evidence showing that the intestinal microbiota influences the brain and behavior. Investigation of the integration of the intestinal microbiota into the GBA could improve the understanding of the pathophysiology of both functional[1] and inflammatory[2] bowel conditions.

The GBA contributes to homeostasis of several systems, including GI function, appetite, and weight control. Because GI motility and epithelial function are critical determinants of the habitat for the microbiota, changes induced by the central nervous system or the GI tract alter the habitat and perturb the intestinal microbiota.[3] The longstanding observation that oral antibiotics and laxatives ameliorate hepatic encephalopathy provides a potent reminder that the intestinal microbiota is capable of influencing behavior, albeit under pathologic conditions.[4] Taken together, these observations provide a framework for considering the integration of the intestinal microbiota into the bidirectional GBA.

The Intestinal Microbiota

The gut contains a vast and complex microbial ecosystem, comprising mainly bacteria, of which most are strict anaerobes; it also includes fungi and viruses,[5–7] but only bacteria are considered in this review. Commensal bacteria instruct the immune and physiologic systems throughout life and are responsible for the presence of inflammatory and immune cells in the healthy gut: so-called "physiologic" or "controlled" inflammation. The term physiologic inflammation refers to the presence of

Abbreviations used in this paper: ACTH, adrenocorticotrophic hormone; GBA, gut–brain axis; GI, gastrointestinal; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; SPF, specific pathogen-free.

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inflammatory cells in the mucosa and submucosa of the healthy GI tract and reflects the presence and immunologic accommodation (rather than immune tolerance) of the intestinal microbiota. The microbiota serves the host by protecting against pathogens, participating in the intake nutrients from the diet, metabolizing certain drugs and carcinogens, and influencing the absorption and distribution of fat.[8,9] The influence of the intestinal microbiota extends beyond the GI tract, contributing to, for example, pain perception in the skin[10] and fat deposition in the liver.[11,12] Disruption of the symbiotic relationship between the microbiota and the GI tract, referred to as dysbiosis,[13] perturbs host functions and, in some cases, causes the expression of overt and serious diseases such as IBD and *Clostridium difficile* colitis.[14–16]

Influence of the Microbiota on the GI Tract

A strategy that is commonly used to investigate interactions between the microbiota and the host is to compare germ-free animals with those colonized with a single strain or multiple strains of bacteria.[17] The microbiota influences expression of a broad array of host genes. A comparison of germ-free mice and mice colonized with *Bacteroides thetaiotaomicron*, a prominent member of the adult mouse and human gut microflora, showed that the microbiota modulate the expression of genes that regulate nutrient absorption, mucosal barrier enhancement, xenobiotic metabolism, and angiogenesis.[18] Colonization with *B. thetaiotaomicron* also induced a 2- to 5-fold increase in mRNA encoding the synaptic vesicle-associated protein-33,[18] which is involved in synaptic neurotransmission.[19] This finding indicates that commensal bacteria can influence the expression of genes whose products influence function in the nervous system.

Comparisons of germ-free and colonized animals indicate that, although crypt villous formation does not require the presence of bacteria, epithelial cell differentiation, including Paneth cell development, depends on the microbiota in a way that serves both the host and resident bacteria (for review, see Falk et al[17]). Similarly, the production and composition of mucin[20] and the development of 5-hydoxy-tryptamine–secreting enteroendocrine cells is influenced by the microbiota.[21] Germ-free rodents have an enlarged cecum, reflecting a gross disturbance in GI motility[22,23]; its prompt reversal to normal size on bacterial colonization identifies the microbiota as a determinant of GI motility.[24–26] The abnormal motility of germ-free animals probably reflects a combination of the lack of a mature enteroendocrine system,[21] changes in neurotransmission,[18] and immaturity of the mucosal immune system. The intestinal microbiota also has an important influence on the imprinting, maturation, and maintenance of the mucosal immune system (for reviews, see Falk et al[17] and Macpherson et al[27]). Inflammatory cells are sparse in the germ-free intestine, and secondary lymphoid structures are not developed. The significant number of inflammatory cells in the lamina propria of the colonized intestine of healthy hosts and the preservation of normal epithelial structure and function are reflections of the delicate and mutually beneficial relationship between the intestinal microbiota and the host. Disruption of this balance, as a result of perturbation of the microbiota by infection or antibiotics, results in dysbiosis. The effect of dysbiosis on the host is determined by the nature and magnitude of change in the bacteria composition of the GI tract, as well as by host susceptibilities.

Another strategy for assessing the effect of the intestinal microbiota on host function is to perturb the commensal bacteria with the use of oral antibiotics.[28] A combination of neomycin and bacitracin altered the microbiota in mice, substantially reducing the *Lactobacillus* population.[29] As shown in Figure 1, this resulted in a small increment in myeloperoxidase (MPO) activity (a measure of granulocytic inflammatory cell activity) without causing tissue damage. This increment in physiologic inflammation was accompanied by an increase in immunoreactive substance P, a sensory neurotransmitter, in the intestinal wall. The functional consequence was an increase in the visceromotor or pseudoaffective response (abdominal wall contraction after colorectal balloon distension), a widely used measure of visceral pain.[30] Thus, perturbation of the microbiota produced a response profile reminiscent of changes seen in some patients with irritable bowel syndrome (IBS): subclinical inflammation or immune activation and visceral hyperalgesia. Interestingly, when the mice were gavaged with *Lactobacillus paracasei*, the antibiotic-induced changes in inflammation, neurotransmitter content, and the visceromotor response improved.[29] Because the antibiotic-induced changes in the visceromotor response, immunoreactive substance P, and myeloperoxidase activity could also be attenuated by the administration of dexamethasone, it was concluded that the changes in visceral perception were secondary to the increase in the inflammatory or immune cell presence induced by the dysbiosis.[29] These results show that commensal bacteria can influence primary afferent nerves in the gut and serve as an example of a functional relationship between the sensory component of nervous system and the intestinal microbiota.

Influence of GI Physiology on the Microbiota

Although the microbiota exert a broad influence on host physiology, the converse is also true. Under normal conditions, the GI tract provides a stable habitat for commensal bacteria that supports its structural and functional integrity (Figure 2*A*). Disturbance of normal GI physiology destabilizes the habitat, resulting in changes in its microbial composition. An example of this is the change in the bacterial composition of the GI tract

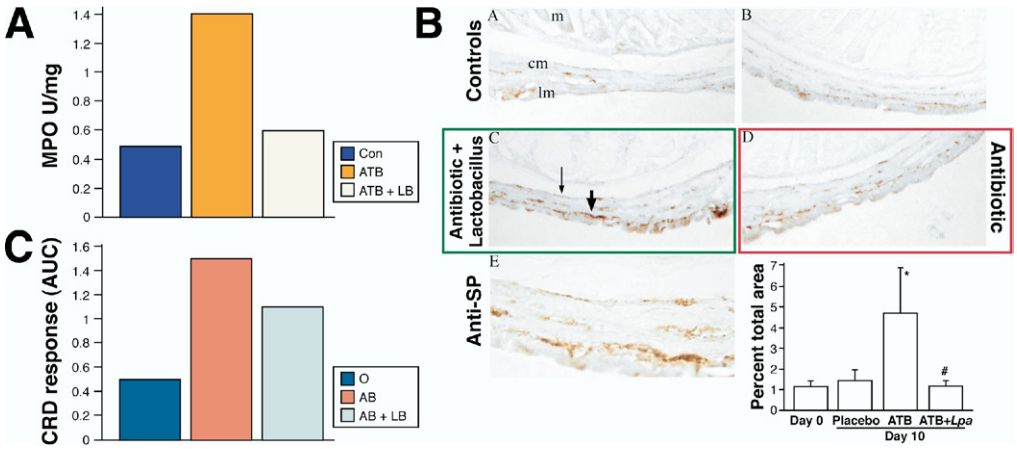

Figure 1. The effect of antibiotic-induced perturbation of the intestinal microbiota on myeloperoxidase activity (*A*), immune-reactive substance P in the intestinal wall (*B*), and visceromotor response to balloon distension in mice (*C*) with or without treatment with *Lactobacillus paracasei*. AB, antibiotic; ATB, antibiotic; AUC, area under the curve; Con, control; CRD, colorectal distension; LB, lactobacillus. Adapted with permission from Verdu et al.[29]

after interruption of normal interdigestive motility in the rat[31] and in human beings.[3] Changes in epithelial cell physiology, mucous secretion, and intestinal barrier function are also likely to affect the mucosal-associated microbial ecosystem.[17] The sympathetic nervous system facilitates the selective presentation of enteric bacteria to the mucosal immune system.[32] Norepinephrine-containing nerve fibers were identified in close proximity to the epithelium that overlies lymphoid follicles in pig jejunum. Application of norepinephrine increased the uptake of pathogenic bacteria into the follicles; this was prevented by the adrenergic antagonist phentolamine. However, the underlying cellular mechanisms were not elucidated but probably involve the interaction of sympathetic nerves with dendritic cells or specialized epithelium to sample luminal bacteria and selectively take up pathogenic bacteria for presentation to the mucosal immune system.[32]

Evidence also suggests that the release of biologic amines, such as norepinephrine, can influence the composition of the intestinal microbiota. This neurotransmitter has been shown to stimulate the growth of pathogenic and nonpathogenic *Escherichia coli* in vitro and to influence their adherence to the mucosa.[33–35] Changes in host physiology, initiated within the GI tract or by the central nervous system, produce changes in the bacterial composition of the GI tract. Alternatively, changes in the microbiota, induced by infection or antibiotics, or other events such as stress (see below) perturb physiologic inflammation and GI physiology. A change in GI physiology provides an altered habitat that in turn supports a different microbiota. We propose that this cycle (Figure 2*B*) could be a basis for maintaining a state of GI dysfunction after perturbation of the microbiota; it could also explain the development and persistence of dysbiosis in conditions in which there is a primary disturbance of GI physiology. So, the optimal therapy for these disorders would stabilize both host physiology and the bacterial composition of the GI tract.

Ability of the Brain to Influence Microbiota

Several animal studies suggest that psychological stress alters GI flora, but each of these studies has limitations. Tannock and Savage[36] reported changes in GI flora of mice stressed by deprivation of food, water, or bedding. However, these environmental changes would be expected to have a direct effect on the microbiota that is independent from a stress response. Bailey and Coe[37] used maternal separation to demonstrate a reduction in lactobacilli for ≤7 days in infant rhesus monkeys; these results should be interpreted cautiously because the stressor limited maternal contact, and the analysis was limited to the identification of culturable bacteria. An interesting finding was that the reduction in lactobacilli appeared to promote the emergence of enteric pathogens such as *Campylobacter jejuni*. Additional studies showed the ability of probiotics to ameliorate stress-induced changes in GI function[38] and to attenuate the observed reduction in lactobacilli in maternally deprived rat pups.[39] A recent study showed that stress during early life (maternal separation) produced changes in the microbiota of the offspring, and this was associated with increased levels of corticosterone and inflammatory cytokines[40]; the investigators speculated that this could reflect cytokine-induced hyperresponsiveness of the hypothalamic–pituitary pathway in response to maternal separation. In another study, maternal separation in mice was accompanied by an increase in intestinal permeability and a vulnerability of the GI tract to inflammatory stimuli.[41] Taken together, research conducted on offspring that had been separated from their mothers show an increased stress response, a systemic cytokine response, increased intestinal permeability, and a shift in the bac-

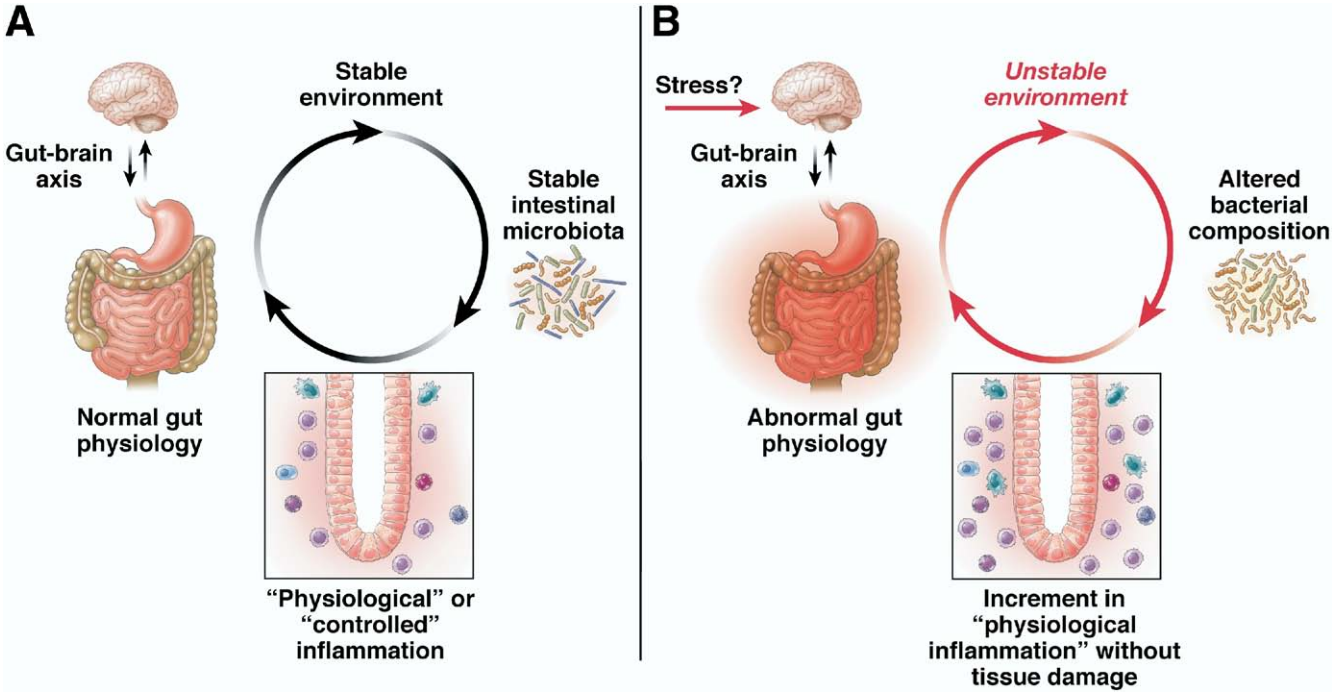

Figure 2. (*A*) The interrelationship of the intestinal microbiota and gastrointestinal physiology and inflammation in health (*A*) and in the presence of intestinal dysbiosis (*B*). (*A*) The gastrointestinal tract under normal conditions provides a stable habitat for commensal bacteria that supports the structural and functional integrity of the gut. The stable bacterial population in turn supports normal gut physiology. (*B*) Disturbance of (1) normal gut physiology or (2) the microbiota destabilizes the habitat, resulting in change in physiologic inflammation which in turn alters physiology.

terial composition of the GI tract; these changes could contribute to the increased susceptibility of the GI tract to chemical and infectious inflammatory stimuli observed in stress models. Because the stressor, separation of mother and offspring, is applied at a time when the GI tract is becoming colonized and host immune and physiological systems are maturing, the extent to which these findings can be applied to adult animals is limited. Reserpine-induced depression in adult mice was also accompanied by a vulnerability to GI tract inflammation,[42] but underlying mechanisms might differ from those observed in the maternal separation model, and the effect of the adult-onset depression on the microbiota has not yet been studied.

There are several mechanisms by which stress can alter the bacterial composition of the GI tract, including changes in epithelial cell function and mucus secretion as well as changes in GI motility.[41,43,44] As described, release of norepinephrine into the GI tract during stress might preferentially stimulate the growth of specific strains of bacteria as well as their ability to adhere to the mucosa.[32–34]

Ability of Microbiota to Influence the Brain and Behavior

The most compelling evidence of a GI microbe–brain interaction is the often dramatic improvement in patients with hepatic encephalopathy after the administration of oral antibiotics and laxatives.[4] Although the mechanistic basis for hepatic encephalopathy is incompletely understood,[45] there is some evidence from a rat model of hepatic failure that certain bacteria can produce a ligand for the benzodiazepine receptor that may contribute to the encephalopathy.[46] Observations in human beings offer provocative but nevertheless weaker evidence of communication between the microbiota and brain. Patients with symptoms of depression have been shown to have abnormal profiles of breath hydrogen excretion after ingesting fructose and other sugars.[47] Eliminating fructose from the diet resulted in an improvement in depression.[48] In addition, fructose malabsorption was accompanied by a reduction in plasma tryptophan.[49] Fructose malabsorption provides substrate for rapid bacterial fermentation, resulting in changes in GI motility, the mucosal biofilm, and the profile of the microbiota.[50] A recent study showed that rats given *Bifidobacteria infantis* for 14 days had increased plasma tryptophan levels, suggesting that commensal bacteria have the ability to influence tryptophan metabolism.[51] Thus, it is possible that the reported linkages between carbohydrate malabsorption and depressive-like behavior reflect bacterial interference with tryptophan metabolism.[51] Culture- and molecular-based analyses have shown changes in the microbiota of a small number of autistic patients, compared with controls, with a greater prevalence of clostridial species in autistic patients.[52,53] In an uncontrolled trial, vancomycin provided transient symptomatic relief to a

limited number of children with late-onset autism.[54] However, the relationship between the microbiota and autistic behavior remains speculative.

Studies have shown that the brain responds to the introduction of noninvasive pathogenic bacteria into the cecum; brain stem nuclei are rapidly activated,[55]and there is expression of anxiety-like behavior in mice.[56,57] This response is thought to be mediated by signals from the afferent vagus nerve to the nucleus of the solitary tract and the lateral parabrachial nucleus.[57] No evidence of inflammation was observed within the short time frame of these studies.[57,58] The composition of commensal bacteria was not assessed in these studies, but persistent and major perturbations of the microbiota were unlikely, given the short duration of the experiments and the absence of an overt inflammatory response to pathogens, which is critical for a sustained disruption of the microbiota.[59,60]

Studies in which mice were chronically infected with *Helicobacter pylori* also show evidence of behavioral changes. This infection produces changes in gastric physiology that gradually improve after successful eradication of *H. pylori*.[61] These changes were accompanied by an alteration in feeding behavior that persisted after eradication of infection and resolution of the changes in gastric physiology. The persistent alteration in feeding behavior was accompanied by changes in the hypothalamic appetite-regulating peptide pro-opiomelanocortin.[62] The mechanisms that mediate changes in the brain and in behavior during and after *H. pylori* infection are unknown but could involve persistent immune activation in response to the infection.[63] A direct effect of *H. pylori* is unlikely, given the persistence of the behavioral changes long after eradication. The microbiota were not characterized in this model, but it is interesting that changes in brain chemistry and behavior were reversed by gavage of *Lactobacillus rhamnosus* and *Lactobacillus helveticus*.[62]

Few studies have examined the brain and behavior in germ-free hosts. However, a study by Sudo et al[64] provided insight into the role of commensal bacteria in the imprinting of the hypothalamic–pituitary response to stress. During mild restraint stress, the investigators observed an increase in adrenocorticotrophic hormone (ACTH) and corticosterone release in young germ-free mice, compared with young, colonized specific pathogen-free (SPF) mice (Figure 3). The response observed in germ-free mice was specific to the stressor and did not occur during ether-induced stress. The increases in stress-induced ACTH and corticosterone release were completely reversed when germ-free mice were colonized with *B. infantis* but only partially reversed when germ-free mice were colonized with flora from SPF mice. This observation is important because it suggests that, within the flora of SPF mice, there are bacteria that contribute to suppression of the ACTH response and bacteria that increase this response; *B. infantis* clearly belongs to the former category. The investigators also

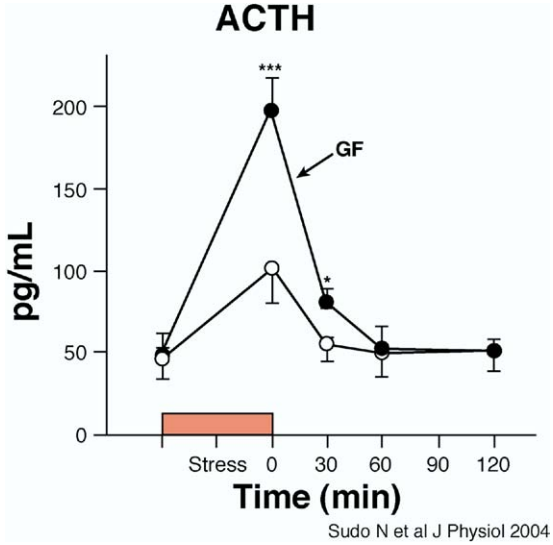

Figure 3. The effect of mild restraint stress on plasma adrenocorticotrophic hormone (ACTH) concentrations in germ-free and colonized young mice. Adapted with permission from Sudo et al.[64]

showed that enteropathogenic *E. coli* could increase the stress response after mono-colonization of germ-free mice. This effect of *E. coli* appears to involve attachment of the bacteria to epithelial cells, because the effect was not observed after mono-colonization with mutant *E. coli* that lacked the translocation intimin receptor, which is critical for the successful attachment to the epithelium. Interestingly, the reversibility of the exaggerated stress response was observed only in very young mice, indicating that there is a critical period in which the plasticity of the neural regulation of the stress response is sensitive to input from the microbiota. Another important finding of Sudo et al[64] was the reduction in brain-derived neurotrophic factor expression and protein levels in the cortex and hippocampus of germ-free mice compared with SPF mice. The brain-derived neurotrophic factor regulates several aspects of brain activity, including mood and cognitive function. In a preliminary report, McVey–Neufeld et al[65] identified defects in contextual learning in germ-free mice under stress and nonstress conditions. They also found that germ-free mice exhibited higher levels of anxiety when stressed (an exaggerated stress response). Taken together, these reports demonstrate that the intestinal microbiota influence the development of brain responses to stress and influence cognitive function in young mice.

Experimental perturbation of the intestinal microbiota influences behavior in adult mice (Figure 4). In that study,[66] mice were given antibiotics (neomycin and bacitracin) for 7 days by gavage, along with the antifungal agent primaricin to perturb the microbiota.[28,29] Changes in behavior, as assessed by the step-down test and the light box–dark box test, were observed in the antibiotic-treated mice; these tests measure anxiety-like behavior or timidity.[67] Antibiotic-treated Balb/c mice showed a re-

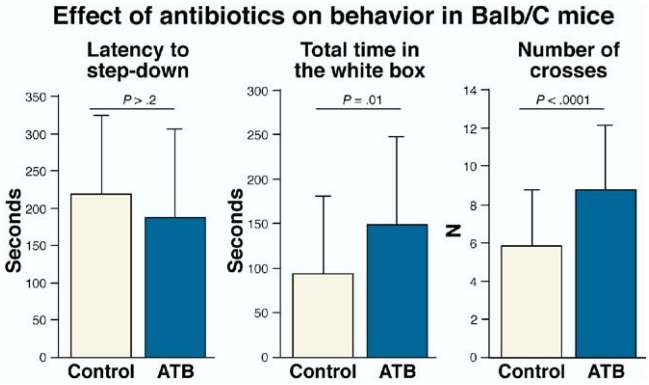

Figure 4. The effect of antibiotic-induced perturbation of the microbiota in mice. Mice were treated with bacitracin, neomycin, and primaricin[28] by gavage for 7 days. Behavior was assessed in the step-down test (*left*), and in the light box–dark box test (*middle* and *right*). Responses of control mice are shown in the *yellow bars* and those of antibiotic-treated mice are shown in the *blue bars*. Antibiotic-treated mice showed a significantly lower latency to step-down, greater time spent in the white box, and an increased number of crossovers between the light and dark boxes. ATB, antibiotics.

duced latency to step-down and increased activity in the light box–dark box test (Figure 4). In addition, the mice given antibiotics spent more time exploring the light box, compared with control mice. These findings indicate that perturbation of the microbial content of the GI tract in adult mice results in measurable changes in behavior. The mice did not show fear or anxiety, but rather greater activity after perturbation of the microbiota; this is not consistent with a malaise effect of the gavaged antibiotics. On the basis of these findings, we propose that perturbation of the microbiota can influence behavior. This is consistent with a recent study reporting behavioral changes in mice in which the microbiota had been perturbed by dietary alterations.[68]

The mechanisms by which the microbiota influence behavior are unknown but could include immune-mediated, neural, or humeral mechanisms. These mechanisms are by no means mutually exclusive; it is likely that they occur in series or in parallel. Immune mechanisms include activation of the innate immune response in the GI tract. Toll-like receptors-2, -4, and -5 are down-regulated in germ-free mice and become up-regulated during colonization, implying interactions between these receptors and the microbiota.[69] Dendritic cells of the GI tract have processes that breach the epithelial layer and interact with commensal bacteria to induce the production of immunoglobulin A by B lymphocytes and plasma cells.[70] Secreted immunoglobulin A limits the penetration of the epithelium by the microbiota. These mechanisms restrict the inflammatory response to commensal bacteria to the level seen under normal conditions (physiologic inflammation). Dendritic cells are in close proximity to nerves in the GI tract[71]; the sensory neuropeptide calcitonin-gene-related peptide modulates dendritic cell function[72] and might signal the presence of commensal bacteria to the brain by the vagus nerve.[71] There is close integration of innate and adaptive responses, and the integrity of the adaptive immune response is important for normal cognitive function. Specifically, Kipnis et al[73] showed that, in mice, a deficit in peripheral T cells can result in cognitive and behavioral impairment, although the origin of these cells, and whether they cross the blood–brain barrier or signal from the periphery to influence behavior, is not known.

The vagus nerve has an important role in signaling from the GI tract to the brain and can be stimulated by bacteria products such as endotoxins or inflammatory cytokines such as interleukin-1β and tumor necrosis factor α.[74] The vagal response to stimulation by peripheral inflammatory events is the suppression of proinflammatory cytokine release from intestinal macrophages mediated by the α-7 subunit of the nicotinic acetylcholine receptor on these cell.[74,75] Interestingly, this response is attenuated by the induction of depression in the mouse.[42] The introduction of noninvasive pathogens is rapidly signaled to the brain, reflected by increased activity of vagus nuclei in the brain stem, and this is accompanied by anxiety-like behavior in mice.[55,57,58] It is possible that perturbation of the microbiota is signaled in a vagus-dependent manner, resulting in altered behavior. For example, introduction of lactobacilli to the duodenum of rats has been shown to increase gastric vagal activity within minutes.[76]

How is the presence of commensal bacteria communicated to the brain and how does it induce behavior changes? Some studies indicate that soluble factors are involved. Factor-S is a sleep-inducing substance that accumulates in the brain and body fluids of sleep-deprived animals. It is unique because of its bacterial origin and is derived from the bacterial cell wall. Studies suggest that GI bacteria are an important source of Factor-S because normal sleep patterns were disrupted after perturbation of the microbiota with oral antibiotics.[76,77] Commensal bacteria also produce precursors of benzodiazepine receptor ligands that could contribute to encephalopathy in a rat model of liver failure.[46] Cognitive function improved in patients with minimal hepatic encephalopathy given *Bifidobacterium longum* with fructo-oligosaccharide for 9 weeks. Although the mechanism of action is poorly understood, *B. longum* might inhibit the activity of urease-positive commensal bacteria, which would reduce ammonia levels or the production of other substances of bacterial origin, including mercaptans and thioles.[78]

Probiotics

Probiotics are microbes (bacteria or yeast) that confer health benefits to the host when administered in sufficient quantity. They have been shown to influence function in a variety of organs, including the nervous

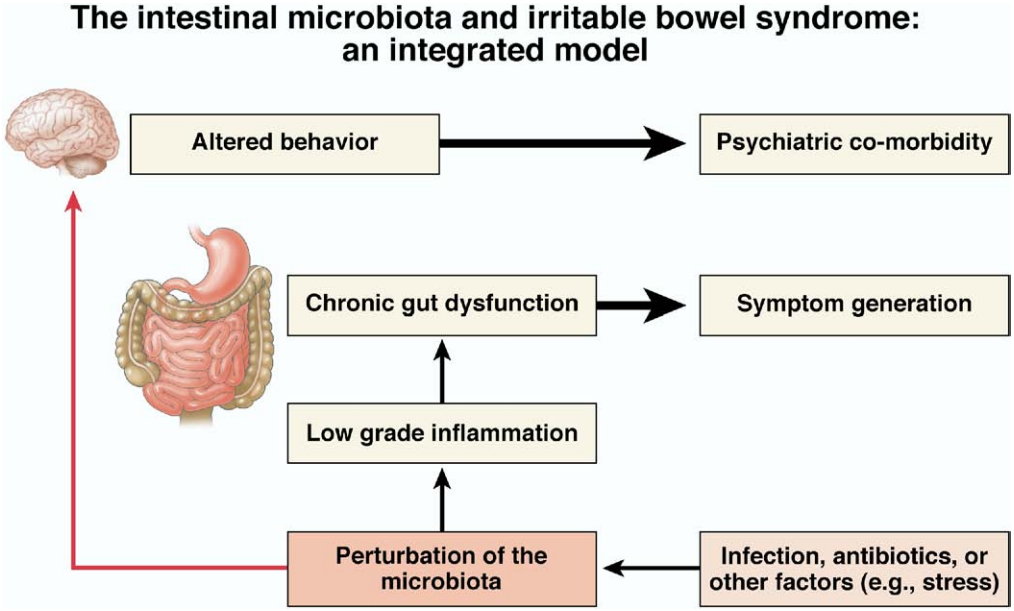

Figure 5. A hypothetical model describing the role of the intestinal microbiota in the pathogenesis of irritable bowel syndrome. In this model, known risk factors for IBS that include gastroenteritis, antibiotic use, and stress produce changes in commensal bacteria and an increment in physiologic inflammation. This leads to changes in gut function as a basis for abdominal symptom generation. Perturbation of the microbiota may contribute to the behavioral profile seen in this condition.

system. Several studies report the effects of probiotics on the GBA and in models of altered behavior.

In rats subjected to water-avoidance stress, intestinal barrier function was reduced, and bacterial adherence to the epithelium was increased. In addition, commensal bacteria translocated across a leaky epithelial barrier, ultimately reaching the mesenteric lymph nodes.[79] Exposure of rats to the probiotics *L. helveticus* and *L. rhamnosus* prevented the stress-induced increase in adherence as well as the translocation of commensal bacteria. Although it was observed that the probiotics improved epithelial function, it is not clear exactly how the probiotics functioned in this model of the stress response.

Rat pups that experience maternal deprivation have a significant increase in the visceromotor response to colonic distension and increased GI paracellular permeability, compared with controls. The probiotic *L. paracasei* significantly improved stress-induced visceral pain and restored normal GI permeability in the stressed rats. The investigators concluded that this was mediated, at least in part, by a soluble factor that was derived from the probiotic. However, the mechanisms of action were not identified.[80]

Another study investigated the antidepressant potential of a probiotics *B. infantis* given for 14 days to rats that were chronically subjected to the forced swim test as a stressor.[51] This probiotic therapy resulted in a reduction in levels of interferon γ, tumor necrosis factor α, and interleukin-6 after mitogen stimulation of peripheral blood monocytes. In addition, there was a marked and significant increase in plasma tryptophan and kynurenic acid in the probiotic-treated rats, compared with controls. However, there was an unexplained reduction in the concentration of 5-hydroxy-indole-acetic acid in the frontal cortex and a decrease in dihydroxyphenylacetic acid, a metabolite of dopamine, in the amygdaloid cortex in the rats given the probiotics, and no improvement in the forced swim test was observed. Although treatment failed to influence behavior, the results of this study are important because they show the antidepressant potential of the bacterium *B. infantis,* primarily by virtue of its ability to increase the serotonergic precursor, tryptophan.[51]

Microbiota–GBA and Disease

Evidence is increasing for a role of the GBA in the pathogenesis of IBD. Imbalance between the sympathetic and parasympathetic outflow from the central nervous system has been reported in patients with IBD[81–83] and may be associated with behavioral change. For example, depression has been correlated with Crohn's disease, stress, and ulcerative colitis in separate groups of patients.[84] A controlled study found an increased prevalence of depression in patients with IBD.[85] It is difficult to ascertain from human studies whether behavioral changes are primary or occur as a result of the morbidity of these conditions. Animal studies show that stress exacerbates experimental colitis and that depression increases susceptibility to inflammatory stimuli by impairing vagal parasympathetic outflow to the gut.[41,42,86] Given the experimental evidence that perturbation of the microbiota alters behavior and that dysbiosis occurs in

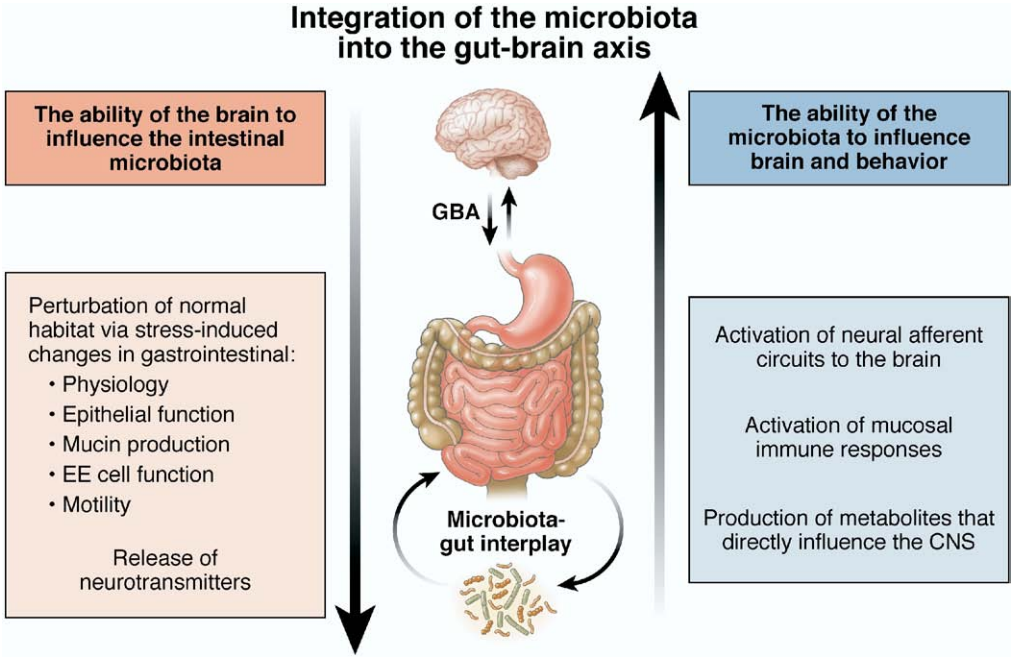

Figure 6. The integration of the intestinal microbiota into the brain–gut axis (GBA). Shown are the putative mechanisms whereby the brain may influence the composition of the intestinal microbiota, and whereby the microbiota may influence the brain. The communication between these systems is bidirectional. These are the components of the proposed bidirectional microbiota–gut–brain axis. CNS, central nervous system; EE, entero-endocrine.

IBD, we speculate that dysbiosis could also contribute to the behavioral changes reported in some patients with IBD.

IBS is considered to be a disorder of the GBA, and evidence is emerging of dysbiosis in patients with IBS. Factors that are known to predispose individuals to IBS include enteric infection, antibiotic use, and stress and are also known to alter the bacterial composition of the GI tract. Acute bacterial gastroenteritis is the strongest risk factor known for the development of IBS.[87–90] Antibiotic usage in children or in the context of postinfective IBS is also a risk factor for the development of IBS.[91,92] Patients with IBS have an increased response to stress,[93] and stress is also a predictor of IBS[94] as well as a determinant of symptom severity.[95]

Indirect evidence of dysbiosis in patients with IBS was based on the analysis of fermentation profiles on stool or breath samples. Some patients with IBS have demonstrable qualitative or quantitative changes in fermentation profiles compared with healthy controls,[96–99] but the interpretation of these findings remains controversial.[100,101] With the use of 16s rRNA analysis of stool samples, a recent study found significant changes, particularly in *Lactobacillus* species, in patients with IBS patients compared with controls.[102] A reduction in lactobacilli was also observed in patients with diarrhea-predominant IBS, whereas patients with constipation-predominant IBS had increases in *Veillonella* species.[103] Another study showed a greater temporal instability of the microbiota during a 6-month period in patients with IBS,[104] but, unfortunately, the investigators did not correlate these findings with symptom fluctuation. Nevertheless, taken together, these findings support the existence of intestinal dysbiosis in IBS.

In animal studies, dysbiosis has been shown to induce low-grade inflammation that is not accompanied by tissue damage,[29] and it is plausible that dysbiosis is the cause of the low-grade inflammation found in mucosal biopsies in subsets of patients with IBS. The longstanding notion that IBS represents a low-grade inflammatory disorder[105] is now supported by several lines of evidence, including genetic studies,[106,107] semiquantitative histologic studies,[108] as well as studies reporting increased mediator production from inflammatory cells in the GI wall[109] and systemic circulation.[110] We speculate that dysbiosis is a determinant of immune activation and low-grade inflammation in this subset of patients with IBS.

The potential for the microbiota to produce small increments in physiologic inflammation and thereby perturb GI and brain function prompts consideration of a unifying hypothesis for IBS. Historically, IBS has been viewed as a psychosomatic disorder, with emphasis on psychiatric comorbidity and symptom reporting by patients with IBS, a central model of the pathogenesis of IBS. During the past decade, another school of thought has emerged, implicating gastroenteritis and low-grade inflammation as mechanisms that underlie GI dysfunction and the symptoms of IBS, a peripheral model of IBS. However, psychiatric comorbidity also occurs in patients

with postinfective IBS, and low-grade inflammation is found in patients with IBS with or without a history of gastroenteritis. These observations prompt consideration for a role of microbiota–GBA interactions in the pathophysiology of IBS, particularly in patients with demonstrable intestinal dysbiosis.

On the basis of the experimental data reviewed in this article, we propose a model to incorporate intestinal dysbiosis into a conceptual framework of IBS, illustrated in Figure 5. In this model, recognized IBS risk factors, such as acute gastroenteritis, antibiotic therapy, or stress, produce intestinal dysbiosis and an incremental increase in physiologic inflammation in the colon that is subclinical but sufficient to alter neuromuscular function (and thus produce GI symptoms). In addition, intestinal dysbiosis may contribute to the behavioral profile of patients with IBS. This construct requires testing in clinical studies.

In conclusion, experimental data and clinical observations support the integration of the intestinal microbiota into the GBA (Figure 6). These include the bidirectional interactions between the microbiota and GI physiology and the associations between the microbiota and behavior. Future research should focus on the contributions of immunologic, neural, and biochemical or metabolic pathways to the microbiota–GBA relationship. A better understanding of these relationships will improve our understanding of functional and inflammatory conditions of the GI tract and of hepatic encephalopathy. This knowledge may also prompt further exploration of the role of the intestinal microbiota in behavioral illnesses such as depression.

References

1. Chang L, Sundaresh S, Elliott J, et al. Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis in irritable bowel syndrome. Neurogastroenterol Motil 2009 l21:149–159.
2. Stasi C, Orlandelli E. Role of the brain-gut axis in the pathophysiology of Crohn's disease. Dig Dis 2008;26:156–166.
3. Vantrappen G, Jannssens J, Hellemans J, Ghoos Y. The interdigestive motor complex of normal subjects and patients with bacterial overgrowth of the small intestine. J Clin Invest 1977; 59:1158–1168.
4. Morgan MY. The treatment of chronic hepatic encephalopathy. Hepatogastroenterology 1991;38:377–387.
5. Eckburg PB, Bik EM, Bernstein CN, et al. Diversity of the human intestinal microbial flora. Science 2005;308:1635–1638.
6. O'Hara AM, Shanahan F. Gut microbiota: mining for therapeutic potential. Clin Gastroenterol Hepatol 2007;5:274–284.
7. Marchesi J, Shanahan F. The normal intestinal microbiota. Curr Opin Infect Dis 2007;20:508–513.
8. Hooper LV, Gordon JI. Commensal host-bacterial relationships in the gut. Science 2001;292:1115–1118.
9. Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science 2005; 307:1915–1920.
10. Amaral FA, Sachs D, Costa VV, et al. Commensal microbiota is fundamental for the development of inflammatory pain. Proc Natl Acad Sci U S A 2008;105:2193–2197.
11. Dumas ME, Barton RH, Toye A, et al. Metabolic profiling reveals a contribution of gut microbiota to fatty liver phenotype in insulin-resistant mice. Proc Natl Acad Sci U S A 2006;103:12511–12516.
12. Backhed F, Ding H, Wang T, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 2004;101:15718–15723.
13. Hawrelak JA, Myers SP. The causes of intestinal dysbiosis: a review. Altern Med Rev 2004;9:180–197.
14. Lepage P, Colombet J, Marteau P, Sime-Ngando T, Dore J, Leclerc M. Dysbiosis in inflammatory bowel disease: a role for bacteriophages? Gut 2008;57:424–425.
15. Jacobs NF Jr. Antibiotic-induced diarrhea and pseudomembranous colitis. Postgrad Med 1994;95:111–120.
16. McFarland LV. Epidemiology, risk factors and treatments for antibiotic-associated diarrhea. Dig Dis 1998;16:292–307.
17. Falk PG, Hooper LV, Midtvedt T, Gordon JI. Creating and maintaining the gastrointestinal ecosystem: what we know and need to know from gnotobiology. Microbiol Mol Biol Rev 1998;62: 1157–1170.
18. Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI. Molecular analysis of commensal host-microbial relationships in the intestine. Science 2001;291:881–884.
19. Skehel PA, Armitage BA, Bartsch D, et al. Proteins functioning in synaptic transmission at the sensory to motor synapse of Aplysia. Neuropharmacology 1995;34:1379–1385.
20. Wold JK, Khan R, Midtvedt T. Intestinal glycoproteins of germfree rats. Chemical composition of intestinal and fecal mucus from germfree rats fed a chemically defined diet. Acta Pathol Microbiol Scand [B] Microbiol Immunol 1971;79:525–530.
21. Uribe A, Alam M, Johansson O, Midtvedt T, Theodorsson E. Microflora modulates endocrine cells in the gastrointestinal mucosa of the rat. Gastroenterology 1994;107:1259–1269.
22. Abrams GD, Bishop JE. Effect of normal microbial flora on gastrointestinal motility. Proc Soc Exp Biol (N Y) 1967;126: 301–304.
23. Gustafsson BE, Midtvedt T, Strandberg K. Effects of microbial contamination on the cecum enlargement of germfree rats. Scand J Gastroenterol 1970;5:309–314.
24. Husebye E, Hellstrom PM, Sundler F, Chen J, Midtvedt T. Influence of microbial species on small intestinal myoelectric activity and transit in germ-free rats. Am J Physiol Gastrointest Liver Physiol 2001;280:G368–G380.
25. Husebye E, Hellstrom PM, Midtvedt T. Intestinal microflora stimulates myoelectric activity of rat small intestine by promoting cyclic initiation and aboral propagation of migrating myoelectric complex. Dig Dis Sci 1994;39:946–956.
26. Caenepeel P, Janssens J, Vantrappen G, Eyssen H, Coremans G. Interdigestive myoelectric complex in germ-free rats. Dig Dis Sci 1989;34:1180–1184.
27. Macpherson AJ, Geuking MB, McCoy KD. Immune responses that adapt the intestinal mucosa to commensal intestinal bacteria. Immunology 2005;115:153–162.
28. van der WD, Sturm CA. Antibiotic decontamination of the digestive tract of mice. Technical procedures. Lab Anim Care 1968; 18:1–10.
29. Verdu EF, Bercik P, Verma-Gandhu M, et al. Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. Gut 2006;55:182–190.
30. Burton MB, Gebhart GF. Effects of intracolonic acetic acid on responses to colorectal distension in the rat. Brain Res 1995; 672:7782.
31. Scott LD, Cahall DL. Influence of the interdigestive myoelectric complex on enteric flora in the rat. Gastroenterology 1982;82: 737–745.

32. Green BT, Lyte M, Kulkarni-Narla A, Brown DR. Neuromodulation of enteropathogen internalization in Peyer's patches from porcine jejunum. J Neuroimmunol 2003;141:74–82.
33. Chen C, Brown DR, Xie Y, Green BT, Lyte M. Catecholamines modulate *Escherichia coli* O157:H7 adherence to murine cecal mucosa. Shock 2003;20:183–188.
34. Freestone PP, Williams PH, Haigh RD, Maggs AF, Neal CP, Lyte M. Growth stimulation of intestinal commensal *Escherichia coli* by catecholamines: a possible contributory factor in trauma-induced sepsis. Shock 2002;18:465–470.
35. Lyte M. The biogenic amine tyramine modulates the adherence of *Escherichia coli* O157:H7 to intestinal mucosa. J Food Prot 2004;67:878–883.
36. Tannock GW, Savage DC. Influences of dietary and environmental stress on microbial populations in the murine gastrointestinal tract. Infect Immun 1974;9:591–598.
37. Bailey MT, Coe CL. Maternal separation disrupts the integrity of the intestinal microflora in infant rhesus monkeys. Dev Psychobiol 1999;35:146–155.
38. Eutamene H, Bueno L. Role of probiotics in correcting abnormalities of colonic flora induced by stress. Gut 2007;56:1495–1497.
39. Gareau MG, Jury J, MacQueen G, Sherman PM, Perdue MH. Probiotic treatment of rat pups normalises corticosterone release and ameliorates colonic dysfunction induced by maternal separation. Gut 2007;56:1522–1528.
40. O'Mahony SM, Marchesi JR, Scully P, et al. Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. Biol Psychiatry 2009;65:263–267.
41. Varghese AK, Verdu EF, Bercik P, et al. Antidepressants attenuate increased susceptibility to colitis in a murine model of depression. Gastroenterology 2006;130:1743–1753.
42. Ghia JE, Blennerhassett P, Collins SM. Impaired parasympathetic function increases susceptibility to inflammatory bowel disease in a mouse model of depression. J Clin Invest 2008;118:2209–2218.
43. Groot J, Bijlsma P, Van Kalkeren A, Kiliaan A, Saunders P, Perdue M. Stress-induced decrease of the intestinal barrier function. The role of muscarinic receptor activation. Ann N Y Acad Sci 2000;915:237–246.
44. Rubio CA, Huang CB. Quantification of the sulphomucin-producing cell population of the colonic mucosa during protracted stress in rats. In Vivo 1992;6:81–84.
45. Williams R. Review article: bacterial flora and pathogenesis in hepatic encephalopathy. Aliment Pharmacol Ther 2007;25(Suppl 1):17–22.
46. Yurdaydin C, Walsh TJ, Engler HD, et al. Gut bacteria provide precursors of benzodiazepine receptor ligands in a rat model of hepatic encephalopathy. Brain Res 1995;679:42–48.
47. Ledochowski M, Widner B, Sperner-Unterweger B, Propst T, Vogel W, Fuchs D. Carbohydrate malabsorption syndromes and early signs of mental depression in females. Dig Dis Sci 2000;45:1255–1259.
48. Ledochowski M, Widner B, Bair H, Probst T, Fuchs D. Fructose- and sorbitol-reduced diet improves mood and gastrointestinal disturbances in fructose malabsorbers. Scand J Gastroenterol 2000;35:1048–1052.
49. Ledochowski M, Widner B, Murr C, Sperner-Unterweger B, Fuchs D. Fructose malabsorption is associated with decreased plasma tryptophan. Scand J Gastroenterol 2001;36(4):367–371.
50. Gibson PR, Newnham E, Barrett JS, Shepherd SJ, Muir JG. Review article: fructose malabsorption and the bigger picture. Aliment Pharmacol Ther 2007;25(4):349–363.
51. Desbonnet L, Garrett L, Clarke G, Bienenstock J, Dinan TG. The probiotic *Bifidobacteria infantis*: an assessment of potential antidepressant properties in the rat. J Psychiatr Res 2008;43:164–174.
52. Finegold SM, Molitoris D, Song Y, et al. Gastrointestinal microflora studies in late-onset autism. Clin Infect Dis 2002;35(Suppl):S6–S16.
53. Song Y, Liu C, Finegold SM. Real-time PCR quantitation of clostridia in feces of autistic children. Appl Environ Microbiol 2004;70(11):6459–6465.
54. Sandler RH, Finegold SM, Bolte ER, et al. Short-term benefit from oral vancomycin treatment of regressive-onset autism. J Child Neurol 2000;15(7):429–435.
55. Wang X, Wang BR, Zhang XJ, Xu Z, Ding YQ, Ju G. Evidences for vagus nerve in maintenance of immune balance and transmission of immune information from gut to brain in STM-infected rats. World J Gastroenterol 2002;8:540–545.
56. Goehler LE, Lyte M, Gaykema RP. Infection-induced viscerosensory signals from the gut enhance anxiety: implications for psychoneuroimmunology. Brain Behav Immun 2007;21:721–726.
57. Gaykema RP, Goehler LE, Lyte M. Brain response to cecal infection with *Campylobacter jejuni*: analysis with Fos immunohistochemistry. Brain Behav Immun 2004;18:238–245.
58. Lyte M, Li W, Opitz N, Gaykema RP, Goehler LE. Induction of anxiety-like behavior in mice during the initial stages of infection with the agent of murine colonic hyperplasia *Citrobacter rodentium*. Physiol Behav 2006;89:350–357.
59. Stecher B, Robbiani R, Walker AW, et al. *Salmonella enterica serovar Typhimurium* exploits inflammation to compete with the intestinal microbiota. PLoS Biol 2007;5:2177–2189.
60. Lupp C, Robertson ML, Wickham ME, et al. Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. Cell Host Microbe 2007;2:204.
61. Bercik P, De Giorgio R, Blennerhassett P, Verdu EF, Barbara G, Collins SM. Immune-mediated neural dysfunction in a murine model of chronic *Helicobacter pylori* infection. Gastroenterology 2002;123:1205–1215.
62. Bercik P, Verdu EF, Foster JA, et al. Role of gut-brain axis in persistent abnormal feeding behavior in mice following eradication of *Helicobacter pylori* infection. Am J Physiol Regul Integr Comp Physiol 2009;296:R587–R594.
63. Verdu EF, Bercik P, Huang XX, et al. The role of luminal factors in the recovery of gastric function and behavioral changes after chronic *Helicobacter pylori* infection. Am J Physiol Gastrointest Liver Physiol 2008;295:G664–G670.
64. Sudo N, Chida Y, Aiba Y, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J Physiol 2004;558:263–275.
65. McVey–Neufeld KA, Bienenstock J, JA Foster. The impact of intestinal microbiota on anxiety like behavior [abstract]. Neurogastroenterol Motil 2008;20(Suppl2):125.
66. Denou E, Bercik P, Collins SM. Perturbation of the intestinal microbiota alters behavior in mice. Gastroenterology DDW (in press).
67. Crawley JN. Behavioral phenotyping of rodents. Comp Med 2003;53:140–146.
68. Li W, Dowd SE, Scurlock B, Acosta-Martinez V, Lyte M. Memory and learning behavior in mice is temporally associated with diet-induced alterations in gut bacteria. Physiol Behav 2009;96:557–567.
69. Lundin A, Bok CM, Aronsson L, et al. Gut flora, Toll-like receptors and nuclear receptors: a tripartite communication that tunes innate immunity in large intestine. Cell Microbiol 2008;10:1093–1103.
70. Macpherson AJ, Slack E. The functional interactions of commensal bacteria with intestinal secretory IgA. Curr Opin Gastroenterol 2007;23:673–678.

71. Goehler LE, Gaykema RP, Nguyen KT, et al. Interleukin-1beta in immune cells of the abdominal vagus nerve: a link between the immune and nervous systems? J Neurosci 1999;19:2799–2806.
72. Hosoi J, Murphy GF, Egan CL, et al. Regulation of Langerhans cell function by nerves containing calcitonin gene-related peptide. Nature 1993;363:159–163.
73. Kipnis J, Cohen H, Cardon M, Ziv Y, Schwartz M. T cell deficiency leads to cognitive dysfunction: implications for therapeutic vaccination for schizophrenia and other psychiatric conditions. Proc Natl Acad Sci U S A 2004;101:8180–8185.
74. Borovikova LV, Ivanova S, Zhang M, et al. Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature 2000;405:458–462.
75. Ghia JE, Blennerhassett P, Kumar-Ondiveeran H, Verdu EF, Collins SM. The vagus nerve: a tonic inhibitory influence associated with inflammatory bowel disease in a murine model. Gastroenterology 2006;131:1122–1130.
76. Tanida M, Yamano T, Maeda K, Okumura N, Fukushima Y, Nagai K. Effects of intraduodenal injection of *Lactobacillus johnsonii* La1 on renal sympathetic nerve activity and blood pressure in urethane-anesthetized rats. Neurosci Lett 2005;389(2):109–114.
77. Brown R, Price RJ, King MG, Husband AJ. Are antibiotic effects on sleep behavior in the rat due to modulation of gut bacteria? Physiol Behav 1990;48:561–565.
78. Malaguarnera M, Greco F, Barone G, Gargante MP, Malaguarnera M, Toscano MA. *Bifidobacterium longum* with fructo-oligosaccharide (FOS) treatment in minimal hepatic encephalopathy: a randomized, double-blind, placebo-controlled study. Dig Dis Sci 2007;52:3259–3265.
79. Zareie M, Johnson-Henry K, Jury J, et al. Probiotics prevent bacterial translocation and improve intestinal barrier function in rats following chronic psychological stress. Gut 2006;55:1553–1560.
80. Eutamene H, Lamine F, Chabo C, et al. Synergy between *Lactobacillus paracasei* and its bacterial products to counteract stress-induced gut permeability and sensitivity increase in rats. J Nutr 2007;137:1901–1907.
81. Lindgren S, Stewenius J, Sjölund K, Lilja B, Sundkvist G. Autonomic vagal nerve dysfunction in patients with ulcerative colitis. Scand J Gastroenterol 1993;28:638–642.
82. Lindgren S, Lilja B, Rosën I, Sundkvist G. Disturbed autonomic nerve function in patients with Crohn's disease. Scand J Gastroenterol 1991;26:361–366.
83. Ganguli SC, Kamath MV, Redmond K, et al. A comparison of autonomic function in patients with inflammatory bowel disease and in healthy controls. Neurogastroenterol Motil 2007;19:961–967.
84. Maunder RG, Levenstein S. The role of stress in the development and clinical course of inflammatory bowel disease: epidemiological evidence. Curr Mol Med 2008;8:247–252.
85. Walker JR, Ediger JP, Graff LA, et al. The Manitoba IBD cohort study: a population-based study of the prevalence of lifetime and 12-month anxiety and mood disorders. Am J Gastroenterol 2008;103:1989–1997.
86. Qiu BS, Vallance BA, Blennerhassett PA, Collins SM. The role of CD4+ lymphocytes in the susceptibility of mice to stress-induced reactivation of experimental colitis. Nat Med 1999;5:1178–1182.
87. Marshall JK, Thabane M, Garg AX, Clark WF, Salvadori M, Collins SM. Incidence and epidemiology of irritable bowel syndrome after a large waterborne outbreak of bacterial dysentery. Gastroenterology 2006;131:445–450.
88. Parry SD, Stansfield R, Jelley D, et al. Does bacterial gastroenteritis predispose people to functional gastrointestinal disorders? A prospective, community-based, case-control study. Am J Gastroenterol 2003;98:1970–1975.
89. Spiller RC. Estimating the importance of infection in IBS. Am J Gastroenterol 2003;98:238–241.
90. Rodriguez LA, Ruigomez A. Increased risk of irritable bowel syndrome after bacterial gastroenteritis: cohort study. BMJ 1999;318:565–566.
91. Ruigomez A, Garcia Rodriguez LA, Panes J. Risk of irritable bowel syndrome after an episode of bacterial gastroenteritis in general practice: influence of comorbidities. Clin Gastroenterol Hepatol 2007;5:465–469.
92. Mendall MA, Kumar D. Antibiotic use, childhood affluence and irritable bowel syndrome (IBS). Eur J Gastroenterol Hepatol 1998;10:59–62.
93. Mayer EA, Naliboff BD, Chang L, Coutinho SV. V. Stress and irritable bowel syndrome. Am J Physiol Gastrointest Liver Physiol 2001;280:G519–G524.
94. Fujii Y, Nomura S. A prospective study of the psychobehavioral factors responsible for a change from non-patient irritable bowel syndrome to IBS patient status. Biopsychosoc Med 2008;2:16.
95. Blanchard EB, Lackner JM, Jaccard J, et al. The role of stress in symptom exacerbation among IBS patients. J Psychosom Res 2008;64:119–128.
96. King TS, Elia M, Hunter JO. Abnormal colonic fermentation in irritable bowel syndrome. Lancet 1998;352:1187–1189.
97. Lin HC. Small intestinal bacterial overgrowth: a framework for understanding irritable bowel syndrome. JAMA 2004;292:852–858.
98. Pimentel M, Lezcano S. Irritable bowel syndrome: bacterial overgrowth—what's known and what to do. Curr Treat Options Gastroenterol 2007;10:328–337.
99. Treem WR, Ahsan N, Kastoff G, Hyams JS. Fecal short-chain fatty acids in patients with diarrhea-predominant irritable bowel syndrome: in vitro studies of carbohydrate fermentation. J Pediatr Gastroenterol Nutr 1996;23:280–286.
100. Dear KL, Elia M, Hunter JO. Do interventions which reduce colonic bacterial fermentation improve symptoms of irritable bowel syndrome? Dig Dis Sci 2005;50:758–766.
101. Vanner S. The small intestinal bacterial overgrowth. Irritable bowel syndrome hypothesis: implications for treatment. Gut 2008;57:1315–1321.
102. Kassinen A, Krogius-Kurikka L, Makivuokko H, et al. The fecal microbiota of irritable bowel syndrome patients differs significantly from that of healthy subjects. Gastroenterology 2007;133:24–33.
103. Malinen E, Rinttila T, Kajander K, et al. Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. Am J Gastroenterol 2005;100:373–382.
104. Maukonen J, Satokari R, Matto J, Soderlund H, Mattila-Sandholm T, Saarela M. Prevalence and temporal stability of selected clostridial groups in irritable bowel syndrome in relation to predominant faecal bacteria. J Med Microbiol 2006;55:625–633.
105. Collins SM. Is the irritable gut an inflamed gut? Scand J Gastroent Suppl 1992;192:102–105.
106. Gonsalkorale WM, Perrey C, Pravica V, Whorwell PJ, Hutchinson IV. Interleukin 10 genotypes in irritable bowel syndrome: evidence for an inflammatory component? Gut 2003;52:91–93.
107. van der Veek PP, van den BM, de Kroon YE, Verspaget HW, Masclee AA. Role of tumor necrosis factor-alpha and interleukin-10 gene polymorphisms in irritable bowel syndrome. Am J Gastroenterol 2005;100:2510–2516.
108. Barbara G, Stanghellini V, De Giorgio R, et al. Activated mast cells in proximity to colonic nerves correlate with abdominal pain in irritable bowel syndrome. Gastroenterology 2004;126:693–702.

109. Barbara G, Wang B, Stanghellini V, et al. Mast cell-dependent excitation of visceral-nociceptive sensory neurons in irritable bowel syndrome. Gastroenterology 2007;132:26–37.
110. O'Mahony L, McCarthy J, Kelly P, et al. Lactobacillus and bifidobacterium in irritable bowel syndrome: symptom responses and relationship to cytokine profiles. Gastroenterology 2005; 128:541–551.

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Conflicts of interest

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