

The neurobehavioral implications of the brain and microbiota interaction

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1. ABSTRACT

In the past, microorganisms were not considered to be particularly important in brain development and functioning. However, recent evidence shows the existence of a bidirectional, and possibly multidimensional relationship between the body microbiota and the brain. The microbiota influence brain behavior in health or disease, by utilizing endocrine, neurocrine and immunologic signaling pathways. Also, the chemical mediators involved range from known neurotransmitters to small peptide molecules. Here, we discuss the evidence that currently exists in experimental animals and/or humans in support of the existence of a relationship involving the skin/gut microbiome, the brain, and behavior; and

the mechanisms involved in such interactions. The implications of such interactions for shifts in behaviors, and the pathogenesis of behavioral and neurodegenerative disorders are also discussed. Finally, the possible clinical applications of deliberate manipulations of the microbiota composition and density for the management or prevention of behavioral and neurodegenerative disorders is discussed.

2. INTRODUCTION

Humans and animals share a life-long relationship with a number of microbial species resident on or within their body. A number of these

microbes maintain a symbiotic relationship with the host, and have been shown to regulate host's nutrition/ metabolism, ensure the development/functioning of the immune system, maintain general health/wellbeing (1-2), and modulate the development of diseases (3).

The mammalian microbiota is a unique ensemble of microorganisms that include bacteria, fungi, and viruses resident in the different niches (skin, gut, genitalia) on and within the body (4). The diversity of these microbes, and the significant inter-individual variations in their composition in health (1) and with aging (5) is reshaping our understanding of the extent of these relationships, as well as the implications for the development of chronic diseases (6) and brain disorders (4-5).

In recent times, the term gut microbiota has been used to describe the microbial population resident in the gastrointestinal tract. Research exploring the gut microbiota has continued to expand our understanding of the widespread systemic implications of gut microbiota dysbiosis as it relates to the development of obesity, diabetes mellitus (7), inflammatory bowel diseases (8), skin diseases (9), and neurodevelopmental/neuropsychiatric (10-11) disorders.

Recent advances demonstrate the importance of the microbiota (especially the gut microbiota) in the maintenance of brain structure and function, and the development of neurological disorders (5). The local and systemic benefits of the skin in regulating the homeostasis of epidermal keratinocytes and the host immune network are also being revealed (12). Ongoing research into the dynamics and molecular mechanisms of the gut-brain axis suggests the existence of a skin-gut-brain axis (13-14), which may also (directly or indirectly) influence the development of the immune system and/or the brain (15-17). In this review, we discuss the evidence that currently exists in experimental animals and/or humans in support of the existence of a relationship involving the skin and/or gut microbiome, the brain, and behavior; and mechanisms involved in such interactions.

2.1. The microbiota

2.1.1. The microbiota in health

The human body contains about a thousand different species of bacteria carrying at least 150 times more genes than exists in the entire human genome (18). The bacterial composition in and on the human body varies across subjects, and with multiple health conditions. Studies have shown that these variations are influenced by environmental factors including geographical locations, sex, race (19), dietary factors (20), social interaction (21-22) and genetics (23). The evolution of the microbiota with advancing age, as well as the important role it plays in human health maintenance and the development of diseases (24-26) have also been reported.

In humans, earlier studies had reported that bacterial colonization of the 'germ-free' infant begins at birth, and gut microbial communities become demonstrable as early as the 1st week of life (27). Thereafter, the bacterial composition continues to fluctuate until about 1-3 years of age when it begins to approach the adult microbial composition (27-28). This notion that human microbial colonization begins at birth (in the birth canal for vaginally-delivered babies) since the uterus was considered sterile (29) was entertained for years. However, in the last few years, researchers have begun to question the sterility of the uterus (30-31), with suggestions that microbial colonization in humans (like in a number of other organisms) could possibly begin *in-utero* (29, 32). The presence of bacteria in close to a third of the placentas obtained following life-birth (33), evidence of bacteria in meconium (34), the demonstration of microbial DNA in the placenta (32) and amniotic fluid (31, 35) are evidence in support of this recent view. Evaluation of the microbiome of human placentas revealed the presence of microbial colonies consisting of non-pathogenic microbiota including *Fusobacteria*, *Tenericutes*, *Firmicutes*, *Bacteroidetes* and *Proteobacteria*. This microbial composition was closely-related to the microbiota of the human oral cavity (32). Also, similarities between the microbial populations in the placenta, amniotic fluid and neonate's meconium are all accumulating evidence supporting the prenatal colonization of the infant.

An initial contact with microbial colonies provides a nidus for a gradual evolution of these communities into a diverse eco-system, which is maintained throughout the host's lifetime (36). A symbiotic relationship develops between the host and the microbial community, and this relationship may be critical for the sustenance of health and the prevention of disease. The rapid colonization of the infant skin within days of delivery has been shown to coincide with functional changes, including maturation of skin structure and function (37). Also, while the composition of microbial communities on the skin evolves with age and environment (39), proper and timely establishment of a healthy skin microbiome may be crucial in preventing the invasion of the body by potentially-harmful microbes (37). Failure of this could predispose to the development of cutaneous diseases, allergies, and inflammatory non-communicable diseases (12). Contact with microbial communities also contributes to the development of cutaneous homeostasis and immune system (37,39-40). The proper development of the immune system is also crucial to the development and proper functioning of most organ systems, including the human brain (12). The skin microbiome contributes significantly to the development of host immunity by influencing the host cells to produce endogenous antimicrobial peptides. It also modulates the host's innate and adaptive defense system. In healthy skin, *Staphylococcus epidermidis* is a commonly-isolated microbe that protects humans from colonization by pathogenic bacteria (41). This shows that a balance between the host cells and resident bacteria is crucial for optimal skin defense and body health (42).

The gut microbiota includes a diverse community of microbes that inhabit the bowel (8). Over 100 trillion bacteria (made up of about one thousand species) reside in the human gastrointestinal tract (43-44). The gut microbiota is fairly more stable than the skin microbiota, although, like the skin microbiota, it shows significant variations amongst healthy subjects (45). It varies with age, aging, nutrition, antibiotic therapy and illness (43). The gut microbiota is also important in the development and proper functioning of the immune system, and the synthesis of vitamins (44). Bacterial colonies in the gut also aid in metabolizing

indigestible fibers and in the defense against colonization by infectious and/or opportunistic pathogens. They also contribute to the formation of the architecture of the intestine (46).

The crucial role played by the microbiota in proper development and functioning of body organs have also been highlighted by studies that have evaluated the beneficial effects of prebiotics or probiotics supplements in humans (47-48). Supplementation of the milk of formula-fed infants with prebiotics oligosaccharides have been shown to promote the growth of probiotics members of the *Bifidobacterium* and *Lactobacillus* species; and inhibit the growth of pathogenic members of the *Clostridium* species (48). This has been associated with a decreased incidence of colic in these children (48). In rats, probiotic supplementation with *Lactobacillus reuteri* has been shown to increase the number of action potentials and the excitability of the enteric nervous system; with suggestions that this could modulate gut motility and pain perception (49). Prenatal probiotics interventions have also been shown to modulate host-microbe interactions via their ability to influence the expression of Toll-like receptor-related genes in the placenta and fetal microbiome, thereby reducing the risk of the development of diseases like atopic dermatitis (50-51).

2.1.2. The microbiota in disease

A complex symbiosis develops between the microbiota and the human body; its disruption could be detrimental to health and well-being. Microbial dysbiosis has been linked to a myriad of causes, including physiological alterations that occur due to antibiotic therapy, diet, and hygiene (44, 52). Also, physical activity (53) and environmental factors like temperature, humidity, ethnicity and cultural habits (54-55) can significantly alter the microbial composition of the human microbiota.

A number of diseases have been linked to gut microbiota dysbiosis (Table 1), including metabolic diseases (obesity, type 2 diabetes mellitus and cardiovascular disease), digestive system disorders (inflammatory bowel disease, coeliac disease and colorectal cancers), skin conditions (acne, dermatitis and eczema), respiratory system

Table 1. Chronic diseases and alterations in bodily microbial density

Disorder	Host	Microbial composition	Reference
Obesity	Humans	↑ <i>Firmicutes</i> ↓ <i>Bacteroidetes</i>	(62,63)
Type 2 diabetes mellitus	Human	↑ <i>Faecalibacterium prausnitzii</i> and <i>Roseburia intestinalis</i>	(67)
Dysmetabolism	Murine	administration of <i>Akkermansia muciniphila</i> improved glucose control	(68)
Coeliac disease	Human	a decrease in the ratio of gram-positive to gram-negative bacteria, lower concentrations of <i>Clostridium histolyticum</i> , <i>Faecalibacterium prausnitzii</i> , <i>Bifidobacterium</i> and <i>C. lituseburense</i> with coeliac disease	(69)
Psoriasis	Human	high concentrations of <i>Actinobacteria</i> and <i>Firmicutes</i>	(39)
Leprosy	Human	↑ <i>Proteobacteria</i> with a reduction in <i>Actinobacteria</i>	(72)
Atopic dermatitis	Human	The decrease in microbiome diversity, alteration in microbial diversity correlates with disease severity and linked to mutations in the filaggrin genes. ↑ <i>Firmicutes</i> and <i>staphylococci</i> , <i>Staphylococcus aureus</i> and <i>Staphylococcus epidermidis</i>	(42)
Acne	Human	↑ <i>Propionibacterium acnes</i>	(73)

diseases (cystic fibrosis, chronic obstructive pulmonary disease and asthma), and neurological/neurodegenerative disorders such as autism spectrum disorders, schizophrenia, bipolar disorder, Parkinson's disease, and Alzheimer's disease (56–61). Studies in obese rodents and humans have demonstrated a predominance or relative abundance of certain microbes like the *Firmicutes*, or a reduction in the colonization by *Bacteroidetes* (62–63). Also, studies in germ-free mice have demonstrated the role of intestinal microbiota in fat storage (64–66). The absence of intestinal microbes in these mice was protective against the development of diet-induced obesity (64–66).

In humans, metagenome-wide association studies have shown that specific intestinal bacterial colonies or bacterial genes correlate significantly with type 2 diabetes mellitus. These studies also demonstrated low concentrations of certain bacteria, especially butyrate- and/or short-chain fatty acid producing bacteria like *Faecalibacterium prausnitzii* and *Roseburia intestinalis* in subjects with type 2 diabetes mellitus (67). The importance of gut microbiota in the control of blood glucose levels in diet-induced dysmetabolism was demonstrated in mice that were fed high-fat diet, in which oral administration of *Akkermansia muciniphila* was associated with improved glucose tolerance and attenuation of inflammation within the adipose tissue

(68). The administration of metformin to these mice was also associated with an increase in the concentration of *Akkermansia*, a mucin-degrading bacterium, suggesting that modulation of the gut microbiota could impact the antidiabetic effects of metformin (68).

Gastrointestinal diseases like coeliac disease and inflammatory bowel disease have also been linked to dysbiosis of the gut microbiota. De Palma *et al.* (69) reported a decrease in the levels of immunoglobulin (Ig) A-coated fecal bacteria in treated and untreated subjects with celiac disease (CD) compared to healthy controls. Levels of Ig G and Ig M-coated bacteria were also significantly lesser in treated CD compared to untreated CD subjects. Bacterial counts also revealed a decrease in the ratio of gram-positive to gram-negative bacteria in treated and untreated CD patients compared to healthy controls; while lower concentrations of *Clostridium histolyticum*, *Faecalibacterium prausnitzii*, *Bifidobacterium* and *C. lituseburense* were observed in untreated CD patients compared to healthy controls (69).

Dysbiosis of the skin microbiome has also been observed in a few systemic diseases (70); however, how this contributes to disease pathogenesis and/or pathophysiology remains unclear (39,71). In patients with psoriasis, the skin microbiome has been reported to have high

concentrations of *Actinobacteria* and *Firmicutes* (37). Also, in subjects with leprosy, (specifically lesional forms) the microbiome contained an abundance of *Proteobacteria*, with a reduction in *Actinobacteria* (72). Patients with atopic dermatitis (AD) have a decrease in microbiome diversity, compared to healthy controls (42). This loss of diversity correlated with disease severity, and was also linked to mutations in the filaggrin genes (42). In patients with AD, the skin microbiome has also been shown to harbor higher concentrations of *Firmicutes* and *staphylococci* such as *Staphylococcus aureus* and *Staphylococcus epidermidis* (42). Acne is another skin condition which is associated with the presence of *Propionibacterium acnes* (73).

2.1.2.1. Antibiotic use and the microbiota

The use of antibiotics has extended man's life expectancy by an average of 20 years (74). However, it is very unfortunate that these agents have been indiscriminately used and/or abused. As a result of this, microbes are becoming increasingly-resistant to antibiotics through a number of mechanisms, including enzymatic inactivation of the antibiotics, modification of the targets of the antibiotics and prevention of the accumulation of lethal intra-cellular concentrations of the antibiotic through efflux pumps (75). Antibiotic resistance may also occur through mutations and horizontal transfer of resistance genes.

Uncontrolled or unguarded use of antibiotics has been shown to cause dysbiosis within the human microbiota. Gut dysbiosis has been associated with significant reduction in taxonomic richness, diversity, and evenness (76,77). Depletion of bacterial diversity, loss of potential competitors, lower expression of natural antibacterial, as well as decreased neutrophil-mediated killing, may enhance host's susceptibility to exogenous pathogens or opportunistic members of the microbiota (78,79). Beyond alteration of composition, antibiotics can also affect gene expression, protein activity, and overall metabolism of the gut microbiome (80); allowing changes in the nutritional landscape of the gut which directs the expansion of pathogenic bacteria (81). Alterations in the gut microbiome that occur as a result of antibiotics use or abuse predisposes the host to infections that are caused by newly-acquired

or opportunistic pathogens. The accumulation of resistance genes by members of the gut microbiota can also follow abuse of antibiotics.

Antibiotic-induced dysbiosis may also adversely impact brain function. Antibiotics that are strong enough to kill off gut bacteria can also impede the growth of new brain cells in the hippocampus, a section of the brain associated with memory (82).

3. THE BRAIN AND THE MICROBIOTA

3.1. Brain development and the microbiota

In recent times, researchers have begun to ask questions about the presence of a microbiome in the brain, especially following reports demonstrating the presence of microbes in the brain specimens of HIV-infected subjects (83). While the healthy human brain continues to be considered a sterile environment, the role of the microbiome in other regions of the body in modulating brain development, neurochemistry and behaviors (Table 2) have been examined (84–88).

Human brain development begins *in-utero* and extends through adolescence into early adulthood. It is an intricate process that involves the migration of cells over long distances (*in-utero*), with extensions of their cell processes over even greater distances (4,89). The protracted course of pre- and post-natal brain development and the complexity of the process increase its vulnerability, providing a window of opportunity for environmental factors to exert influences that modulate brain structure, function and neurotransmitter/receptor expression (4,76, 87). In the last decade or more, there have been suggestions that the microbiota (especially the gut) influences brain development and maturation (86,90).

While examining the impact of normal gut microbiota on brain development in mice, Diaz Heijtz et al. (86) concluded that early-life but not late-life exposure of germ-free mice to normal gut microbiota was associated with the modulation of motor activity and anxiety-like behaviors (86). These modulatory effects on behaviors occurred via their ability to influence canonical signal pathways, and release of

Table 2. Brain development and the microbiota

Model	Neurodevelopmental changes	References
Mice	Exposure of germ-free mice to normal gut microbiota early in life influenced brain development, motor activity and anxiety-like behaviours by modulating canonical signal pathways, and the expression of genes synaptic-related proteins like synaptophysin and PSD-95 in the striatum.	(86)
Mice	Germ-free mice had alteration in levels of noradrenaline, dopamine and serotonin	(86)
Mice	Post-natal reconstitution of germ-free mice with normal gut microbiota flora containing <i>Bifidobacterium infantis</i> improved the stress response of the germ-free mice by influencing adrenocorticotrophic hormone levels and development of the hypothalamopituitary axis	(90)
Mice	Absence of normal gut microbial flora as observed in germ-free mice was associated with a decrease in the expression of the mRNA of N-methyl-D-aspartate receptor subunit in the amygdala	(86,87)
Mice	A decrease in the expression of serotonin receptor in the hippocampus and increased hippocampal expression of brain-derived neurotrophic factor has also been reported in germ-free mice	(102, 103)
Rat	<i>Bifidobacterium infantis</i> colonization was associated with antidepressant effects and a ↓serotonin levels in frontal cortex and an ↑ kynurenic acid and tryptophan	(91-92)
Mice	Antibiotic-induced depletion of gut microbiota from early adolescence caused cognitive deficits, alteration in tryptophan metabolism and ↓oxytocin, ↓brain derived neurotrophic factor and ↓vasopressin in the adult brain	(106)

brain neurotransmitter (including serotonin, dopamine and noradrenaline) and synaptic-related proteins like synaptophysin (86). Sudo et al (90) reported that post-natal reconstitution of germ-free mice with normal gut microbiota flora containing *Bifidobacterium infantis* improved the response of germ-free mice to stress by influencing adrenocorticotrophic hormone levels in mice (90). Studies in rodents have also demonstrated the antidepressant effects of *Bifidobacterium infantis* via its ability to normalize the concentration of pro-inflammatory cytokines and tryptophan (91-92). Gut microbiota have also been reported to influence the expression and synthesis of receptors and neurotransmitters (including serotonin) which are important in brain development (93). Desbonnet et al. (91) also reported that in rat pups of dams colonized with *Bifidobacterium infantis* a decrease in frontal cortex concentration of serotonin, and an increase in plasma levels of kynurenic acid and tryptophan were observed, when compared to pups of non-colonized controls (91).

During the first three years of life, microbial colonization of the different niches on and within the human body occurs rapidly. There is an initial domination of the microbiota by members of the *Actinobacteria* and *Proteobacteria* phyla, and then a gradual shift towards *Firmicutes* and *Bacteroidetes* domination as adulthood is reached (94-95). This

process also coincides with the period of brain development (spanning from early-life to adolescence) during which synaptogenesis and pruning goes on in the brain (94-97). In the growing infant, there is increasing advocacy for the consideration of the gut microbial communities as modifiable influencers of brain development and host behavior (98–101) The involvement of the gut microbiota in the programming of the brain circuitries that modulate a number of behavioural responses including stress, anxiety, cognition, motor coordination and social interaction during early brain maturation (90, 99,102–105) have also been reported.

Gut microbiota depletion (due to use of antibiotics from early adolescence) was associated with cognitive deficits, alteration in tryptophan metabolism, and a significant decrease in the expression of oxytocin, brain derived neurotrophic factor and vasopressin in the adult brain (106). There have been suggestions that gut microbial depletion or dysbiosis may play a role in the pathogenesis of attention-deficit-hyperactivity and autism spectrum disorders. A number of studies have also implicated the central nervous system in the pathogenesis and pathophysiology of some functional gastrointestinal tract diseases including inflammatory bowel disease and irritable bowel syndrome (107-108). Studies have also demonstrated the beneficial effects of

prenatal prebiotics and probiotic interventions in disease prevention (50-51,109) suggesting that these could be effective novel methods in preventing and/or managing neurodevelopmental disorders like autism and attention deficit hyperactivity disorder. Studies demonstrating the concurrence of postnatal neurodevelopment and the establishment of the gut microbiota provide evidence of a possible bi-directional modulation or regulation of the maturation of one another (110).

The constitution of the skin microbiome (like that of the gut) is a reflection of the synergy of the functional metabolic activity of the host tissues and resident microbes, which are greatly influenced by host behavior and the surrounding environment. In the last decade, there have been suggestions that the brain interacts with the skin, like in a “skin-brain” axis. Also, the extensive innervation of the skin has led researchers to consider it a sort of “diffuse brain” (111). The occurrence of skin disorders like psoriasis and atopic dermatitis is now being associated with the development of behavioral disorders like posttraumatic stress disorder, anxiety, and depression (112–114). Some studies have also shown that the colonization, virulence, and adhesion of skin microbes could be modulated by peptide neurohormone, neurotransmitters and steroid hormones (14). These neuropeptides such as vasoactive intestinal peptide, catecholamines, calcitonin gene-related peptide, nerve growth factor and substance P, can be secreted by some skin cells. However, while the idea of a skin-brain or brain–skin axis is plausible, the full extent of this relationship (unidirectional or bidirectional) remains largely speculative.

3.2. Neurobehavioral phenotypes and the microbiome

Gut microbiota influences on the central nervous system were first reported following observations that laxatives and/or oral antibiotics could mitigate the progression of hepatic encephalopathy (87). In the symbiotic relationship between man and microbes, the human body provides lodgings and nutrition, while microbes repay this favor by ensuring the maintenance of health/well-

being, and prevention of outsider invasion (115). The roles of microbes in ensuring optimal brain development and functioning have been reported (86, 87, 90,103,1116). Also, current research is beginning to report the importance of the microbiota in modulating host behaviors (Table 3).

Studies in germ-free or antibiotic-treated fruit-fly (*Drosophila melanogaster*) showed hyperlocomotion and increased walking speed, which was reversed by mono-colonization with the fruit-fly commensal *Lactobacillus brevis* (117). In another study by Akami *et al* (118), increase in foraging behaviors of Oriental flies (*Bactrocera dorsalis*) was determined by the intestinal microbiota consisting mainly of members of the *Enterobacteriaceae* family (118). In mammals, the importance of the microbiota in modulating dietary behaviors and metabolic profile been reported. In a study in which cultured and uncultured human fecal microbiota were transferred from weight-discordant twins (obese and lean) to germ-free mice, it was observed that the germ-free mice colonized with obese fecal microbiota showed significantly-higher weight increases compared to mice colonized with microbiota from the lean twin (66). Also, co-habitation of these two mouse groups was associated with a transformation of the metabolic profile of the obese microbiota mouse groups to a lean-like state (66). There have been reports associating obesity and related dysmetabolism with an increase in the incidence of mood disorders, depression and cognitive decline (119-120). The possibility of replicating these features in murine models of obesity (121) has allowed researchers to examine the role of diet and dysmetabolism on neurobehavior (122-123). Bruce-Keller and colleagues (122) reported that mice colonized with high-fat diet microbiota (with a preponderance of bacteria from the phylum *Firmicutes* and a depletion of *Bacteroidetes*) had alteration in exploratory activity, cognition and stereotypic behavior compared to controls (122).

Diaz Heijtz *et al.* (86) reported increased locomotor activity and rearing behaviours in germ-free mice, while Desbonnet *et al* (104) reported evidence of increased self-grooming and alteration in social interaction evidenced by avoidance of

Neurobehavioral impact of the microbiome

Table 3. Neurobehavioral phenotypes and the microbiota

Model	Microbial composition/procedure	Behavior	Phenotype	reference
<i>Drosophila melanogaster</i>	Germ-free(GF) antibiotic-treated flies	Motor	↑locomotion ↑walking speed	(117)
<i>Drosophila melanogaster</i>	<i>Lactobacillus brevis</i>	Motor	Reversal of ↑locomotion ↑walking speed	(117)
<i>Bactrocera dorsalis</i>	Enterobactereacea	Foraging behavior	Basal Increase in foraging	(118)
Mice	High-fat diet microbiota	Exploratory activity, cognition	↑Locomotor activity, and ↓cognition	(122)
Mice	GF	Locomotor activity	↑Locomotor and rearing	(86)
Mice	GF	Stereotypy, social interaction	↑grooming, ↓social investigation, avoidance of conspecifics,	(104)
Mice	GF	Memory	↓short term recognition memory and working memory	124
Mice	Oral antibiotic depletion of microbiota	Exploratory activity	↑exploratory activity	(84)
Mice	GF	anxiety	↓Basal anxiety	(87, 102)
Mice	GF	depression	↓ time spent immobile	(125)
Mice	<i>Lactobacillus rhamnosus</i>	Anxiety, depression	Increased open-arm time and decreased immobility time	(128)
Mice	Colonization with <i>Citrobacter rodentum</i>	cognition	↑Stress-induced memory loss	(124)
Human	Age-associated dysbiosis (↑Bacteroidetes ↓Firmicutes)	cognition	↓cognition	(126)
Humans	↑Alcaligenaceae and Porphyromonadaceae, Veillonellaceae in cirrhotics with hepatic encephalopathy	cognition	Memory loss	(127)
Mice	Maternal high-fat diet-induced microbiota low in	Social interaction	↓social interaction, ↓preference for social novelty	(132)
Mice	<i>Lactobacillus reuteri</i>	Social interaction	↑social interaction,	(132)
Rat	Peri-conceptual use of antibiotic	Social interaction, anxiety	↓social interaction, ↑anxiety	(133)
Mice	Use of antibiotics to deplete microbiota during adolescence	Social memory	↓social memory in adulthood	(106)
Mice	<i>Bacteroides fragilis</i>	Cognition, social interaction and anxiety	↑social interactions, ↓stereotypy, ↓anxiety and ↓sensorimotor deficits	(3)
Rat	<i>Bifidobacterium infantis</i>	Depression	decreased immobility time	(92)
Mice	<i>Bifidobacterium breve</i>	Cognition	↑cognitive abilities	(135)
Rats	<i>Lactobacillus helveticus</i> and <i>Bifidobacterium longus</i>	anxiety	↓anxiety in the conditioned defensive burying test	(136)
Mice	Prebiotic administration of human milk oligosaccharides 3'Sialyllactose or 6'Sialyllactose	anxiety	↓stress-induced anxiety	(137)
Human	<i>Lactobacillus casei</i>	Anxiety and depressive-like behaviors	↓anxiety and depressive-like behaviours	(138,139)

contd...

Table 3. Contd...

Model	Microbial composition/procedure	Behavior	Phenotype	reference
Human	Bifidobacterium longum and Lactobacillus helveticus	Psychological stress indices	↓ somatization, anger, anxiety and depression	(136)
Human	Administration of prebiotic trans-galactooligosaccharide to irritable bowel syndrome sufferers	anxiety	↓ anxiety	(140)

conspecifics, decreased preferences for novel conspecifics, and decreased time spent in social investigation (104). In another study using germ-free mice Gareau et al (124) reported impairment of short-term recognition memory and working memory (124), while Zheng et al (125) reported antidepressant-like response (125). Gareau et al (124) also observed that colonization of mice with pathogenic bacteria *Citrobacter rodentum* was associated with accentuation of stress-induced memory loss (124).

In humans, there have been reports of a loss of microbial diversity with aging (with a predominance of *Bacteroidetes* and a decrease in *Firmicutes*) and the presence of an age-associated cognitive decline in the same age group (126). In a study examining gut microbial diversity of cirrhotics with or without hepatic encephalopathy (HE), it was reported that (compared to healthy controls), cirrhotics had a preponderance of commensals from the phyla *Enterobacteriaceae*, *Alcaligenaceae*, and *Fusobacteriaceae*, with low concentrations of *Lachnospiraceae* and *Ruminococcaceae* (127). However, in cirrhotics (with HE) a higher concentration of *Alcaligenaceae* and *Porphyromonadaceae* *Veillonellaceae* was observed, which correlated positively with cognitive-deficits, high levels of pro-inflammatory markers (TNF- α , IL-2, IL-6 and IL-13) and endotoxemia (127).

Oral antibiotics-induced microbiota depletion in mice have also been reported to transiently alter the microbial composition of the gut, resulting in an increase in exploratory behavior and an increase in the hippocampal expression of brain-derived neurotropic factor (84). These changes did not occur with intraperitoneal administration of the antibiotics (84). A few studies have also reported that in germ-free mice, the basal behavior was anxiolysis (86, 102) compared to the expected aversion for

open spaces (a protective feature) in mice with normal gut microbiota. In the germ-free mice, this alteration in anxiety-related behaviors was accompanied by an increase in the expression of brain derived neurotropic factor (BDNF) in the hippocampus (dentate gyrus), a decrease in the expression N-methyl-D-aspartate receptor subunit in the central amygdala, and a decrease in serotonin receptor expression by the dentate gyrus region of the hippocampus (102). In another study, Bravo et al (128) reported that long-term treatment of healthy mice with *Lactobacillus rhamnosus* probiotic was associated with anxiolysis in the elevated plus-maze and decreased immobility time in the forced swim test; there was also a significant decrease in the levels of stress-induced corticosterone levels and region-specific increase in the expression of γ -amino butyric acid receptor mRNA. These features were however abolished with vagotomy (128), suggesting that these effects were vagus nerve dependent.

Currently, the role of the gut microbiota in social interaction and the development of social behavior has been studied extensively (123,129–132), using either germ free mice, or following colonisation with probiotic (132). Peri-conceptual exposure to antibiotics has also been shown to alter social behavior and induce anxiety (133). The use of antibiotics to deplete mouse gut microbiota during adolescence was associated with impairment of social memory in adulthood (106), features which corresponded with a reduction in mRNA levels of vasopressin and oxytocin in the mouse hypothalamus (106). In general, alterations in social memory and neurobehavior could also be attributed to the ability of gut microbiota to modulate myelination of neurons in the prefrontal cortex (134). Hoban *et al.* (134) also reported evidence of abnormal hypermyelination of axons in male germ-free mice (134). Studies in the mouse model of

autism have also revealed that oral treatments with *Bacteroides fragilis* were associated with improvements in social interactions, stereotypic behaviors, anxiety-related behaviors and sensorimotor deficits (3).

The role of the microbiota in the modulation of neurobehavior and/or brain neurochemistry has also been demonstrated by studies using prebiotics or probiotic interventions. In rodents, probiotic intervention with *Bacteroides infantis* was associated with decreased immobility time in the forced swim paradigm (92). Buffington et al. (132) demonstrated that a maternal diet that is high in fat caused microbiota dysbiosis, deficits in social interaction, preference for social novelty and a decrease in the neuronal oxytocin immunoreactivity in the paraventricular nucleus of the hypothalamus in the maternal high-fat diet offspring, compared to normal diet fed controls. However, there was restoration of behavioral deficits, microbiota dysbiosis and oxytocin immunoreactivity following reconstitution with *Lactobacillus reuteri* probiotic (132), demonstrating that these deficits were possibly due to low-concentrations of *Lactobacillus reuteri* in mouse pups (132). Colonization with specific members of the *Bifidobacterium breve* species have been associated with alteration of the fatty-acid composition of the brain in favor of fatty-acids that improve cognitive abilities (135). Also, in rats, probiotic formulations of *Lactobacillus helveticus* and *Bifidobacterium longus* were associated with a decrease in anxiety-related behaviors measured in the conditioned defensive burying test (136). The use of prebiotic intervention to promote selective proliferation of commensal bacteria has also been reported to modulate neurobehavior. Tarr et al (137) reported that a diet rich in human milk oligosaccharides 3'Sialyllactose or 6'Sialyllactose reduced stress-induced anxiety-like behavior in the open field and light/dark preference paradigms (137).

In humans, the benefits of probiotic intervention on the modulation of behavior have also been demonstrated. In healthy adults with baseline depressive symptoms or patients with chronic fatigue syndrome, the probiotic administration of *Lactobacillus casei* was associated with improvement in symptoms of depression and anxiety

respectively (138-139). Probiotic formulations of *Bifidobacterium longum* and *Lactobacillus helveticus* administered to otherwise healthy subjects was also associated with a decrease in psychological distress including somatization, anger, anxiety and depression, using relevant clinical indices (136). Administration of prebiotic trans-galactooligosaccharide to irritable bowel syndrome sufferers was associated with significant reduction in anxiety and also an increase in the concentration of bifidobacteria in fecal samples (140).

A number of other studies have also reported the possible influences behavioral modification can exert on microbial density or diversity (141-142). Exposure to social disruption stress in rodents was associated with a decrease in the relative abundance of bacteria of the genus *Bacteroides*, and an increase in those belonging to the genus *Clostridium*. There was also an associated increase in the circulating levels of interleukin 6 (IL-6) and monocyte chemo-attractant protein (MCP)-1 (142). Increased levels of IL-6 and MCP-1 have also been observed to correlate positively with increased concentration of bacteria from 3 genera including *Coprococcus* spp, *Pseudobutyrvibrio* sp, and *Dorea* spp (142). In mice exposed to chronic restraint stress, a significant increase in *Citrobacter rodentium* colonization with an associated increase in the gene expression for tumor necrosis factor alpha in colonic tissue has been demonstrated (141). Accumulating evidence of the ability of gut microbiota to influence neurobehaviour and the alteration of microbiota diversity and density due to influences from neurobehavioural modifications further confirm the possible existence of a bidirectional communication that allows brain signals to influence sensory, motor and secretory functions of the gut, while visceral messages from the gut can in turn influence neurobehavior, social behavior and brain chemistry (123).

Also the relationships that link the gut microbiome, skin microbiome, and the brain could be crucial in understanding the contributions of bacteria to the development of odor cues, which is known to contain important information about the host's social relationships and social behaviors. Studies have shown that the intensity of the human odor and/or its

chemical composition is closely-linked to the skin's microbial diversity and composition (143–145). The interaction of the skin microbiota, the sweat glands, and the brain is important in the creation of human odors that allows kin recognition of kin, the detection of fear in animal prey, and sexual behaviors in animals (146). There have been reports suggesting that *Staphylococcus epidermidis* which is part of the normal skin microbial flora has the ability to degrade leucine content of sweat causing foot odor. Also, colonization of the plantar skin by *Bacillus subtilis* has been associated with strong foot odor (143). Absence of axillary odor has also been linked to paucity of gram positive bacteria (145).

3.2.1. Signaling pathways/mediators and molecular alterations involved in behavior/microbiota interactions

The communication between the gut microbiota and the brain affects behavior. This communication occurs via neural, immune and endocrine pathways that connect the microbiota to the central nervous system (147). The interactions (Table 4) between microbiota and brain alter neurobehavior, signal pathways, neurotransmitters and hormones (Figure 1); it also modulates the expression of receptor mRNA in different regions of the brain (86, 90, 102, 124, 147).

Neural pathways involve efferents from the central nervous system to the gut, and afferents from the gut to the central nervous system. Efferent signals that are conducted from the central nervous system to the gut modulate gut motility, secretion, and permeability, which in turn affect microbiota composition. The efferent pathway relies on neurotransmitters such as acetylcholine. The afferent signals are transmitted from the gut to the central nervous system via the vagus nerve which is capable of recognizing microbial products and cellular components. The changes that had been observed in the brain include region-dependent alterations in levels of gamma-aminobutyric acid (GABA) mRNA gene expression (128). Bravo et al (128) reported that colonization with *Lactobacillus rhamnosus* was associated with a decrease in the expression of GABA_{B1b} mRNA in the amygdala, hippocampus and locus coeruleus; with an increase in the prefrontal cortex and cingulate cortex. The expression of

GABA_{Aα2} mRNA was decreased in the cortical region, and increased in the hippocampus/amygdala (128). The role of GABA in maintaining central excitation/inhibition balance has been reported (148). Studies examining the impact of gut microbiota on neurotransmitters concentration in germ-free mice have reported that hippocampal concentrations of serotonin and 5-hydroxyindoleacetic acid (metabolite of serotonin) were elevated compared to control (103); while a decrease in the expression of the serotonin receptor mRNA was observed in the dentate gyrus (102). The expression of brain derived neurotrophic factor (BDNF) increased in the dentate gyrus (102), and decreased in the cornu ammonis (CA) 1 region of the hippocampus, amygdala and cingulate cortex (86, 90, 124). While the content and expression of BDNF is modulated by the gut microbiota, brain derived neurotrophic factor itself regulates neurogenesis, supporting the result of a recent study that demonstrated increased hippocampal neurogenesis in adult germ-free mice (149). There was also reduced expression of different subunits of the N-methyl-D-aspartate receptor; the NR1 in the cortex (90), NR2a in the cortex and hippocampus (90) and NR2b in the central amygdala (102). Diaz Heijtz et al (86) reported a decrease in the expression of nerve growth factor-inducible clone A and dopamine receptor in the striatum, and an increase expression of dopamine receptor in the hippocampus of germ-free mice (86).

Antibiotic-induced depletion of gut microbiota was associated with increased expression of BDNF in the hippocampus (84). Matsumoto et al (150) also reported increase in dopamine concentration in the cerebral cortex of germ-free mice compared to mice colonized by normal microbial flora (150), which supports the increased exploratory activity reported by previous studies (86, 102). Colonization of mice with non-invasive parasite *Trichuris muri* was associated with colonic inflammation and anxiety-like behavior, while *in-situ* hybridization revealed a decrease in hippocampal expression of BDNF mRNA (151). Gareau et al (124) also reported decreased BDNF expression in mice colonized with *Citrobacter rodentium* following induction of stress (124). Intervention with probiotic *Bifidobacterium infantis* was associated with a decrease in the frontal cortex concentration of 5–

Table 4. Microbiota-mediated neurochemical and molecular alterations

Model	Microbial Composition/Mediators	Brain region/tissue	Neurochemical/Molecular alteration	Reference
Mice	<i>Lactobacillus rhamnosus</i>	Hippocampus, amygdala, LC Prefrontal cortex, cingulate gyrus	↑ GABA _{Aα2} mRNA ↓ GABA _{B1b} mRNA ↓ GABA _{Aα2} mRNA ↑ GABA _{B1b} mRNA	(128)
Mice	GF	Hippocampus	↑ serotonin and 5-hydroxyindoleacetic	(103)
Mice	GF	Dentate gyrus	↓ serotonin receptor mRNA	(102)
Mice	GF	Dentate gyrus Cornu ammonis 1 region of the hippocampus, amygdala and cingulate cortex Hippocampus	↓BDNF ↑BDNF ↑neurogenesis	(102) (86, 90, 124) (149)
Mice	GF	Cortex Cortex and hippocampus Central amygdala	↓ N-methyl-D-aspartate receptor; the NR1 ↓NR2a ↓NR2b	(90) (90) (102)
Mice	GF	Striatum Hippocampus	↓ nerve growth factor-inducible clone A ↓Gopamine receptor ↑dopamine receptor	(86) (86)
Mice	GF	Cerebral cortex	↑Dopamine	(150)
Mice	Antibiotic-induced depletion of microbiota	Hippocampus	↑BDNF	(84)
Mice	<i>Trichuris muri</i>	Hippocampus	↓ BDNF mRNA	(151)
Mice	<i>Citrobacter rodentum</i>	CA1 region of the hippocampus	↓BDNF expression	(124)
Mice	<i>Bifidobacterium infantis</i>	Frontal cortex Amygdala Paraventricular nucleus	↓5-hydroxyl indole acetic acid ↓3,4-Dihydroxyphenylacetic acid ↓cFos expression	(92) (90)
Mice	GF	Hypothalamus	↑corticotropin-releasing factor gene expression and protein levels ↑Plasma ACTH	(90)
Rats	<i>Bifidobacterium pseudocatenulatum</i> <i>Bifidobacterium animalis</i> subsp <i>lactis</i> and <i>Propionibacterium jensenii</i>	Hypothalamus	↓dopamine and adrenaline ↑ACTH (reversal of maternal-separation stress induced derangements) reversal of maternal-separation stress induced biochemical derangements and microbial density	(157) (155)
Rats	IBS microbiota	Colonic	colonic genes involved in glucocorticoid receptor signaling	(156).
Mice	GF or mice deficient of SCFAs receptor FFAR2a (GPR43)	Microglia	↓ <i>Fcgr2β</i> , <i>Mapk8</i> , <i>IL-1α</i> , and <i>Cd86</i> ., Janus kinase 3 and the signal transducer and activator of transcription 1 (<i>Stat1</i> ↑ <i>Nfkbia</i>) and the central microglia transcription and survival factors (<i>Sipi1</i> and <i>Csf1r</i>)	(166)
Mice	GF	Hippocampus, hypothalamus	↑Apoptosis,	(153)
Mice	GF	Prefrontal cortex	Hypermyelination, overexpression of myelin-related genes	(134)

hydroxyl indole acetic acid and amygdaloid cortex concentration of 3, 4-Dihydroxyphenylacetic acid, metabolites of serotonin and dopamine respectively (92); and a decrease in the expression of cFos in the

paraventricular nucleus (92), neurotransmitter modulations that could be related to the antidepressant effects observed in the forced swim test. A number of these studies confirm previous

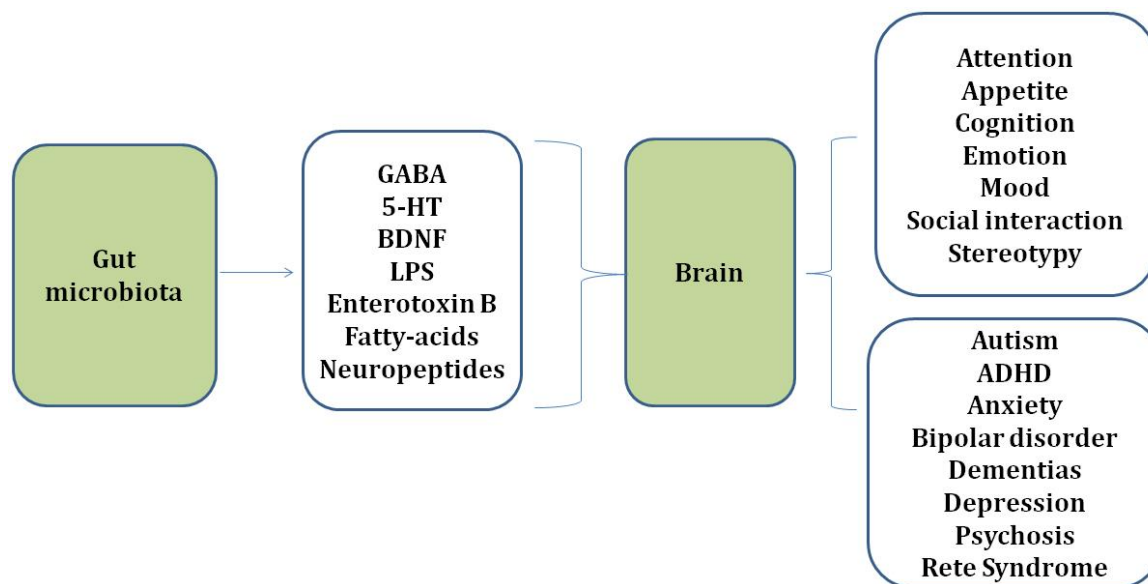


Figure 1. Showing how the gut microbiota via neural, homonal or endocrine mechanisms modulates brain function and chemistry.

reports associating hippocampal depletion of neurotransmitters with the development of depression and depressive-like symptoms (152). Neuronal apoptosis has also been linked to the gut microbiota. In the hypothalamus and some regions of the hippocampus, increased apoptosis was observed in neonatal germ-free mice when compared with control mice, this was also accompanied by increase microbial count and density (153). Neuron myelination and myelin plasticity have also been reported to be impacted by the gut microbiota; in germ-free mice, evidence of hypermyelination of the axons in the prefrontal cortex as well as over-expression of myelin-related genes have been reported (134).

The endocrine pathway involves the hypothalamo-pituitary-adrenal (HPA) axis, and the secretion of hormonally-active peptides such as peptide YY. Changes in the HPA axis response can be triggered by impulses originating from the brain (e.g. due to stress), or from the gut microbes who can positively or negatively regulate the HPA axis (90). In germ-free mice, restraint stress has been observed to cause increased HPA response, leading to an elevation of hypothalamic corticotropin-releasing factor gene expression/protein levels, a decrease in cortical/hippocampal brain-derived neurotrophic factor, and an elevation of plasma adrenocorticotrophic

hormone (ACTH)/corticosterone (90). However, colonization by specific microbiota at certain periods in their life can either normalize their HPA response to stress, or make them to continue to show exaggerated responses (90). This influence has been suggested to profoundly impact behaviors like anxiety, attention, and certain aspects of memory.

Probiotic intervention (from postnatal day 2 to 21) with a strain of *Bifidobacterium pseudocatenulatum* reversed neuroendocrine and neurobehavioural changes induced by maternal deprivation induced stress (154). The neuroendocrine response included an attenuation of stress-induced increase in corticosterone levels, and catecholamine levels in the hypothalamus. A down-regulation of maternal-deprivation stress-induced increase in intestinal levels of interferon gamma and catecholamine (dopamine and adrenaline) was also demonstrated (154).

Maternal probiotic supplementation with *Bifidobacterium animalis* subsp *lactis* and *Propionibacterium jensenii* has also been shown to reverse the changes induced by maternal-separation stress or the adult-stress protocol on biochemical parameters and microbial density (155). In rats pups exposed to maternal-separation stress, an increase

in plasma levels of ACTH and fecal concentration of enterococci, *Escherichia coli*, and clostridia has been shown. In adult animals exposed to stress, an increase in both ACTH and corticosterone levels was observed; there was also a decrease in the density of Bifidobacteria, and an increase in *Escherichia coli* and *Bacteroides* (155). Transplantation of fecal microbiota from patients with irritable bowel syndrome into germ free mice has also associated with anxiety-related behavior, increased gastrointestinal transit time, alteration in the intestinal barrier (156). There was also increased expression of colonic genes involved in glucocorticoid receptor signaling (156).

In line with the bidirectional communication that occurs in the gut-brain axis, the neurons of the autonomic nervous system and neuroendocrine mediators carry outputs from the brain to the gut. Biologically active peptides including peptide YY (PYY), neuropeptide Y and pancreatic polypeptide are expressed at distinct levels of the gut-brain axis (157). Peptide YY and pancreatic polypeptide are expressed exclusively by the endocrine cells of the digestive system, while NPY is expressed at all levels of the gut-brain and brain-gut axis (157). PYY is synthesized in the gut by enteroendocrine cells with the G protein-coupled receptor FFAR3 (Gpr41) which sense dietary proteins/fats, and microbial-derived short-chain fatty acids (158). The PYY that is transported to the brain affects feeding behavior, while in the GIT, it triggers satiety, reduces food intake, and slows the GI motility (158).

The immune pathway involves the influence of microbial antigens and metabolites on the immune system, which in turn affects the central nervous system. It is known that specific microbes and their products can use different mechanisms to influence the development and functions of particular subsets of immune cells (159). Microbial antigens might trigger the immune system and contribute to the pathogenesis of neurodegenerative disorders such as multiple sclerosis which has profound behavioral components (160-161). On the other hand, the microbial metabolites that affect immune function include chemical mediators such as small chain fatty-acids (acetic acid, propionic acid and butyric acid) and tryptophan metabolites. The short

chain fatty acids (SCFAs) are mainly produced by large-intestinal bacterial fermentation of the complex carbohydrates. They modulate gut motility, enhance intestinal epithelium integrity, increase mucus production, promote regulatory T-cells, and inactivate nuclear factor kappa B (162-164). In the gastrointestinal system, SCFAs induce the release of neuropeptides and hormones including glucagon-like peptide 1 and PYY from intestinal enteroendocrine cells (165), via the activation of the G protein-coupled receptors (GPRs; GPR41, GPR43, and GPR109A), and also by acting as epigenetic regulators. SCFAs modulate gene expression through the inhibition of histone deacetylases.

In the central nervous system, SCFAs are important in the modulation of microglial maturation and homeostasis (166). The microglia in germ-free mice exhibited global defects including altered cell size, immature phenotypes and impaired innate immune response. These features were also evident in mice deficient of SCFAs receptor FFAR2a (GPR43) (166). Genome-wide mRNA studies revealed significant differences in mRNA profiles of microglia genes of germ-free compared to specific-pathogen (SPF) mice (166). A total of 198 genes were observed to be downregulated while about 173 genes were unregulated in microglia from germ-free mice, compared controls (166). A number of the downregulated genes were genes that had been linked to cell activation including *Fcgr2b*, *Mapk8*, *IL-1 α* , and *Cd86*. Also diminished were genes such as Janus kinase 3 and the signal transducer and activator of transcription 1 (*Stat1*), which are linked to signaling of type I IFN receptors (166). Upregulated genes in the germ-free mice microglia include a number of genes responsible for the inhibition of transcription (*Nfkbia*) and the central microglia transcription and survival factors (*Sfpi1* and *Csf1r*) (166), which are usually downregulated in microglia (167-168).

Bacterial populations in the gut have also been reported to be capable of producing hormones and/or neurotransmitters similar to that produced by humans (169). Members of the *Bifidobacterium* species produce gamma-amino butyrate (GABA), *Lactobacillus* species secrete acetylcholine and GABA, while *Escherichia* produce norepinephrine,

Table 5. Brain disorders and alterations in microbiota density

Disorder/Behavioural alterations	Host	Microbial composition	Reference
Autism spectrum disorder	Humans	↑Clostridia, Bacteroidetes, <i>Bacteroides vulgates</i> , <i>Desulfovibrio</i> species, Proteobacteria ↓ Actinobacteria and Firmicutes ↓microbial diversity ↓ <i>Coprococcus</i> , <i>Prevotella</i> and <i>Veillonellaceae</i> species	(178,183–185)
Autism spectrum disorder	Murine	↑Bacteroidetes, ↓ Firmicutes	(186)
Attention deficit hyperactivity disorder	Human	↑ <i>Bifidobacterium</i>	(187)
Rete syndrome	Human	↑ <i>Bifidobacterium</i> , <i>Actinomyces</i> , Clostridia <i>Enterococcus</i> , <i>Lactobacillus</i> , <i>Escherichia</i> , and <i>Shigella</i> species	(180)
Major depressive disorder	Human	↑Bacteroidetes, ↓ <i>Lachnospiraceae</i>	(188).
Major depressive disorder	Human	↑ Proteobacteria, Bacteroidetes and Actinobacteria	(189)
Major depressive disorder	Human	↑ Actinobacteria ↓ Bacteroidetes	(125)
Parkinson's disease	Human	↓ <i>Enterobacteria</i> and <i>Prevotella</i> species	(179)
Alzheimer's disease	Murine	↓ <i>Akkermansia</i> and <i>Allobaculum</i> ↑ <i>Rikenellaceae</i>	(182)
Anxiety, anhedonia and depressivelike behaviour	Rat	Fecal transplantation of microbiota from depressed patients	(125, 190)
ASD: Autism spectrum disorders, ADHD: Attention deficit Hyperactivity disorder, MDD: Major depressive disorder, PD: Parkinson's disease, AD: Alzheimer's disease.			

dopamine and serotonin. Serotonin is also produced by the *Streptococcus* and *Enterococcus* spp, while dopamine and norepinephrine is produced by members of the *Bacillus* species. These neurotransmitters that are produced by microbes could also impact neurobehaviour (169-170).

3.3. Brain disorders and the microbiota

The microbiota contributes to the maintenance of the central nervous system structure and function through direct interactions between the enteric nervous system and the gut microbiota (171-172), or indirectly through the modulation of endocrine, immunological, and neural pathways (173). Gut microbiome dysbiosis has been associated with the development of neuropsychiatric disorders, including bipolar disorder, anxiety disorder, schizophrenia, autism, chronic fatigue syndrome, major depressive disorder and stress (174–176). Neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, and stroke have also been linked to microbiome dysbiosis (177).

A number of studies (Table 5) have demonstrated the presence of alterations in specific

microbes (or their metabolites) with the occurrence of central nervous system disorders (55,178–182). Following the examination of fecal samples of children with autism, Finegold and colleagues (183-184) reported low levels of microbes within the phyla *Actinobacteria* and *Firmicutes*, and an increase in the concentration of *Proteobacteria* and *Bacteroidetes*, when compared to healthy controls (183-184). The presence of high loads of *Bacteroidetes*, *Bacteroides vulgatus*, and *Desulfovibrio* species were observed in the feces of severely- autistic children (183). Song *et al.* (178) reported an abundance of *Clostridia* in children with autism who had gastrointestinal disorders (178), while Kang *et al* (185) reported evidence of a decrease in microbial diversity and the presence of lower levels of *Coprococcus*, *Prevotella*, and *Veillonellaceae* in stool samples of autistic children (185). In the valproic acid model of autism in rodents, alterations in the concentrations of *Firmicutes* and *Bacteroidetes* occurred alongside autism-like social behaviors (186). In children with attention deficit hyperactivity disorder, an increase in fecal *Bifidobacterium* has also been reported (187). In Rete syndrome, alteration of the gut microbiota has also been described; and examination of stool samples from patients revealed a decrease in

microbial diversity and an increase in levels of *Bifidobacterium*, *Bacteroidaceae*, *Actinomyces*, *Clostridium*, *Enterococcus*, *Lactobacillus*, *Escherichia*, and *Shigella* species (180). In patients with major depressive disorder (MDD), there have been reports suggesting a preponderance of Bacteroidetes phyla and a decrease in Lachnospiraceae (188). Jiang et al. (189) reported an increase in the concentration of Proteobacteria, Bacteroidetes and Actinobacteria in depressed patients; while Zheng et al (125) reported a relative abundance of Actinobacteria and a decrease in Bacteroidetes in patients with MDD compared to healthy controls (125). A cause-effect relationship has also been demonstrated between the microbiota dysbiosis and mood disorders, using fecal transplantation studies (125, 190). Transplantation of fecal microbiota from depressed patients to rodents was associated with the induction of depression-like behavior (125). Anxiety and anhedonia were also reported in rats colonized with fecal microbiota from depressed patients (190).

A number of neurodegenerative disorders have been associated with microbiota dysbiosis. In Parkinson's disease, it had been suggested that the pathogenesis may have actually arisen from the abnormalities in the gut before spreading via the gut-brain axis to the brain (191). However, what is generally obvious is increasing evidence in support of the existence of a brain-gut interaction (107,110), and possibly the involvement of the brain-skin cross talk (192) in the pathophysiology of a number of neurodegenerative disorders. Examination of fecal samples from Parkinson's disease patients revealed a decrease in the population of *Enterobacteria* and *Prevotella* species (179). The transfer of microbiota from new-onset, treatment-naïve PD patients to groups of germ-free mice has also been shown to lead to worsening motor deficits (193). In Alzheimer's disease, an association has been established between the presence of beta-amyloid plaques and microbial dysbiosis (182). Alzheimer's disease has also been associated with alterations in microbial diversity and population. Studies using transgenic mice revealed Alzheimer's disease-induced alteration of microbial diversity, with alterations in the populations of *Bacteroidetes* and *Firmicutes*; specifically lowering the population of *Akkermansia*

and *Allobaculum*, and increasing the population of *Rikenellaceae* (182).

In recent times, the brain-skin crosstalk has been proposed, as reported by studies that have demonstrated over-activation of neuropeptides in skin disorders like psoriasis and atopic dermatitis; and revealed skin conditions related to neuroimmunological stress (194–197). Mijouin *et al.* (113) also reported that exposure of the skin microbe *Bacillus cereus* to substance P was associated with increased cytotoxicity, increased caspase-1 activity, and morphological changes in the actin cytoskeleton (113). In neurodegenerative disorders like Parkinson's disease and Alzheimer's disease, direct alteration of the skin microbial density or diversity is rarely reported. Instead, the evidence of skin involvement would include an increase in melanoma and non-melanoma skin cancers in Parkinson's disease (198), or altered skin physiology in Alzheimer's disease (199). However, the precise role of the skin microbiome in all these is still a subject of research.

3.4. Behavioral disorders, the microbiota, and future therapeutic implications

Based on available evidence, it is easy to deduce that manipulation of the microbiota may be a therapeutic tool in the management or prevention of behavioural disorders. Along this line, the study of how this knowledge can be used specifically for the management of behavioral disorders is an advancing field that holds a lot of promise. Human behavioral disorders that have been linked to microbiota dysbiosis include ADHD, ASD, schizophrenia, bipolar disorders, major depression, anxiety disorders, obsessive-compulsive disorder, eating disorders; and specific neurological disorders such as AD, PD, and MS (200). However, beyond the recognition or identification of a link, the questions is to what extent can manipulation of the microbiome be used in the therapy of these disorders, and what the future holds if therapy is steered in this direction. Also, while the global market for agents that may be of benefit in the prevention or management of dysbiosis continues to expand, the extent to which they may be used in the clinical management of behavioral disorders remains largely unknown. Again, the multifactorial origin of a

number of disorders implies that targeting the microbiome alone is not likely to be enough for therapy. However, some scientists are in support of the idea of targeting the microbiota as novel therapeutic options in the management of neuropsychiatric and neurodegenerative diseases. Their support for the exploration of this novel approach to therapy has been backed by a number of theories, including: a) the “old friend” hypothesis, b) the gut microbiota hypothesis, and c) the leaky-gut theory.

The old friend theory suggests that a symbiotic relationship has existed between man and microbes from time immemorial (201), this relationship is crucial to the maintenance of the balance of nature as well as the health and well-being of humans. The transformations that have occurred as a result of modernization (leading to changes in lifestyle, diet and healthcare) have greatly reduced man’s exposure to microbes, which has in turn altered man’s immune system and brain development (202). The gut microbiota theory of brain disorders initially arose from the results of a 2002 study that demonstrated resistance to disease and improvement in health and brain function following the feeding of *Lactobacillus*–fermented fodder to pigs; and subsequently from accumulating evidence linking the gut microbiota to neurobehavioural dysfunction and the development of mood disorders and mental illness (202-203). The leaky gut theory is based on the hypothesis that the human body has two main barriers, the blood brain barrier (BBB) and the gut barrier. The gut barrier regulates the movement of nutrient and signal molecules into the body, and prevents the entry of microorganisms. However, the BBB with the aid of tight junctions regulates the entry and exit of molecules in central nervous system. The maintenance of the integrity of these barriers is critical to health and wellbeing (204-205). The importance of the gut microbiota in the development of the BBB was demonstrated by Braniste et al. (206), when they reported that in germ-free mice an increase in the permeability of the BBB was observed when compared to mice with normal gut flora. This was observed to persist to adulthood and was also associated with a decrease in the expression of tight junction proteins (occludin and

claudin-5) (206).

These theories portray the important role the microbiota–gut-brain axis could potentially play in the prevention of and development of drugs for the management of brain disorders; also, accumulating preclinical evidence continues to support these views. A number of methods directed at improving the microbiota such as the transplantation of fecal microbiota, and the use of probiotics or prebiotics have been shown to have significant benefits in health and disease. In children with ASD, seven-to-eight weeks of microbiota transfer therapy altered the gut microbiome and improved both gastrointestinal and behavioral symptoms (207). These improvements were associated with increased overall bacterial diversity and an increase in the abundance of *Bifidobacterium*, *Prevotella*, and *Desulfovibrio* species (207). Also, several systematic reviews have indicated that probiotics can effectively improve mood in humans (208-209), offering promise in the management of depression. In a clinical trial involving patients with mania, 24 weeks of adjunctive probiotics (using *Lactobacillus rhamnosus* strain GG and *Bifidobacterium animalis* subsp. *lactis* strain Bb12) were shown to prevent psychiatric re-hospitalizations after discharge, especially in individuals with elevated levels of baseline systemic inflammation (210).

From the foregoing, while it is becoming increasingly-evident that manipulations of the microbiome can be clinically-beneficial for a limited number of behavioral disorders, for the vast majority, research is yet to substantiate their benefit in humans. Therefore, more effort is needed towards conducting research in this area, especially, considering the fact that orthodox medications have not proven to be fully-satisfactory in the management of a number of such disorders.

4. CONCLUSION

The increase in the knowledge of the microbiota and how they influence brain development and behavior has revolutionized our perception of the microbes that live in and on our bodies. Presently, we are aware that the brain and the gut microbiota can communicate with each other via neural, endocrine

and immunological pathways; and that these communications might be important in the pathogenesis of neurological and behavioral disorders. Also, a number of chemical mediators that act as messengers along such pathways are already being identified. However, while novel therapies that target these pathways are being investigated, only limited progress has been made in the area of microbiome manipulation for the prevention and management of behavioral/neurological disorders. As more resources are dedicated to research in this area, the coming years are likely to yield more interesting findings and possible clinical applications that might revolutionize the management of behavioral and neurological disorders.

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