JOURNAL OF MEDICINAL FOOD
*J Med Food* 17 (12) 2014, 1–12

DOI: 10.1089/jmf.2014.7000

**REVIEW**

# The Gut Microbiome and the Brain

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ABSTRACT The human gut microbiome impacts human brain health in numerous ways: (1) Structural bacterial components such as lipopolysaccharides provide low-grade tonic stimulation of the innate immune system. Excessive stimulation due to bacterial dysbiosis, small intestinal bacterial overgrowth, or increased intestinal permeability may produce systemic and/or central nervous system inflammation. (2) Bacterial proteins may cross-react with human antigens to stimulate dysfunctional responses of the adaptive immune system. (3) Bacterial enzymes may produce neurotoxic metabolites such as D-lactic acid and ammonia. Even beneficial metabolites such as short-chain fatty acids may exert neurotoxicity. (4) Gut microbes can produce hormones and neurotransmitters that are identical to those produced by humans. Bacterial receptors for these hormones influence microbial growth and virulence. (5) Gut bacteria directly stimulate afferent neurons of the enteric nervous system to send signals to the brain via the vagus nerve. Through these varied mechanisms, gut microbes shape the architecture of sleep and stress reactivity of the hypothalamic-pituitary-adrenal axis. They influence memory, mood, and cognition and are clinically and therapeutically relevant to a range of disorders, including alcoholism, chronic fatigue syndrome, fibromyalgia, and restless legs syndrome. Their role in multiple sclerosis and the neurologic manifestations of celiac disease is being studied. Nutritional tools for altering the gut microbiome therapeutically include changes in diet, probiotics, and prebiotics.

**KEY WORDS:** • ***D-lactic acid*** • ***endotoxin*** • ***microbial endocrinology*** • ***microbiome*** • ***prebiotics*** • ***probiotics*** • ***short-chain fatty acids*** • ***trimethylamine oxide (TMAO)***

## INTRODUCTION

THE MOST SURPRISING revelation of the Human Genome Project is the small size of the human gene pool—about 26,000 functioning units[1]—compared with the genomes of much simpler organisms. Rice (*Oryza sativa*), for example, has about 46,000 functioning genes that have evolved over 15 million years.[2] Researchers call this the ''genome-complexity conundrum,''[3] and some speculate that human physiologic and behavioral complexity may depend on the large number of microbial genes present in the human body.

The term gut microbiome, in its strictest sense, describes the composite microbial genome found in the mammalian gastrointestinal tract. The hundred trillion bacteria in the body of an adult human contain about 4 million distinct bacterial genes, with more than 95% of them located in the large intestine.[4] Since most of these genes encode for enzymes and structural proteins that influence the functioning of mammalian cells, the gut microbiome can be viewed as an anaerobic bioreactor programmed to synthesize molecules which direct the mammalian immune system,[5] modify the mammalian epigenome,[6] and regulate host metabolism.[7]

Manuscript received 9 September 2014. Revision accepted 9 October 2014.

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A study of germ-free (GF) mice found that the vast majority of chemicals circulating in blood are dependent on the microbiome for their synthesis, although many are subsequently modified by the host.[8] These chemicals have a profound effect on mammalian behavior and neuroendocrine responses. This review will focus on research done in humans, but work done with GF rodents signals the evolutionary importance of the microbiome in shaping mammalian behavior, with important implications for human health. The developmental abnormalities found in GF mice are totally reversible by colonization with intestinal bacteria early in life but not in adulthood, suggesting that the microbiome influences brain development.[9,10]

When compared with conventional mice, GF mice show greater exploratory activity in an open-field activity box, suggesting less vigilance and caution.[11] Similar behavioral changes are produced in conventional mice by administration of a mixture of nonabsorbed antibiotics for 7 days.[12] Although some researchers have attributed these behavioral changes to diminished anxiety, elevation of striatal norepinephrine, dopamine, and serotonin turnover in the brains of GF mice[11] and elevated plasma levels of adrenal corticotrophic hormone and corticosterone in response to restraint stress[13] demonstrate heightened stress reactivity in GF mice, an effect also seen in GF rats, who are, however, less active and more cautious than conventional rats.[14] It appears that

both timidity, a behavior pattern associated with mice, and aggressiveness, a behavior pattern associated with rats, require a microbiome for their characteristic expression. Either behavioral deviation—the increased risk taking of GF mice or the withdrawal of GF rats—can significantly impair survival in the wild, where the need to gather food should be balanced against the need to avoid predators. These rodent studies suggest that the gut microbiome has strategic evolutionary importance by modulating stress responses and influencing behaviors that impact the survival of species.[15]

Studies with different strains of laboratory mice indicate that there may be specific behavioral effects induced by specific microbiota. Balb/C mice, for example, are more susceptible to stressors and to the effects of the anxiogenic neurohormone corticotrophin-releasing factor than are NIH Swiss mice.[16] When GF variants of either strain are colonized by gut microbes of the other strain, they begin behaving similar to conventional versions of the strain whose microbiome they have received.[12] Balb/C mice become less stress reactive, and NIH Swiss mice become more stress reactive than their conventional counterparts.

Central nervous system (CNS) effects of the microbiome may be produced by immunologic, biochemical, or neuroendocrine mechanisms.[17]

## IMMUNOLOGIC MECHANISMS

### *The innate immune system*

Structural components of the microbial cell wall continually stimulate the innate immune system to produce cytokines, creating a basal state of immune activation that begins at the intestinal mucosal surface and impacts the entire body.[18]

The gut microbiome interacts with the hypothalamic-pituitary-adrenal (HPA) axis to shape the normal architecture of sleep. Bacterial peptides induce intestinal macrophages and T-cells to produce the cytokines interleukin-1beta (IL-1b) and tumor necrosis factor alpha (TNFa)[19]; bacterial cell wall lipopolysaccharides (LPS) induce synthesis of IL-18.[20] The adult human gut is believed to contain about one gram of LPS.[15] IL-1b,[21] TNFa[22], and IL-18[23] are inducers of nonrapid eye movement (nREM) sleep. Cortisol inhibits immune cell synthesis of these cytokines. IL-1b and TNFa show a circadian rhythm in human blood, with peak levels at midnight, when cortisol is the lowest, and trough levels in the early morning, as plasma cortisol surges.[24] The cortisol-induced decline in microbiome-stimulated circulating IL-1b may orchestrate the normal shift from early sleep, which is predominantly nREM, to late sleep, which is dominated by REM.[25]

Although cytokine secretion induced by low-level exposure of immune cells to bacterial cell wall components contributes to normal sleep patterns, excessive cytokine levels are associated with disrupted sleep.[26] Parenteral administration of LPS to humans in nanogram quantities (0.4 ng/kg body weight) increases plasma concentration of pro-inflammatory cytokines IL-6 and TNFa and the anti-inflammatory cytokines IL-10 and IL-1 receptor antagonist, along with salivary and plasma cortisol and plasma norepinephrine. These changes are accompanied by depressed mood, increased anxiety, and impaired long-term memory for emotional stimuli.[27] In addition, visceral pain sensitivity thresholds are reduced and visceral pain (provoked by rectal distension) is rated as more unpleasant after administration of low-dose LPS.[28]

Increased exposure to gut microbiome-derived LPS (endotoxemia) may occur in the elderly, in whom it is diminished by yogurt consumption,[29] as a consequence of small intestinal bacterial overgrowth (SIBO),[30,31] and secondary to increased intestinal permeability resulting from extreme physiologic stress,[32] ethanol exposure,[33] or a ''fast-food style'' Western diet, high in both carbohydrate and saturated fat.[34]

LeClercq *et al.*[35] have reported increased intestinal permeability, elevated blood LPS and peptidoglycan levels, and low-grade systemic inflammation associated with psychological symptoms of alcohol dependence in alcohol-dependent subjects. They tested inflammatory responses of peripheral blood mononuclear cells (PBMCs) to gut-derived bacterial products in healthy controls and in chronic alcoholics before and during ethanol detoxification. They found activation of Toll-like receptors by LPS and peptidoglycans in PBMCs of alcoholics, associated with increased messenger RNA and plasma levels of IL-8, IL-1$\beta$, and IL-18. Levels of IL-8 and IL-1$\beta$ were positively correlated with alcohol consumption and alcohol-craving scores. Using Cr51-EDTA as a probe of intestinal permeability, they divided their population of chronic alcoholics into those with high and normal permeability.[36] The high permeability group had higher scores of depression, anxiety, and alcohol craving than the low permeability group, as well as a distinct pattern of changes in the gut microbial population, characterized by decreased colonization with bacteria known to have anti-inflammatory effects, *Bifidobacterium* species and *Faecalibacterium prausnitzkii* in particular. Those alcoholics who showed persistence of intestinal hyperpermeability after 3 weeks of ethanol withdrawal also demonstrated persistence of depression, anxiety, and alcohol craving. Their theory is that for some alcoholics (probably 30–50% of the total), ethanol consumption alters the gut microbiome to deplete protective bacteria, increasing intestinal permeability and producing systemic inflammation provoked by absorption of bacterial peptidoglycans and LPS, which amplifies the psychopathology of ethanol addiction.

Increased intestinal permeability has also been described in patients with chronic fatigue syndrome (CFS),[37] fibromyalgia, and complex regional pain syndrome.[38] SIBO by itself can increase intestinal permeability[39]; SIBO is associated with fibromyalgia[40] and restless legs syndrome (RLS),[41] with treatment of SIBO producing clinically significant improvement in a small group of patients with RLS.[41] The CNS effects of elevated gut-derived LPS or peptidoglycan exposure might contribute to the pathogenesis of these disorders.

Researchers at Johns Hopkins University School of Medicine found evidence of increased gut bacterial translocation in schizophrenic patients, unrelated to antipsychotic treatment. Presence of the translocation marker soluble CD14

tripled the risk of schizophrenia and was positively associated with C-reactive protein (CRP) but not with LPS-binding protein (LBP), suggesting that gut bacterial components other than LPS may be stimulating monocyte activation and inflammation in schizophrenics.[42]

In summary, the gut microbiome stimulates a chronic state of low-level activation of the innate immune system in humans, which is influenced by the circadian pattern of adrenal cortical function. Altered exposure to structural components of the microbiome, which may occur because of increased intestinal permeability or SIBO, may disrupt normal neuroendocrine regulation and has been associated with several disorders linked to abnormal CNS function.

### *Adaptive immunity*

The adaptive immune system responds to specific microbes with antibodies or antigen-specific cellular immune responses and can produce CNS dysfunction through auto-immune reactions caused by molecular mimicry between bacterial and self proteins. Although this is an area of ongoing investigation, there is presently little evidence for a link between the gut microbiome, the adaptive immune system, specific autoimmunity, and disorders of the CNS in humans.[43,44] However, in a laboratory model of multiple sclerosis, mice sensitized to the autoantigen, myelin oligodendrocyte glycoprotein, only developed experimental autoimmune encephalitis in the presence of commensal bacteria.[45]

Celiac disease (CD) is a notable exception, although the mechanism is indirect. Alterations in the gut microbiome may play a primary role in the pathogenesis of CD,[46] a gluten-sensitive disease in which the adaptive immune system damages not only the gut but also the brain. The most common CNS manifestations of CD are ataxia (with or without myoclonus), headache, and cognitive dysfunction. Gastrointestinal symptoms are often absent in neurologic CD, as are the usual marker of intestinal CD, transglutaminase (TG) antibodies. The autoimmune target in neurologic gluten sensitivity is TG6 rather than TG2, which is the target for autoantibodies measured in commercial tests.[47] Most studies, but not all,[48] have found significant differences between healthy children and children with CD in the duodenal[49,50] and oral[51] microbial populations. Some of these differences are the result of inflammation and disappear during a gluten-free diet, but reduced levels of *Bifidobacterium* species, a replicable finding, do not become normal with a gluten-free diet.[52,53] Infants at high risk of developing CD because of family history and personal genotype show a reduction in *Bifidobacteria* before the onset of illness.[54] *Bifidobacteria* protect human intestinal cells from the toxic effects of gliadin peptides, the inflammatory triggers of CD, by altering their structure.[55] They also induce an anti-inflammatory response in stimulated human mononuclear cells in tissue culture.[56] Destruction of protective *Bifidobacteria* can explain the association between incident CD and previous antibiotic exposure.[57] Loss of *Bifidobacteria* may play a pathogenetic role in CD and contribute to its rising prevalence. Administration of *Bifidobacterium longum* ameliorates an animal model of gluten enteropathy,[58] and *Bifidobacteria* have been proposed as potential therapeutic agents for prevention of CD in high-risk individuals.[59]

## BIOCHEMICAL MECHANISMS

Intestinal bacteria produce numerous metabolites with potential encephalotoxicity. The most studied are D-lactic acid[60] and ammonia.[61] Their role in common clinical syndromes will be briefly reviewed, followed by a discussion of the conflicting roles of short-chain fatty acids (SCFA), which may inhibit inflammation but contribute to the pathogenesis of autistic spectrum disorders (ASD).

### *D-lactic acid*

A product of microbial fermentation of carbohydrate, D-lactate is usually produced in excess when small bowel resection allows delivery of a high carbohydrate load to the colon. Elevation of D-lactate in plasma may also occur after other types of abdominal surgery, as a result of increased intestinal permeability and bacterial translocation across the intestinal mucosal barrier.[62] Nonsurgical causes of intestinal hyperpermeability also increase absorption of D-lactate from the intestinal lumen.[63,64]

Increased levels of D-lactate producing bacteria in stool were found in a study of patients with CFS and neurocognitive dysfunction, raising the possibility that microbial D-lactate might contribute to symptoms of patients with CFS.[65] Maes *et al.* found increased intestinal permeability to be common among patients with CFS[37] and to improve in response to administration of glutamine, N-acetylcysteine, and zinc along with adoption of a ''leaky gut'' diet. Improved permeability was demonstrated by reduction in titers of antibodies directed against intestinal flora and was directly related to improvement of symptoms.[66] Pimentel *et al.* demonstrated that eradication of SIBO with antibiotics improved symptoms of patients with CFS and SIBO,[67] but did not measure D-lactate production or absorption. Taken together, these studies suggest that increased intestinal permeability or SIBO in patients with CFS may permit excessive absorption of compounds such as D-lactate produced by the gut flora that have direct or indirect neurotoxic effects, contributing to chronic fatigue.

Probiotics and prebiotics may limit production of D-lactic acid in the gut but should be chosen carefully. Some species of *Lactobacillus* are D-lactate producers[68,69] and high-dose beta-glucan (found in oats and barley) can increase intestinal permeability.[70] In a single case report, a man with recurrent D-lactic acidosis due to short bowel syndrome, who had grown unresponsive to antibiotics and dietary restriction, was rescued from repeated neurotoxicity by a combination of *Bifidobacterium breve Yakult* and *Lactobacillus casei Shirota* as probiotics and galacto-oligosaccharide as a prebiotic. The combination, called a symbiotic, allowed reduction in colonic absorption of D-lactate by limiting the growth of D-lactate-producing bacteria and stimulating intestinal motility.[71] No dietary restrictions were needed. *Bifidobacteria* and galacto- or fructo-oligosaccharides

(FOS) favor acetate over lactate as an end-product of carbohydrate metabolism. Horses who had barley added to their diets experienced a change in fecal flora characterized by increased concentrations of lactic acid bacteria belonging to the genera *Lactobacillus* and *Streptococcus*, associated with an increase in D-lactate concentration in the stool. These changes were prevented by administration of FOS.[72]

### *Ammonia*

Ammonia is a well-known neurotoxin, produced in the intestinal tract from urea by the action of bacterial ureases. Gut-derived ammonia is taken up by the liver and consumed in the urea cycle. By creating portosystemic shunts, cirrhosis allows absorbed ammonia to escape hepatic metabolism, increasing blood ammonia, which contributes to the pathogenesis of hepatic encephalopathy (HE).[61] In addition to direct neurotoxic injury, ammonia alters function of the blood–brain barrier, impairing intracerebral synthesis of serotonin and dopamine and producing abnormal neurotransmitters such as octopamine.[73]

Minimal HE (MHE) is a common neurocognitive disorder that occurs in 80% of cirrhotic patients[74] and often evades diagnosis.[75] It is characterized by subtle intellectual deficits and psychomotor abnormalities that have a significant negative impact on health-related quality of life, impair motor vehicle operation, and increase the incidence of vehicular accidents.[76] Failure to diagnose MHE in apparently ''normal'' patients with chronic liver disease is considered a medical error.[77]

Cognitive dysfunction in patients with cirrhosis is associated with altered composition of the gut microbiome, which differs between cirrhotics with or without HE.[78,79] Levels of urease-producing bacteria are positively associated with cognitive dysfunction in cirrhotic patients.[80] The nonabsorbed antibiotic rifaximin, when added to conventional therapy with lactulose, increases the rate of total reversal of HE from 51% to 76% and reduces mortality from 49.1% to 23.8%,[81] demonstrating the importance of gut flora in HE pathogenesis. Changing the gut microbiome with synbiotics has also been shown to alleviate cognitive dysfunction in patients with cirrhosis. A combination of *B. longum* and FOS[82] or a cocktail of four freeze-dried, non-urease-producing bacteria (*Pediacoccus pentoseceus, Leuconostoc mesenteroides*, *Lactobacillus paracasei* ssp. *paracasei*, and *Lactobacillus plantarum*) mixed with beta glucan, inulin, pectin, and resistant starch[83] had similar effects. Each regimen reduced serum ammonia and improved cognitive performance when compared with placebo. Administration of synbiotics has been proposed for all patients with cirrhosis as a way to prevent MHE.[82]

### *Short-chain fatty acids*

Volatile fatty acids with a chain length of two to four carbon atoms (acetate, propionate, and butyrate) are produced in abundance through bacterial fermentation of indigestible carbohydrate in the normal colon. Health benefits of high fiber consumption have been linked to increased synthesis of SCFA.[84,85] Butyric acid, for example, supplies 70% of energy requirements of the colonic epithelium[86] and has direct anti-inflammatory effects, inhibiting activation of nuclear factor kappa-B (NFkB).[87] Propionic acid also inhibits NFkB and may improve insulin sensitivity by activating peroxisome proliferator-activated receptor gamma.[88]

In addition, SCFA impact at least two systems of molecular signaling that have widespread regulatory effects throughout the body: histone deacetylation (HDAC) and G-protein-coupled receptors (GPCRs).[89] SCFA are natural inhibitors of histone deacetylases and activators of specific GPCRs. Acetylation and deacetylation of the histone proteins around which DNA coils is a fundamental process in the epigenetic regulation of gene expression. An imbalance in the direction of excessive HDAC has been found in Parkinson's disease,[90] depression, and schizophrenia.[91] Inhibition of HDAC has beneficial effects in cancer and a number of animal models of CNS disease, including brain trauma, dementia, and autoimmune encephalitis.[92,93] Histone deacetylase inhibitors have been proposed for enhancement of cognitive function.[94]

GPCRs are transmembrane proteins that recognize molecules in the extracellular milieu and transmit information within cells to regulate cell behavior.[95] They represent a major gateway through which cells convert external cues into intracellular signals and respond with appropriate actions. GPCRs are implicated in the pathophysiology of many types of disease, including neurodegenerative disorders. Approximately 40% of clinically approved drugs act by modulating GPCR signaling pathways.[96] SCFAs activate two specific GPCRs (GPR41 and GPR43) that have no other known ligands.[97] GPR41 is abundant in human sympathetic ganglia, where its activation by propionic acid increases sympathetic nervous system outflow, and one potential mechanism by which dietary fiber can increase basal metabolic rate and help control obesity.[98]

Despite evidence of anti-inflammatory effects of propionic acid[88] and the recommendation of some researchers that increasing propionic acid synthesis in the colon may be of therapeutic value for metabolic disorders,[99] MacFabe has identified potential neurotoxicity of propionate and studied its possible role in autism.[100] His group found that pathological changes in the brains of animals exposed to intraventricular propionic acid were identical to abnormalities found in the brains of autistic children and adults. Depletion of glutathione and increased markers of oxidative stress accompanied neuroinflammation. Butyrate demonstrated similar but much milder effects. MacFabe believes that gut-derived propionate contributes to the pathogenesis of autism and that SCFA-induced neurotoxicity explains the sensitivity to dietary carbohydrates noted by physicians treating children with ASD.

In support of MacFabe's hypothesis are the findings of Wang *et al.* of elevated SCFA[101] and propionate[102] in the stool of autistic children. Since the most abundant carbohydrate fermenting bacteria are unchanged or reduced in stools of autistic children,[103,104] Wang speculates that unusual fermenters, perhaps Clostridial species that are often elevated in

the stools of autistic children,[105,106] may be responsible for increased propionate production.[107] Autism is associated with early weaning from breast milk to infant formula.[108] Compared with breast milk, infant formula feeding increases fecal concentration of propionate and butyrate.[109]

Williams *et al.* examined ileal biopsies of autistic children with gastrointestinal (GI) complaints and found a deficit of genes encoding disaccharidases and hexose transport enzymes, indicating impairment of the primary pathway for carbohydrate digestion and absorption in enterocytes,[110] a finding which suggests that bacterial dysbiosis results from an underlying impairment of digestion and absorption. In a subsequent report, they observed the presence of a unique genus of aerobic gram-negative rods, *Sutterella*, in ileal biopsies of autistic children with GI complaints but no children with GI complaints who were not autistic.[111] Western immunoblots revealed plasma IgG or IgM antibody reactivity to the species *Sutterella wadsworthensis* in the majority of children with positive biopsies. *S. wadsworthensis* is a gastrointestinal pathogen that may be mistaken for *Campylobacter jejuni* and may also be found in the stool of healthy individuals.[112] Following the report by Williams *et al.*, Wang *et al.* confirmed an association between abundance of *Sutterella* and the presence of autism. They studied stool specimens, not ileal biopsies, so they were able to examine the relationship between *Sutterella* and GI complaints. There was none. Levels of *Sutterella* were related to autism only.[113]

A role for the gut microbiome and its metabolites in ASD is one of the leading areas in autism research these days,[114,115] but the findings do not yet permit a single coherent theory on which to base therapeutic decisions. A recent report in the *New England Journal of Medicine* describes structural brain abnormalities in autistic children that began during prenatal brain development,[116] indicating that the roots of autism may be found *in utero*. Perhaps greater focus should be placed on the maternal gestational microbiome. Immune activation of pregnant mice (maternal immune activation [MIA]) can create behavioral changes similar to ASD in their offspring.[117] Administration of a single probiotic, *Bacteroides fragilis*, corrects excessive gut permeability, alters gut microbial composition, and ameliorates defects in communication and stereotypic, anxiety-like, and sensorimotor behaviors in the MIA model.

## NEUROENDOCRINE MECHANISMS

Bacteria can synthesize and respond to hormones and neurotransmitters. *Lactobacillus* species produce acetylcholine and gamma-amino butyrate (GABA); *Bifidobacterium* species produce GABA; *Escherichia* produce norepinephrine, serotonin and dopamine; *Streptococcus* and *Enterococcus* produce serotonin; and *Bacillus* species produce norepinephrine and dopamine.[17] These organisms are responsive to human hormones and neurotransmitters,[118] which impact their growth and virulence. Lyte[119] has reviewed research indicating that growth of *Escherichia coli* and other *Proteobacteria* is greatly enhanced by physiologic concentrations of norepinephrine, explaining a direct impact of stress responses on infection, independent of the effect of stress on host immunity.

The interbacterial communication system known as quorum sensing utilizes hormone-like compounds referred to as inducers to regulate bacterial gene expression. Enterohemorrhagic *Escherichia coli* (EHEC) serotype O157:H7 is responsible for outbreaks of bloody diarrhea. Sperandio *et al.* showed that exogenous epinephrine is an inducer of the 0157:H7 virulence factor.[120] EHEC growing in a stressed host may be more virulent than in a non-stressed host.

In addition to specific effects on potential pathogens, host stress responses may provoke widespread changes in gut microbial composition. Bailey *et al.* stressed mice with a process called Social Disruption (SDR), which significantly alters bacterial community structure in the cecum, especially when the microbiota are assessed immediately after exposure to the social stressor. SDR reduces the relative abundance of bacteria from the genus *Bacteroides*, while increasing the relative abundance of bacteria from the genus *Clostridium*. It also increases circulating levels of inflammatory cytokines, IL-6 in particular, which are significantly correlated with stressor-induced changes in microbiome composition. Pretreatment of mice with antibiotics alters the changes in community structure and attenuates the cytokine response after SDR.[121]

A study of college students undergoing the stress of final examinations found a decrease in the relative concentration of lactic acid bacteria in feces after the examination[122] (speciation was not performed). Since lactic acid bacteria have immunomodulating effects[123,124] and may influence the broader composition of the gut microbiome,[125] it seems likely that humans respond to psychosocial stress with responses that are comparable to, if distinct from, the reactions of laboratory animals.

Since gut microbes modify stress responses in laboratory animals, several human clinical trials have been conducted using probiotics to study their impact on stress reactivity and mood. In the most frequently cited study, healthy French adults were administered a combination of *Lactobacillus helveticus* R0052 and *B. longum* R0175 (PF) for 30 days in a double-blind, placebo-controlled, randomized parallel group study. They were assessed with the Hopkins Symptom Checklist (HSCL-90), the Hospital Anxiety and Depression Scale (HADS), the Perceived Stress Scale, the Coping Checklist (CCL), and 24 h urinary-free cortisol (UFC). The probiotic combination significantly reduced psychological distress as measured by the HSCL-90 scale (with significant reductions in global severity index, somatization, depression, and anger-hostility scores), the HADS (significant reductions in the global severity index and anxiety), and the CCL (significant increase in problem solving). There was a significant reduction in UFC.[126] When administered to laboratory rats subjected to experimental myocardial infarction, the same probiotic combination reduced the increase in intestinal permeability[127] and stress-induced cerebral apoptosis[128] found in animals that underwent infarction without probiotic pretreatment.

Tillisch *et al.* administered a fermented dairy product containing *Bifidobacterium animalis* ssp. *Lactis*, *Streptococcus thermophiles*, *Lactobacillus bulgaricus*, *and Lactococcus lactis* ssp. *Lactis* to a group of healthy women for 4 weeks. Participants underwent functional magnetic resonance imaging before and after to measure resting brain activity and response to an emotional reactivity test.[129] A control group received the same dairy product without the probiotics. Use of the probiotic drink was associated with changes in midbrain connectivity and a reduced task-related response in brain regions that control central processing of emotion and visceral sensation.

In an earlier study, researchers in Wales administered a probiotic beverage containing *Lactobacillus casei* to healthy elderly men and women. Those who began the study with depressed mood reported improved mood after 3 weeks of the probiotic but not the placebo beverage. Paradoxically, their memory performance was negatively impacted by the probiotic.[130] The same preparation was administered for 8 weeks by a different research team to adults with CFS. There was no effect on depression, but those receiving the probiotic demonstrated significant improvement on the Beck Anxiety Inventory compared with the placebo group.[131] In an uncontrolled study, 15 patients with CFS received a mixture of *L. paracasei* ssp. *paracasei* F19, *Lactobacillus acidophilus* NCFB 1748, and *Bifidobacterium lactis* Bb12 for 4 weeks. Patients reported improvement in memory and concentration but not in fatigue or physical activity.[132] In a study of volunteers with stress-related irritable bowel symptoms, another probiotic combination, *L. acidophilus Rosell*-52 and *B. longum Rosell*-175, reduced abdominal pain and nausea but had no effect on psychological symptoms or sleep disturbances.[133]

Dinan and coworkers have reviewed the pathways by which probiotic supplements may improve depression or anxiety. Studies in mice and rats support the following interrelated mechanisms: (1) decrease in intestinal permeability resulting in reduced absorption of LPS and reduced production of inflammatory cytokines, (2) downregulation of the HPA axis in responding to stressors, and (3) direct effects on neurotransmission. Gut bacteria and their secretions influence neuronal excitation in the enteric nervous system (ENS), regulating both gut motility and sensory afferent signaling to the brain.[134] Intrinsic primary afferent neurons (IPANs) are cellular targets of neuroactive bacteria and transmit microbial messages to the brain via the vagus nerve.[135,136] Live bacteria may not be needed for these effects; in the case of *B. fragilis*, a lipid-free polysaccharide is both necessary and sufficient for IPAN activation.[137] Although the vagus nerve is a critical route for communication between gut microbes and the CNS in some experimental systems, it is not the only route. Both behavior and CNS levels of brain-derived neurotrophic factor can be altered in mice by manipulation of the gut microbiome without vagal involvement.[12]

Most research on the neuroendocrine effects of gut microbes takes a pharmacologic rather than ecologic approach: A specific intervention is undertaken, and certain results are measured. Unlike pharmacologic agents, however, gut microbes exist in a series of interconnected and highly structured living communities. Administering a probiotic does more than just introduce a new bacterial species, which may or may not be able to establish a niche in the community. It may change community structure in unexpected ways, and these changes may or may not alter community function.[138] Human studies have unveiled substantial differences in the gut microbial composition among individuals[139–141] that depend on age, genetic background, physiological state, microbial interactions, environmental factors, and diet.[142–144] Moreover, the microbiota of the effluent from the ileum is both simpler and less stable than colonic fecal microflora and is dominated by different bacterial phyla.[145] This complexity implies that the application of clinical and laboratory research on the health effects of manipulating the microbiome will need to be tailored to specific characteristics of each individual patient.[146]

## DIET AND THE GUT MICROBIOME

Since diet has a significant impact on composition and function of the human gut microbiome, dietary patterns should be considered in attempts to understand the impact of gut microbes on the brain, especially when interventions are designed. Sequence analysis of amplified microbial ribosomal RNA-encoding genes (16S ribosomal DNA) reveals that the human adult microbiota consists of five bacterial phyla: *Firmicutes* and *Bacteroidetes* predominate, with *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* comprising just 2% of organisms. Most belong to the genera *Faecalibacterium*, *Bacteroides*, *Roseburia*, *Ruminococcus*, *Eubacterium*, *Coprabacillus*, and *Bifidobacterium.*[147] A diet high in animal protein and fat favors abundance of *Bacteroides.* A vegetarian diet or one high in monosaccharides favors abundance of *Prevotella* species.[148] High consumption of oligosaccharides favors growth of *Bifidobacteria*, which is the dominant genus of breast-fed infants, who receive most of their carbohydrate in the form of breast milk oligosaccharides.[149]

The impact of diet on the microbiome is an area of intense study at present, with most research focused on metabolic effects as they relate to obesity, diabetes, and cardiovascular disease. A systematic discussion is outside the scope of this review. There is almost no published research that describes actual diet— >microbiome— >CNS effects in humans, just allusions to such an effect. A role for dietary restriction in the treatment of D-lactic acidosis was previously mentioned. In a single case report, restriction of monosaccharides and sucrose was shown to decrease D-lactate production in a patient with short bowel syndrome, preventing neurotoxicity.[150]

Several aspects of the diet/microbiome relationship deserve further research for their potential importance to brain health in the care of individual patients:

(1) Bacteria can feed or inhibit the growth of each other. Metabolic interactions among components of the

microbiome (the microbial metabolome) is at the cutting edge of microbiome research.[151] Although inter-bacterial inhibition has been understood for a long time, inter-bacterial growth synergy may be as important. *Propionibacterium freudenreichii*, a bacterium found in Swiss cheese, produces substances that enhance growth of *Bifidobacteria.*[152] Administration of this bifidogenic substance to patients with ulcerative colitis produced an increase in fecal butyrate associated with clinical improvement.[153]

(2) Most studies indicate that both health and decreased adiposity are associated with increased diversity of the gut microflora. Dietary restriction increases diversity; dietary excess tends to reduce it.[154]

(3) Extreme dietary changes, such as adoption of a ketogenic diet, produce immediate profound changes in the human gut microbiome. Less dramatic interventions produce mild to moderate changes that vary from person to person and tend to be less than inter-individual variability.[155]

(4) A normal microbiome increases nutrient bioavailability. In order to maintain their health, GF mice should be fed a diet of higher nutrient quantity and diversity than conventional mice.[156] The effect of diet change on the microbiome is not likely to be unidirectional. An altered microbiome may change the effect of food on the host.

(5) Alterations of the microbiome may alter the physiologic effect of nutrients. A critical example of this phenomenon is revealed in the work of Hazen and colleagues at The Cleveland Clinic, who found that higher plasma levels of the vascular toxin trimethylamine-N-oxide (TMAO) conferred an increased risk of major cardiovascular events during a 3-year follow-up.[157] They also demonstrated that plasma TMAO is the product of gut microbial metabolism of dietary choline to trimethylamine (TMA), followed by hepatic oxidation of TMA to TMAO. The same team demonstrated that the gut microbiome of human vegetarians produces significantly less TMA than the gut microbiome of omnivores when fed L-carnitine, another substrate for TMA synthesis.[158] In this way, an essential dietary nutrient (choline) is converted to a vasculopathic substance by the action of a microbiome whose composition is determined by a previous dietary pattern.

(6) Diet involves more than nutrients. Polyphenols are bioactive non-nutrient plant compounds whose bioavailability and physiologic effects greatly depend on their transformation by components of the gut microbiota. Polyphenols, in turn, alter microbial growth patterns. The polyphenol composition of an individual's diet may be more important than macronutrient composition for determining growth effects on gut microbes.[159]

(7) Colonic bacteria have been found in biofilms formed around food particles. These organize gut microbes into distinct communities that behave differently from their planktonic counterparts. Bacteria living in food-associated biofilms produce unique signaling molecules and may represent a new dimension in the relationship between food, microbes, and human health.[160]

## CONCLUSION

Experimental studies with human volunteers and with small mammals demonstrate effects of commensal intestinal bacteria on behavior and brain function that are contextually meaningful and which appear to be biologically significant. Gut bacteria influence reactivity of the HPA axis and the induction and maintenance of nREM sleep. They may influence mood, pain sensitivity and normal brain development.

Clinical studies have demonstrated distinct pathological CNS effects of commensal gut bacteria in hepatic cirrhosis and short bowel syndrome and have led researchers to speculate on possible adverse effects of gut microbes in alcohol dependence, CFS, fibromyalgia, RLS, ASD, schizophrenia, mood disorders, and degenerative or autoimmune neurologic disease. Adverse effects have been attributed to alterations in bacterial community structure (dysbiosis), SIBO, and increased intestinal permeability.

Several mechanisms, none mutually exclusive, may enable commensal gut bacteria to influence function or dysfunction in the CNS: (1) stimulation of host immune responses leading to diverse patterns of systemic cytokine activation; (2) synthesis of absorbable neuroactive metabolites, including neurotransmitters; and (3) alterations in neuronal circuitry by direct microbial effects on the ENS, with CNS transmission through vagal and other routes. The only mechanisms with a high level of proof in humans are the neurotoxic effects of ammonia in HE and of D-lactic acid in short bowel syndrome.

CNS and neuroendocrine activity, stress responses in particular, may, in turn, influence the composition of the gut microbiome by differentially altering the growth of bacterial species and the production of bacterial virulence factors. *Enterobacteriaceae*, a family that includes most of the aerobic Gram-negative pathogens, is especially well tuned to exploiting host stress responses for enhancing bacterial growth and virulence.

Dietary patterns also modify microbiome composition and function, in complex ways that vary among individuals and cultures and are the subject of intense ongoing research. Prebiotics, probiotics, and fermented foods such as yogurt may influence the impact of the gut microbiome on the CNS and have shown significant effects on brain function in a number of experimental trials and clinical studies. Along with diet, these functional food components may offer future opportunities for altering the microbiome to enhance cognitive or emotive function and prevent or treat neurologic disorders.

## AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

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