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# Brain Gut Microbiome Interactions and Functional Bowel Disorders

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## Abstract

Alterations in the bidirectional interactions between the gut and the nervous system play an important role in IBS pathophysiology and symptom generation. A body of largely preclinical evidence suggests that the gut microbiota can modulate these interactions. Characterizations of alterations of gut microbiota in unselected IBS patients, and assessment of changes in subjective symptoms associated with manipulations of the gut microbiota with prebiotics, probiotics and antibiotics support a small, but poorly defined role of dybiosis in overall IBS symptoms. It remains to be determined if the observed abnormalities are a consequence of altered top down signaling from the brain to the gut and microbiota, if they are secondary to a primary perturbation of the microbiota, and if they play a role in the development of altered brain gut interactions early in life. Different mechanisms may play role in subsets of patients. Characterization of gut microbiome alterations in large cohorts of well phenotyped patients as well as evidence correlating gut metabolites with specific abnormalities in the gut brain axis are required to answer these questions.

## INTRODUCTION

Alterations in bidirectional brain gut interactions have been considered a likely pathophysiological construct underlying IBS and related functional GI disorders for some time.[1,2] However, considerable controversy remains regarding the involved molecular mechanisms and the precise targets within the brain gut axis that are responsible for such alterations. Similarly, it remains unclear which of the reported changes are primary and which are secondary in the development of symptoms. The gut microbiota and their metabolic products have recently been proposed as one such plausible mechanism, given their demonstrated ability primarily in preclinical studies to influence intestinal

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permeability [3] and immune function,[4] activity in the enteric nervous system (reviewed in[5]), the HPA axis,[6] pain modulation systems[7] and the brain (reviewed in [8, 9]). Alterations of the normal gut microbiota ("dysbiosis") have been implicated in putative IBS pathophysiology in terms of enhanced gut permeability,[10–12] mucosal immune activation,[10–14] visceral hypersensitivity[14, 15] and altered intestinal motility.[16] However, there is conflicting evidence regarding alterations in the organization and metabolic products of the gut microbiome in patients with chronic abdominal pain and in adult and pediatric IBS,[12] and on the beneficial effects of gut microbial manipulations with prebiotics, probiotics, and antibiotics in some IBS patients. Furthermore, it remains unclear if the observed IBS related alterations in the gut microbiome are related to altered intestinal function/physiology and/or changes in brain signaling. For example, there are multiple mechanisms by which the brain (via the hypothalamic pituitary [HPA] axis, the autonomic nervous system (ANS), and ANS modulation of the enteric nervous system) can influence the context and the intestinal environment in which the microbiota live, by influencing regional gut motility patterns, epithelial permeability, luminal secretions, mucosal immune function and possibly intraluminal release of neurotransmitters from enteroendocrine and other cells in the gut (reviewed in [1, 2]). There are also a limited number of intriguing original preclinical study reports to support an influence of the gut microbiota on brain development and behaviors and on the adult brain,[6, 17–19] including characterization of neuroactive metabolites which may underlie this influence.[8] A recent study has demonstrated for the first time in healthy human subjects that perturbation of the normal gut microbiota with a probiotic can influence brain function. [20] The knowledge of the healthy human microbiome is rapidly advancing and these reference data sets will enable scientists to distinguish physiological from pathological changes associated with the intestinal microbiome.[21]

In this article, we will review the published literature which supports a role for altered gut microbiota in symptoms and pathophysiology of IBS, the most prevalent and best studied functional GI disorder. We will first focus on findings in human patients by critically reviewing reported evidence in support of alterations in gut microbiota (dysbiosis) and related metabolites in IBS patients. We will review the evidence supporting a possible causative role of the reported dysbiosis in IBS symptoms, based on symptomatic responses to modulation of the gut microbiome by diet, by pre- and probiotics, and by antibiotics. We will then review the possible consequences of dysbiosis on the gut brain axis and resulting IBS symptoms, and the possible causes of the dysbiosis. It needs to be emphasized that given the unique nature of bidirectional brain gut interactions, it remains impossible at this point to determine from published cross sectional studies the causality between observed alterations in gut microbiota, intestinal function, the brain and IBS symptoms. However, with the rapidly evolving technologies to characterize the gut microbiome and its metabolic products, as well as brain signatures which may be related to the dysbiosis, important breakthroughs in the characterization of the role of the gut microbiota in modulating gut brain interactions and in the pathophysiology of functional gastrointestinal disorders (FGIDs) can be expected.

## Clinical evidence for alterations in the gut microbiome in IBS patients

There has been a rapid evolution of analytical techniques to characterize different aspects of the gut microbiome.[22–24] While cultured species represents merely 20–30% of identified gut phylotypes (dominated by Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria in the human colon), these techniques remain crucial for investigating microbial diversity and for the selection of key functional groups.[12] Culture independent approaches include 16S rRNA gene based analyses (identifying which microbes are present in the GI tract), metagenomic approaches (addressing which microbial genes are present), and metatranscriptomics, metaproteonomics, and metabolomic techniques (addressing the functional consequences of the microbiome). Currently published studies in IBS subjects have used a variety of these techniques[12] and Table 1).

### Composition and organization of gut microbiota in IBS

Seven studies have evaluated shifts in microbial small bowel community composition in the upper bowel (summarized in [12, 25]) of a total of 314 subjects meeting IBS diagnostic criteria based on culture results, with two additional studies reporting results from molecular studies (Table 1).[26, 27] A larger number of studies (n=22 in a total of 827 subjects) have reported significant microbial shifts in fecal microbial community composition between healthy controls and IBS patients, based on disease subtypes (IBS-D; IBS-C; IBS-M), age (pediatric versus adult), and compartment (mucosa versus stool).[12] Despite a lack of consensus on the wide range of gut microbial differences between IBS subjects and healthy controls and the specific microbial changes that may be correlated to disease outcome, some general trends are starting to emerge as depicted in Table 1. In contrast to culture based studies of small bowel microbiota where no consistent differences are evident between IBS and controls, recent molecular based methods of mucosal brushings or luminal aspirates suggest decreased diversity in small bowel microbiota with increased abundance of gram negative organisms in IBS.[26, 27] Based on analysis of fecal samples, regardless of analytical methodology used, a number of studies reported decreased relative abundance of the genera Bifidobacterium and Lactobacillus, and increased Firmicutes:Bacteroidetes ratios at the phylum level. If these IBS related microbial patterns can be confirmed in future studies, one may speculate about some of the many possible causes for such changes - stress and diet. As discussed below, a temporary reduction in lactobacilli has been reported in animal models of early life stress, which may be related to stress induced changes in intestinal transit.[28–30] On the other hand, Firmicutes is the dominant phylum in adult microbiota consuming a western diet (high in animal fat and protein), while Bacteroidetes was the dominant phylyum in a pediatric population consuming a plant fiber based, agrarian diet.[31] The dominant genera within Bacteroidetes may differ since Prevotella was more abundant in an African population consuming a plant-based diet, whereas Bacteroides was more abundant in a North American population consuming a different plan-based diet.[32] Long-term food preferences appear to shape enterotypes, which are primarily defined by bacterial genera as the key principal components. Diet induced shifts have also been reported at the genus level: Within the phylum Bacteroidetes, individuals on a Western diet have a Bacteroides-enriched enterotype as opposed to those living on an agrarian diet which have a Prevotella-enriched enterotype. When viewed together with the reported shifts in gut microbial composition in

pediatric and adult IBS patients at the phylum level, one could speculate that the findings of an enhanced Firmicutes: Bacteroidetes ratio may in part be due to factors related to the typical Western diet.[33] However, even though the reported prevalence rates are higher in some countries consuming Western diets, in particular the US, UK and Italy, few reliable data are available to support the concept that IBS prevalence differs significantly between individuals living in countries or cultural settings (urban vs rural) consuming a typical Western diet and those living on an agrarian type diet.[34] The question if dietary habits are associated with IBS patterns of dysbiosis, should be addressed in future studies.

Unfortunately little work to date has examined the mucosa-associated microbiota in health or in IBS. In healthy individuals and patients with IBS, the mucosa-associated microbiota determined from duodenal brushings or rectal biopsies differs from that found in feces.[26, 35] These mucosal-fecal microbiota differences have been described as being greater than the fecal microbial differences between patients with IBS and controls.[36, 37] Differences between microbiota associated with mucosa and those found in feces are likely critical to understanding the relationship between dysbiosis and IBS because of the closer communication between signaling systems between mucosa associated microbiota and the epithelium.[2]

Widely conflicting reports of microbial dysbiosis exist in IBS and a number of significant variables likely contribute to theses discrepancies that limit our understanding of the collective literature. Many clinical studies have used markedly different experimental approaches to define microbial communities. In many cases there are poorly defined clinical cohorts, presence of psychological comorbidities, inadequate numbers, and/or lack of repeat studies in the same patient study population. Generally, there is a lack of temporal stool sampling that coincides with clinical symptoms, and most studies have not considered dietary variations or drug use (e.g. antibiotics, proton pump inhibitors) that may directly influence microbial community composition, or indirectly through alteration of gut motility, immune activity, or other functions such as mucosal permeability. Furthermore, there is currently no way to decide if the observed alterations in microbial communities in IBS are a primary abnormality responsible for IBS related symptoms or if the observed changes are secondary to IBS related alterations in various gut functions (regional motility, secretion), or if both of these mechanisms contribute to the persistence of altered bidirectional brain gut interactions.

Larger and more homogeneous sample sizes, control for differences in physiologic parameters (e.g., diet, transit, gut permeability, immune status), and stratification of patients based on greater phenotypic discrimination (e.g., postinfectious, symptom duration, age, sex) will be necessary to observe reproducible differences between microbial communities from IBS subjects and healthy control subjects. This is supported by a recent study demonstrating that two IBS microbial clusters, not related to IBS-D or IBS-C cohorts, show significant compositional differences to healthy controls, with a third IBS cluster sharing an identical enterotype with controls.[38]

### Gut microbiota related metabolites in IBS

Recent studies suggest that significant changes in microbiota related metabolites can occur without detectable changes in the organizational structure of the gut microbiota[39], and that host genotype (FUT2 gene status) can affect the metabolic response of the gut microbiota to the diet.[40] Despite the central role of the gut microbiota in producing the short chain fatty acids (SCFAs) butyrate, propionate, and acetate, there is disagreement regarding whether their fecal concentrations differ between IBS and controls and whether changes in concentration are related to IBS symptoms.[41–45] In addition to reported IBS related alterations in SCFAs, alterations in other metabolites including choline, taurine and branched chain fatty acids,[42] lysophosphatidylcholine,[46] 2(3H)-furanone, and volatile organic metabolites (VOM)[47] Esters of SCFAs and cyclohexanecarboxylic acid and its derivatives were significantly associated with IBS-D, and key VOMs were able to predict a diagnosis of IBS.[46, 47] Inconsistencies among reported studies on microbial metabolites in IBS likely relate, in part, to such variables as methodology, patient selection and control for diet and concurrent medications.

## Evidence to support a causative role of dysbiosis in IBS symptoms

Even before the recent explosion in knowledge about the gut microbiome and its implication as a factor in IBS pathophysiology, various dietary modifications have been suggested to treat IBS symptoms including the traditional (inconsistent) teaching to avoid fiber rich diets while other recommendations have include the intake of various fiber supplements. Such decade old treatment recommendations, poorly substantiated by controlled trials, can now be reevaluated in the context of how such interventions may affect alterations in gut microbiota in IBS. Of the many dietary recommendations given to IBS patients over the years, the one that has received most recent attention is the low fermentable substrate diet (low FODMAPs concept). This diet recommends reduced consumption of foods containing oligosaccharides, disaccharides, monosaccharides and polyols that are poorly absorbed in the small intestine and are fermented by gut bacteria in the large intestine. A recently published randomized controlled trialin IBS patients comparing a FODMAP diet to a regular diet reported a reduction of IBS symptoms in the FODMAP diet group.[48] Whether a similar mechanism also explains the reported response in IBS to a gluten free diet remains to be determined.[49]

### Prebiotics

There are only four randomized trials (one single blind) of prebiotics in IBS and no systematic reviews or metaanalyses.[50] Given the adverse effects of a high carbohydrate diet, including one containing fermentable oligosaccharides, disaccharides, monosaccharides, and polyols (FODMAPs) in some patients with IBS, it is not surprising that high intake of prebiotics worsened or did not improve symptoms in three studies.[51, 52]

### Probiotics

Based on several meta-analyses probiotics appear to provide some benefit in IBS (Table 2).[53] However, these metaanalyses highlight the problems interpreting results from probiotic studies in IBS. These include inadequate sample size, poor study design (e.g., crossover with inadequate washout between study periods), inclusion of different IBS subtypes, and the use

of multiple strains and doses across studies. Ortiz-Lucas et al.[54] evaluated 10 studies adequate for meta-analysis with a focus on the specific organisms potentially effective. A significant benefit on pain relief was found for *B. breve*, *B. longum*, and *L. acidophilus* (Table 2).[54] No effects on stool frequency or consistency were found.[54] Horvath et al. reviewed three studies of *L. rhamnosus* in children with IBS and reported a significant benefit for pain improvement (Table 2).[55]

### Antibiotics

As demonstrated in both preclinical and clinical studies, the gut microbial composition can be altered by treatment with antibiotics. The best data to evaluate the effect of an antibiotic on IBS symptoms comes from studies with the non-absorbable antibiotic rifaximin. In a recent systematic review and metaanalysis on the use of rifaximin in IBS,[56] with the primary outcome improvement in global IBS symptoms, five studies (n=1803) reported an overall small improvement after treatment (OR = 1.57; 95% CI 1.22 to 2.01) with a NNT of 10.2.[56] Using that NNT, rifaximin would be less effective than peppermint oil, psychotherapy, tricyclic antidepressants, spasmolytics, selective serotonin reuptake inhibitors, and probiotics based on a review of metaanalyses of treatments for IBS in adults.[57] A double blind, placebo controlled trial of rifaximin in children with pediatric Rome-defined abdominal pain functional GI disorders (n=75) found no benefit.[58] Evidence has been put forth suggesting that rifaximin works by treating small intestinal bacterial overgrowth (SIBO) in IBS.[59] Although rifaximin is bacteriocidal against a broad array of enteric pathogens (gram negative/positive, aerobic/anaerobic), two weeks of treatment did not affect fecal coliform median log counts,[60] and other anti-inflammatory mechanisms of actions have been demonstrated.[61]

In summary, given the limited data available from high quality randomized clinical trials to assess the effectiveness of prebiotics, probiotics and antibiotics in IBS patients, published data and metaanalyses to date suggest that alterations in gut microbiota may play a relatively small role in generating GI symptoms. However, the interventional data viewed together with the likely heterogeneity of mechanisms contributing to IBS symptoms, suggest that subsets of patients with specific microbiota-related alterations (e.g. deficiencies or excesses of certain microorganisms or metabolites which play a role in causing distinct brain gut abnormalities) may significantly benefit from specific interventions aimed at normalizing a particular dysbiotic state. The identification of such patient subsets with distinct patterns of dysbiosis will require large scale studies in well phenotyped patients with functional GI disorders.,

## Possible causes of dysbiosis in IBS

### Enteric infections

Persistence of IBS-like symptoms (so called postinfectious IBS) which has been reported in a small (8–15%) percentage of patients following an initial episode of documented bacterial or viral enteric infections supports a possible role of perturbations of the gut microbiome by pathogens in the development of altered brain gut interactions associated with IBS like symptoms. Common bacterial causes of traveler's diarrhea (*E. coli*, Salmonella and

Campylobacter) are particularly strongly associated with postinfectious IBS, and both biological and psychological risk factors have been identified.[62] Biological factors include duration and severity of diarrhea, microbial toxin production, gut pathology and inflammation. Psychological factors include a high somatization score in affected patients (e.g. a high number of somatic symptoms the subject has experienced prior to the infection), trait anxiety, and the presence of a major psychosocial stressor around the time of the infection. When these factors are viewed together, it is intriguing to speculate that increased levels of stress related catecholamines in the stool (as observed in animal models of stress) result in increased virulence of the respective pathogen (reviewed in Rhee, et. al.,[2] thereby increasing the duration and severity of the infection. Such a prolonged infection with or without additional antibiotic treatment may shift the gut microbiota to an "IBS-prone enterotypes." Other possible mechanisms include persistently increased mast cell numbers and alterations in neuropeptide homeostasis as a result of infection induced enteroendocrine cell hyperplasia. Increased serotonin containing cells are a hallmark of IBS-D, and increased serotonin release from such cells is likely to alter signaling within the enteric nervous system and gut to brain signaling (reviewed in Hughes et al.[14]).

### Antibiotics

Both clinical and published evidence suggest that in some individuals, previous antibiotic therapy for non-GI related indications may increase the new onset or symptom flare of existing IBS symptoms,[63, 64] and that antibiotic treatment may increase the development of long term post infectious IBS symptoms.[65] However, in contrast to other health problems that have seen a dramatic recent increase in developed countries and which have been associated with increased antibiotic use in childhood,[66, 67] no such epidemiologic changes in IBS prevalence have been reported, arguing against a major causative role of antibiotic use in IBS pathophysiology.

## Top down modulation of the gut microbiota by the CNS

CNS modulation of the gastrointestinal tract via the autonomic nervous system (ANS) and the hypothalamus–pituitary–adrenal (HPA) axis can influence enteric microbiota both indirectly, via changes in their environment, and directly, via host–enteric microbiota signaling.(reviewed in [2]) Both branches of the ANS have a prominent role in the modulation of gut functions, such as regional motility, secretion of acid, bicarbonates and mucus, epithelial fluid handling, gut permeability and mucosal immune response (Fig. 1AB) (reviewed elsewhere[68]). The majority of these functions, except for sympathetically and cortisol mediated immune modulation are affected via sympathetic and parasympathetic influences on circuits of the enteric nervous system. Regional and global changes in gastrointestinal transit can have profound effects on the delivery of important nutrients to the enteric microbiota (such as prebiotics, including resistant starches and certain dietary fibers) pH, and on the luminal environment in healthy and diseased states. For example, impaired intestinal transit caused by compromised, migrating motor complexes (a motor pattern characteristic of the fasting state of the gastrointestinal tract that is under parasympathetic control), is associated with bacterial overgrowth in the small intestine.[69] A reduced number of giant, migrating contractions in the colon has been reported in slow transit constipation,[70]

and may play a role in the subset of IBS-C patients with slow transit, while accelerated intestinal transit, with an increased number of giant, migrating contractions, is seen in many diarrheal states, including diarrhea-predominant IBS.[71] The ANS mediated modulation of mucus secretion is likely to have important effects on the size and quality of the intestinal mucus layer, an important habitat for the biofilm, where the majority of the enteric microbiota reside.[72] The ANS also affects epithelial mechanisms involved in immune activation of the gut, either directly, through modulation of the response of the gut immune cells (for example, macrophages and mast cells) to luminal bacteria, through secretion of antimicrobial peptides[73] or indirectly, through alteration of the access of luminal bacteria to gut immunocytes. For example, several preclinical studies have demonstrated that stressful stimuli can enhance the permeability of the intestinal epithelium, facilitating translocation of luminal organisms and triggering an immune response in the intestinal mucosa.[74–79]

Considerable evidence supports a role of stress and its mediators in modulating the gut microbiome.[28, 80, 81] Both pre and postnatal stressors in animal models have been been shown to modulate the composition and total biomass of the enteric microbiota.[28, 30] In newborn animals, the reported shedding of lactobacilli may have been related to the stress induced acceleration of intestinal transit, since normal bacterial levels were restored 1 week after separation.[28] In adult mice, a psychosocial stressor decreased the relative abundance of the genus Bacteroidetes, while increasing the relative abundance of the genus Clostridia.[82] It remains to be determined if the reported reductions in the abundance of Lactobacilli/Bifidobacteria and Bacteroidetes reported in several preclinical studies and in several IBS studies (see Table 1) may both be a consequence of stress induced acceleration of intestinal transit, or other stress system mediated effects on the gut microbiota.

### Intraluminal release of neurotransmitters

In addition to CNS-induced changes in the gut environment in which the microbiota reside, there is a another, neuroendocrine communication system which allows for bidirectional signaling with the gut microbes, which has been referred to as microbial endocrinology.[83] Several signaling molecules used by the host for neuronal and neuroendocrine signaling (including but not limited to catecholamines, serotonin, dynorphin, and cytokines) may also be released into the gut lumen by neurons, immune cells, enterochromaffin cells and possibly, gut microbes themselves, with the CNS likely having an important role in the release of these molecules.[84–86] Of particular interest for mechanisms with potential relevance for the pathophysiology of a stress sensitive disorder like IBS is the observation that different types of stressors can result in increased luminal levels of catecholamines, including norepinephrine.[87, 88] Recent evidence suggests that the gut microbiota derived beta-glucuronidase may also play an important role in the generation of free, e.g. unconjugated form of norepinephrine.[89] It has long been known that some pathogens can change their proliferative activity in response to exogenous catecholamines in vitro.[90] For example, norepinephrine can stimulate the growth of several strains of enteric pathogens (reviewed elsewhere[88]) and magnifies the virulent properties of Campylobacter jejuni.[91]

## Bottom up modulation of the gut brain axis by IBS related dysbiosis

There are multiple ways, levels, and signaling mechanisms by which gut microbiota can influence the activity and responsiveness of the gut brain axis, including the brain. Such influences may occur early in life and affect the development of the nervous system, the brain gut axis and the HPA axis, or they may occur in the adult organism and modulate fully developed circuits (reviewed in [5, 8, 9]). However, the fact that more review articles, and reports in the lay press on this topic have appeared in the last 5 years than original articles confirming many of the initial observations, suggests caution when extrapolating from existing data to unsubstantiated speculations.

### Role of gut microbiota in brain development

A limited number of preclinical studies have demonstrated that the development of brain mechanisms related to hyperalgesia,[7] HPA axis,[6] affective behavior[17, 18] and associated brain biochemistry [17] depends on an intact gut microbiome (Table 3). However, as these observations were obtained in non-physiological conditions (germ-free status) which may affect specific maternal rodent behaviors which have been shown to be associated with epigenetic changes in stress related genes[92] required for the normal development of the central nervous system,[93] premature conclusions about similar effects occurring in humans should be avoided.

### Role of gut microbiota derived metabolites and signaling molecules in modulating the gut brain axis in the adult

A number of candidate signaling molecules have been identified by which the gut microbiota may communicate with the host, including communications of the microbiota with the enteric nervous system and the brain. Quorum sensing molecules used by microbes to communicate with each other (including metabolites and neurotransmitter homologues) have also been shown to be recognized by the host and may influence enteroendocrine, immune cells, and nerve endings in the gut (reviewed in [2]). Metabolites produced by gut microbes (including short chain fatty acids (SCFA), many neuroactive substances including GABA, tryptophan, serotonin and catecholamines (reviewed in [94]), and metabolites of bile acids and neurotransmitters), and cytokines released in response microbe host interactions [82] can signal via specific receptors on local cells within the gut, or signal by neurocrine (afferent vagal pathways) and endocrine mechanisms to long distance targets beyond the GI tract, including vagal afferents in the portal vein, and the brain. Thus, regardless of the sequence of events leading to a dysbiotic state in some IBS patients (see preceding section), the altered microbial community is likely to exert a modulating effect on bidirectional communication within the gut brain axis. In the following we will highlight a few metabolites which may have a direct relevance to IBS pathophysiology.

### Short chain fatty acids (SCFA)

Fermentable carbohydrates entering the colon are converted to the three primary SCFAs, acetate, propionate, and butyrate which have been demonstrated to exert a number of physiologic effects including reducing food intake, improving glucose tolerance, enhancing lymphocyte and neutrophil function, and activating pathways important in epithelial cell

signaling.[16, 95–99] Recent data, primarily from animal experiments, suggest that SCFA mediate many of their effects through G-protein-coupled receptors, specifically GPR43, GPR109A, and GPR41 (for reviews see Bindels et al. and Ganapathy et al.[100, 101] Evidence suggests that signaling through these receptors as well as transport of SCFA by SLC5A8, and the resultant physiological effects is affected by dietary intake of fermentable fiber [100] Gut microbiota appear capable of regulating gene expression of SCLC5A8 and GPR109A.[102] GPR43 is found in mast cells and may be one mechanism whereby SCFA can alter serotonin (5-HT) release.[103] Most recently, work suggests that GPR41 and GPR43 serve as sensors for SCFA in enteroendocrine cells, but only GPR41 serves this role in neuronal cells of the submucosal and myenteric ganglia.[104] However, despite the potential relevance of altered SCFAs in IBS pathophysiology, few studies are available to support such a role based on therapeutic interventions aimed at this signaling mechanism.[105, 106]

### Bile acids

Primary bile acids are biosynthesized in the liver by the oxidation of cholesterol, conjugated to either glycine or taurine and secreted into the gut via the bile duct. In the gut, glycine and taurine residues are removed, and some of the bile acids are converted into secondary bile acids by the gut microbiota. It is conceivable that microbial dysbiosis observed in some IBS patients may be associated with a shift in microbes that metabolize and conjugate primary bile acids, as preliminary studies have suggested.[107] Bile acids have been proposed to constitute a primary cause of disease symptoms in approximately 30% of adult IBS-D patients[42, 107] and recent evidence are consistent with a role of bile acids in pediatric IBS-C patients as well. [108].

## Implications/future directions

Despite the exciting new and rapidly growing insights into the interactions of the gut microbiota with the gut, the enteric nervous system and the brain, the role of alterations in these interactions in the elusive pathophysiology of IBS remains to be determined. Current evidence is most supportive of a top down modulation of the gut microbiota by the brain through the ANS, and possibly the HPA axis. However, these brain induced microbial alterations may alter the complex signaling of the microbiota to enteric neurons modulating gut functions (e.g. motility and secretion), and to the brain, modulating back ground emotions and visceral perception. Carefully designed translational studies in both human subjects and preclinical models are required to establish the causality of these events. Combining multimodal brain imaging techniques with detailed characterization of gut microbioal signaling systems in healthy adult and pediatric subjects, and well phenotyped patient populations has promise to answer the question what role the gut microbiome plays in determining brain structure and function. Regardless of the precise causality underlying a dysbiotic state in IBS, the gut microbiome has become a promising target for therapeutic interventions.

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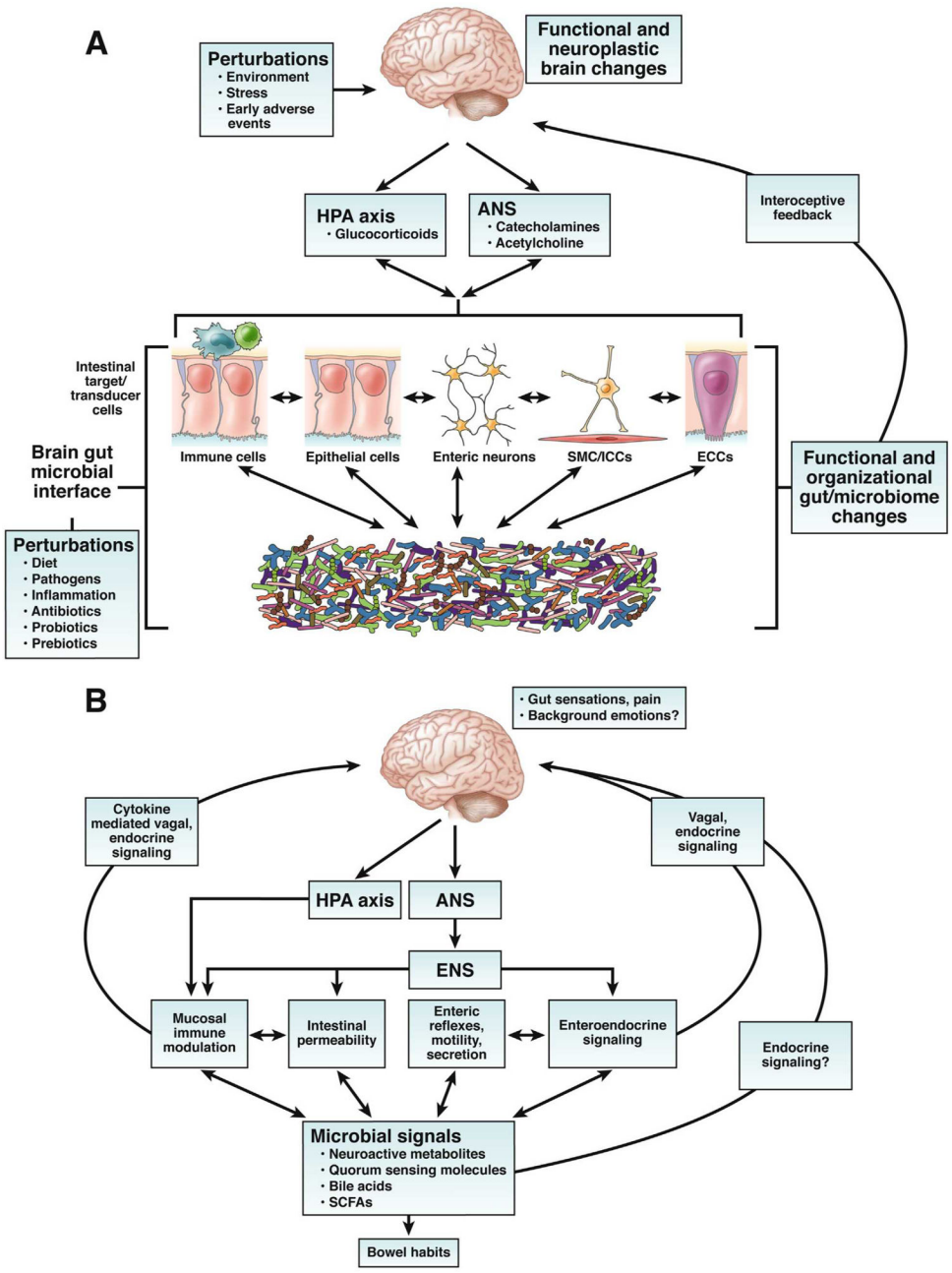


**Figure 1. Bidirectional brain gut microbial interactions**

**A. Key components of brain gut microbial axis**. A network of specialized target/ transducer cells in the gut wall functions as an interface between the organism and the gut lumen. In response to external and bodily demands, the brain modulates individual cells (ECC – enterochromaffin cells; SMC – smooth muscle cells; ICC – interstitial cells of Cajal) within this network via the branches of the autonomic nervous system (ANS) (sympathetic and parasympathetic/vagal efferents) and the hypothalamic pituitary adrenal (HPA) axis. Such modulation can be transient (e.g. in response to transient perturbations) or longlasting (in response to chronically altered brain output). The microbiota are in constant bidirectional

communication with this interface via multiple signaling pathways, and this communication is modulated in response to perturbations of the microbiota, or the brain. The integrated output of the brain gut microbial interface is transmitted to the brain via multiple afferent signaling pathways, including endocrine and neurocrine (vagal, spinal afferents) pathways. While acute alterations in this interoceptive feedback result in transient functional brain changes, chronic alterations are associated with neuroplastic brain changes.

**B. Functional and symptom-related consequences of brain gut microbial interactions**. Several intestinal processes with possible relevance for IBS symptoms can be modulated both the brain (via the ANS, including its enteric nervous system [ENS] branch) and by signals from the microbiota. Microbiota generated molecules can signal to the brain indicrectly via activation of vagal (and possibly spinal) afferent nerve pathways, by microbiota stimulated cytokine and neurotransmitter release from immune or enteroendocrine cells, or such signals may reach the brain via an endocrine route. Microbiota gut brain signaling may contribute to the generation of abdominal pain and discomfort, while microbiota mediated modulation of enteric reflexes is likely to play a role in the pathophysiology of altered bowel habits.

## Table 1

Bacterial classification shifts in human IBS

| Classification Method | Microbiome Shift | Study Subjects |
|---|---|---|
| Culture | ↓ Bifidobacteria (genus which is part of the phylum Actinobacteria); Lactobaccili (which are part of the phylum Firmicutes); Anaerobes | Balsari *et al* (1982)[109] (IBS = 20; Con = 20)<br>Si *et al* (2004)[110] (IBS = 25; Con = 25)<br>Mättö *et al* (2005)[111] (IBS = 26; Con = 25)<br>Carroll et al (2010)[112] (IBS-D = 10; Con = 10) |
| | ↑ Enterobacteria; Aerobes | |
| PCR-DGGE/qPCR | ↓ Anaerobes; Lactobaccilli in IBS-D | Mättö *et al* (2005)[111] (IBS = 26; Con = 25)<br>Malinen *et al* (2005)[113] (IBS = 27; Con = 22) |
| | ↑ Aerobes | |
| FISH | ↓ Bifidobacteria | Kerckhoffs *et al* (2009)[114] (IBS = 41; Con = 26) |
| | ↑ Firmicutes (phylum which includes Lactobacilli) | |
| Microarray | ↓ Bacteroidetes (phylum which includes the genus Bacteroides) ; Bifidobacteria | Rajilic-Stojanoovic (2011) [115] (IBS = 62; Con = 42)<br>Saulnier *et al* (2011)[116] (Pediatric IBS = 22; Con = 22) |
| | ↑ Firmicutes | |
| 16S-yrosequencing | ↓ Bacteroidetes; Bifidobacteria; Actinobacteria | Krogius-Kurikka *et al* (2009)[117] (IBS-D = 10; Con = 23)<br>Rajilic-Stojanoovic (2011)[115] (IBS = 62; Con = 42)<br>Saulnier *et al* (2011)[116] (Pediatric IBS = 22; Con = 22)<br>Jeffery (2012)[38] (IBS = 37; Con = 20) |
| | ↑ Firmicutes; Proteobacteria | |

The table summarizes general bacterial genus and phyla shifts in stool specimens across a range of different classification methods in adult and pediatric IBS cohorts. Data from 13 (out of a total 22) published reports where there is a general consensus on microbiota composition changes in IBS are included. qPCR, quantitative polymerase chain reaction; DGGE, denaturing gradient gel electrophoresis; FISH, fluorescence in situ hybridization.

## Table 2

Meta-analyses of Probiotics in IBS

| Author | Outcome | n | Outcome | NNT |
|---|---|---|---|---|
| Ortiz-Lucas 2013[54] | Abdominal pain | 862 | SMD = −0.24; 95% CI −0.16 to 0.51* | |
| Enck 2010[57] | Global symptoms | Dichotomous - 1838 | OR = 2.24; 95% CI 1.51 to 2.75 | 8 |
| Moayyedi 2010[118] | Global symptoms<br>Abdominal pain | Dichotomous - 918<br>Continuous - 1351<br>Continuous - 834 | RR = 0.71; 95% CI0.57 to 0.88<br>SMD = −0.34; 95% CI −0.06 to −.07<br>SMD = −0.51; 95% − 0.91 to −0.09 | 4 |
| Hoveyda 2009[119] | Global symptoms<br>Abdominal pain | Dichotomous - 895<br>Continuous - 657<br>Dichotomous – 398 | OR = 1.6; 95% CI 1.2 to 2.2<br>SMD = 0.23; 95% CI 0.07 to 0.38<br>OR = 2.88; 95% CI 1.84 to 4.5 | |
| McFarland 2008[120] | Global symptoms<br>Abdominal pain | Dichotomous – 1254<br>Dichotomous - 1039 | RR = 0.77; 95% CI 0.62 to 0.94<br>RR = 0.78; 95% CI 0.69 to 0.88 | 7.3<br>8.9 |
| Horvath 2011[55] | Abdominal pain in children treated with *L. rhamnosus* | No pain or improved pain - 167 | RR = 1.7; 95% CI 1.27 to 2.27 | 4 |

* Average of responses to specific organisms

NNT = number needed to treat, SMD = standardized mean difference, RR = relative risk, OR = odds ratio

## Table 3

Reported effects of gut microbiota on brain and behavior in newborn and adult rodents.

| | |
|---|---|
| Newborn rodents | HPA axis response[6, 19] |
| | Anxiety-like behavior[17, 18] |
| | Neuroplastic changes in emotion regulation systems[17] |
| | Inflammation induced somatic hyperalgesia response[7] |
| Adult rodents | Nociceptive reflexes[121–123] |
| | Emotional behavior[124, 125] |
| | Brain neurochemistry[124, 125] |