

Gut brain axis: diet microbiota interactions and implications for modulation of anxiety and depression

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The human gut microbiome is composed of an enormous number of microorganisms, generally regarded as commensal bacteria. Without this inherent microbial community, we would be unable to digest plant polysaccharides and would have trouble extracting lipids from our diet. Resident gut bacteria are an important contributor to healthy metabolism and there is significant evidence linking gut microbiota and metabolic disorders such as obesity and diabetes. In the past few years, neuroscience research has demonstrated the importance of microbiota in the development of brain systems that are vital to both stress reactivity and stress-related behaviours. Here we review recent literature that examines the impact of diet-induced changes in the microbiota on stress-related behaviours including anxiety and depression.

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Introduction

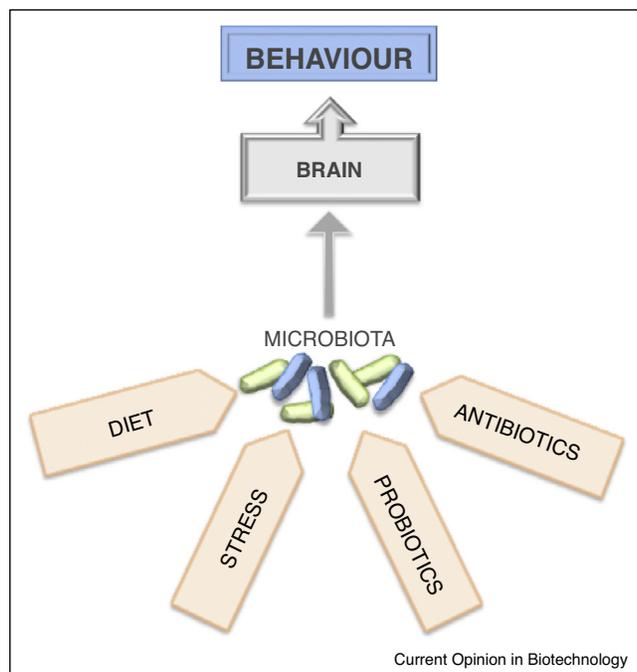
Diet and diet-related changes in gut microbiota influence the gut-brain axis and may in turn influence behaviours including anxiety and depression. A link between gut microbiota and anxiety-related behaviours has recently been established in mice [1–3]. Interestingly, a link between consumption of probiotic bacteria in fermented milk was also shown to influence brain activity in emotional centers in healthy individuals [4**]. This review will cover the latest literature related to microbiota and behaviour, diet-related mechanisms that may influence brain function and behaviour, and implications for modulation of anxiety and depression (Figure 1).

Gut microbiota and behaviour

In the past few years, a link between gut microbiota and stress-related behaviours has emerged in animal studies (see Table 1 for summary). The first experiments followed on the observation that germ free (GF) mice showed enhanced stress-reactivity [5] and sought to determine if this was associated with changes in anxiety-like behaviours. Surprisingly, the results revealed that GF mice showed reduced anxiety-like behaviour in the elevated plus maze (EPM), a well established behavioural test that examines approach and avoidance behaviour in mice, in comparison to specific pathogen free (SPF) mice. The low anxiety-like phenotype was accompanied by long-term changes in plasticity-related genes in the hippocampus and amygdala [6]. Interestingly, the low anxiety-like behavioural phenotype observed in GF mice persisted after colonization with SPF intestinal microbiota, demonstrating that gut–brain interactions influence CNS wiring early in life [2]. Following this initial report, two additional research groups reported reduced anxiety-like behaviour in GF mice using the EPM [3] and using the light dark test, a second approach/avoidance test used to test anxiety-like behaviour in mice [1]. A more recent paper using a different strain of mice (Balb/C compared to Swiss Webster and NMRI) showed that offspring of colonized GF mice (referred to as Ex-GF) had reduced anxiety-like behaviour in the open field test compared to GF mice [7]. In addition, these investigators showed the monoassociation with *Blautia coccooides* in GF mice reduced anxiety-like behaviour in open field, whereas monoassociation with *Bifidobacterium infantis* reduced activity without effecting anxiety-like behaviour in the marble burying test [7] suggesting that the nature of the bacterial species employed influences the impact on behaviour. In GF stress sensitive rats (F344) increase anxiety-like behaviour was observed in a 6 min open field test compared to SPF rats that was associated with enhanced stress reactivity [8]. Both of the recent papers suggest that the interaction of gut bacteria and behaviour relies on strain of mice/rats and experimental design of the behavioural test.

Another important area of study is the relationship between stress, microbiota, and behaviour [9,10]. Using a mouse model of induced anxiety and depression via olfactory bulbectomy, investigators showed that elevated corticotropin-releasing hormone (CRH) expression, increased c-Fos activity, serotonin levels, and colon motility were associated with an altered intestinal microbiome [11], which was suggested to be due to the activation of

Figure 1



Factors influencing the gut–brain axis via microbiota. As reviewed in the article, diet, stress, probiotics, and antibiotics can impact gut microbiota community to influence microbiota to brain pathways and thereby impact behaviour.

the hypothalamic pituitary adrenal (HPA) axis [11]. In a different study that compared the effect of stress and/or antibiotics on the gut microbiome of mice, antibiotics were found to lower overall bacterial counts as expected, and with the addition of stress via the water avoidance stress test, a further reduction in the bacterial load was observed [12]. Analysis of the composition of luminal bacteria using fluorescence *in situ* hybridization revealed that stress alone resulted in a loss of Verrucobacteria, a twofold increase in *Clostridium* spp., and the added presence of a low abundance population of *Lactobacillus/Enterococcus* spp. Antibiotics alone similarly reduced Verrucobacteria but also reduced *Clostridium* spp. and significantly increased Enterobacteria and the *Lactobacillus/Enterococcus* spp. population. The combination of stress and antibiotics created yet another novel environment with a sixfold reduction in *Clostridium* spp. and significant increases in Verrucobacteria, Enterobacteria, and *Lactobacillus/Enterococcus* spp. [12]. The study highlights the dynamic nature of how host–microbiota interactions and stress may modulate microbiome profiles [12]. In a related report from the same research group, changes in the expression of gut sensory markers were shown to be associated with changes in *Bacteroides* spp., *Lactobacillus* spp., and *Bifidobacterium* spp., however changes in gut microbiota composition did not alter expression of toll-like receptors [13]. A similar stress and antibiotic study in

rats also identified a decrease in overall bacterial diversity in distal ileum of the chronic stress group compared to the control group, with no accompanying difference in the overall bacterial load, and this dysbiosis was characterized by the reduction of less-abundant members of the bacterial community [14]. The addition of rifaximin treatment in stressed rats significantly decreased the total bacterial load, altered the microbial community leading to a dominance of Lactobacilli, and prevented the increase of gut permeability induced by the stress trials, a finding that suggests the antibiotic protects intestinal barrier function by modulation of the gut microbiome [14].

In a clinical study focused on further exploration of the link between microbiota composition and depression, researchers observed a general underrepresentation of the Bacteroidetes phylum in depressed patients and an association of the Lachnospiraceae family with the depression group, and interestingly, even with a decrease in Bacteroidetes, specific operational taxonomic units (OTUs) identified as members of the Bacteroidetes phylum correlated with depression [15]. It has been suggested that increased gut permeability and related bacteria translocation may contribute to increased inflammation in depressed individuals [16]. Recently, evidence supporting this suggestion was provided by a clinical study that observed elevated serum levels of IgM and IgA against the lipopolysaccharide (LPS) of gut commensals in patients with depression [17].

Potential mechanisms underlying microbiota-related changes in behaviour

A number of dietary factors have been shown to have an impact on behaviour including anxiety-like and depressive-like behaviours. For example, long-term feeding of a high fat diet increases anxiety-like and depressive-like behaviour in mice and rats [18–20]. Interestingly GF mice, lacking microbiota, are smaller than age-matched SPF mice and have reduced anxiety-like behaviour that may be linked to metabolic changes due to the absence of microbiota. Central changes in brain expression of feeding peptides have been reported in GF mice compared to conventionalized mice [21]. GF mice show reduced body weight and epididymal fat weight, in spite of increased food intake [22] revealing the requirement of microbiota for fat storage. Evidence supports a role for elevated levels of fasting-induced adipose factor (Fiaf), a lipoprotein lipase inhibitor, in this phenotype [22,23]. In conventionally raised mice, low levels of Fiaf are produced by gut epithelium over development, whereas in GF mice, Fiaf expression is upregulated during the transition to weaning (3–4 postnatal week) and circulating levels remain high into adulthood [22]. In adulthood, the peripheral metabolic phenotype of GF mice includes reduced plasma levels of leptin, insulin, and glucose [22,24]. Remarkably, GF mice are resistant to high fat

Table 1

Previous studies exploring the link between microbiota and behaviour

Reference	Model used	Behavioural tests performed	Conclusions
Sudo <i>et al.</i> [5]	GF, SPF, and gnotobiotic mice	Acute restraint test	Microbial exposure at an early developmental stage regulates the development of the HPA stress response
Heijtz <i>et al.</i> [3]	GF and SPF mice	Open field test; light-dark box test; EPM	Microbial colonization at key developmental time points affects brain development and behaviour
Neufeld <i>et al.</i> [6]	GF and SPF mice	EPM	Microbial composition of the gut influences development of behaviour
Nishino <i>et al.</i> [7]	GF, gnotobiotic and commensal fecal-microbiota-associated mice	Open field test; marble burying test	Gut microorganisms have an impact on anxiety and modulate brain development and behaviour
de Theije <i>et al.</i> [32,33]	Murine mouse model of ASD	Social behaviour score (time spent near unfamiliar gender-matched mouse)	Gut microbiome associated with autism-like behaviour found to correlate with decreased serotonin and social behaviour scores
Park <i>et al.</i> [11]	Surgical intervention mouse models — bilateral olfactory bulbectomy or intracerebroventricular infusion pumps	Tail suspension test; step-down test; open field test; water avoidance stress test	Induced chronic depression alters the microbial profile of the colon via activation of the HPA
Aguilera <i>et al.</i> [12]	Broad spectrum antibiotic treated mouse model	Water avoidance stress test; intracolonic capsaicin-evoked visceral pain;	Antibiotics combined with stress decreased total bacterial count, altered community composition, and ultimately led to modulation of visceral sensitivity
Xu <i>et al.</i> [14]	Rat model treated with rifaximin	Chronic water avoidance stress test; repeat restraint stress test	Rifaximin led to an abundance of <i>Lactobacillus</i> spp., with treatment preventing intestinal abnormalities and visceral hyperalgesia in response to chronic stress
Desbonnet <i>et al.</i> [39]	Probiotic trial in mouse model of maternal separation (<i>B. infantis</i>)	Forced swim test	<i>B. infantis</i> reversed some of the behavioural deficits caused by maternal separation
Messaoudi <i>et al.</i> [35,42]	Probiotic trial in rats and humans (<i>L. helveticus</i> and <i>B. longum</i>)	Rats — conditioned defensive burying test; Humans — Hopkins Symptom Checklist; Hospital Anxiety and Depression Scale; Perceived Stress Scale; Coping Checklist	Probiotic combination of <i>L. helveticus</i> and <i>B. longum</i> reduced anxiety-like behaviour in rats and had a beneficial effect on signs of anxiety and depression in humans
Bravo <i>et al.</i> [36]	Probiotic trial in mice (<i>L. rhamnosus</i>)	Stress-induced hyperthermia; EPM; fear conditioning; forced swim test; open field test	Administration of <i>L. rhamnosus</i> reduced anxiety and depression-related behaviours
Ait-Belgnaoui <i>et al.</i> [41]	Probiotic treated rat model (<i>L. farciminis</i>)	Partial restraint stress test	Probiotic treatment attenuated the HPA response to acute stress
Ohland <i>et al.</i> [37*]	Probiotic trial in mice on standard chow or Western diet (<i>L. helveticus</i>)	Barnes maze	The efficacy of <i>L. helveticus</i> on the reduction of stress and anxiety is dependent on diet
Hsaio <i>et al.</i> [34]	MIA model of autism treated with <i>B. fragilis</i>	Open field exploration; marble burying; social interaction; adult ultrasonic vocalization	<i>B. fragilis</i> modulated select gut and behavioural symptoms
Ait-Belgnaoui <i>et al.</i> [40]	Probiotic treated mouse model (<i>L. helveticus</i> and <i>B. longum</i>)	Water avoidance stress test	Pretreatment with probiotics can reduce chronic-stress induced abnormal brain plasticity and reduction in neurogenesis
Bruce-Keller <i>et al.</i> [26]	Microbiome depletion of mice on a normal chow diet with subsequent microbiota transplant from material obtained from high-fat diet mice	EPM; open field test; marble burying test; fear conditioning memory task	The resulting microbiome from a high-fat diet is capable of disrupting brain physiology and function even in the absence of obesity
Naseribafrouei <i>et al.</i> [15]	Adult patients of Innlandet Hospital, Norway with and without a diagnosis of depression	Montgomery-Asberg Depression Rating Scale to rate severity of depression	Sequences identified as <i>Oscillibacter</i> and <i>Alistipes</i> spp. showed a significant association with depression

diet-induced obesity and high fat fed GF mice respond to fasting with increased circulating triglycerides but reduced free fatty acids compared to conventionally raised mice [23]. This metabolic phenotype is explained in part by the role of microbiota in nutrient uptake as GF mice show reduced food efficiency, and when fed a high fat diet, GF mice fail to extract lipid from the diet [24]. In addition, gut–brain communication may be important as high fat feeding in conventional raised mice has been shown to alter expression patterns of feeding peptides in the hypothalamus [25]. A more direct link between diet-related microbiota and behaviour was demonstrated by transferring microbiota from high-fat fed or chow-fed mice to donor mice that had been treated for two weeks with a cocktail of antibiotics to reduce microbiota [26]. Notably, transfer of high-fat diet microbiota led to increased anxiety-like behaviour in the EPM, open field, and marble burying test [26] and a decrease in cued fear memory in comparison to mice that received microbiota from chow-fed donors [26]. Further, these investigators showed increased intestinal permeability and increased inflammatory markers in the medial prefrontal cortex of mice that received the high-fat diet microbiota suggesting, as other reports have, that immune signaling pathways may be a key mediator of microbiota–brain communication [26]. Clearly this study, in combination with the others presented above shows that gut dysbiosis is associated with brain dysfunction and behavioural changes.

Understanding how early life experience contributes to individual differences in vulnerability to psychiatric illness is a leading topic in clinical and behavioural neuroscience. Understanding the developmental trajectory that determines the onset of mood and anxiety disorders is also an important clinical issue. An expanding body of literature provides evidence that exposure to environmental challenges, including diet changes, infection, and stress, during specific developmental windows is critical to the development of stress reactivity and related behaviours. Early postnatal life represents a critical period during which gut–brain communication may influence the developmental trajectory of the brain and thereby contribute to risk of mental illness [27]. Interestingly, early postnatal life is also a critical period for metabolic development and a recent report showed that exposure to low dose antibiotics from birth to weaning in mice resulted in an altered metabolic phenotype that persisted to adulthood; exposure to antibiotics in the post-weaning period had less of an impact on the adult phenotype suggesting greater vulnerability to gut dysbiosis during the early postnatal window [28]. More work linking microbiota and the gut–brain axis to brain development is needed to help understand how these interactions may influence risk of anxiety and depression.

Alterations in the human diet can also dramatically alter the composition of the gut microbiota, and these changes

have been shown to contribute to the development of gastrointestinal disease [29**]. The link between diet-induced changes in microbiota and stress-related behaviour has been studied in animal models. For example, in mice, a beef diet when compared to a standard chow diet led to a different distribution of the major bacterial populations with a greater diversity observed in the beef diet group [30]. In addition, the beef diet group had improved working and reference memory on the hole-board open field test and less anxiety-like behaviour, assessed during the novel encounter in the hole-board open field [30].

Recent reviews of the relationship between microbiota and behaviour reveal several possible humoral, neural, and cellular signaling pathways that may connect microbiota to brain function [9,31]. The immune system is a key player in gut–brain interactions and has long been linked to psychiatric conditions and neurodevelopmental disorders. Some of the most interesting recent findings show a link between microbiota, the immune system, and autism spectrum disorders (ASD). Utilizing a murine mouse model of ASD created by prenatal valproic acid (VPA) exposure, male offspring (compared to females) exhibited increased expression of neuroinflammatory markers with associated intestinal inflammation evidenced by increased neutrophil infiltration, increased levels of butyrate, a decrease in intestinal levels of serotonin, and disturbed social interactions [32*]. A parallel study with more in-depth evaluation of the intestinal tract of these mice also confirmed the loss of the epithelial barrier [33]. In a similar maternal immune activation (MIA) model of ASD, a model that displays both the neurological and gastrointestinal symptoms associated with autism, treatment with *Bacteroides fragilis* corrected intestinal permeability defects through modification of tight junction expression, cytokine production, and alteration of the gut microbiome [34]. While a greater than expected abundance of the Lachnospiraceae family of Clostridia was seen in the MIA offspring, treatment with *B. fragilis* corrects this dysbiosis as well as resolves behavioural symptoms typical of ASD related to communication, stereotypical behaviours, sensorimotor gating, and anxiety [34].

Therapeutic potential of diet and probiotics in normalizing behaviour

Several animal studies have demonstrated an impact of probiotic administration on behaviour, in particular, anxiety-like and depressive-like behaviours; almost all studies have utilized *Lactobacillus* sp. and *Bifidobacteria* sp. Interestingly, probiotic administration to healthy rodents can impact behaviour. For example, 14 day administration of combined *L. helveticus* and *B. longum* reduced anxiety-like behaviour in the defensive marble burying test in Wistar rats [35]. In /C mice, 28 days administration of *L. rhamnosus* reduced both anxiety-like behaviour in

the EPM and depressive-like behaviour in the forced swim test [36] and in 129/SvEv mice, 21 day administration of *L. helveticus* reduced anxiety-like behaviour in the Barnes maze [37*]. In addition, stress-induced anxiety-like and depressive-like behaviour have been shown to be reversed by probiotic administration [38,39]. High fat diet-induced anxiety-like behaviour in 129/SvEv mice was prevented by 21 day administration of *L. helveticus* [37*], however in interleukin-10 (IL10) deficient mice, high-fat diet induced anxiety-like behaviour was not prevented by *L. helveticus* administration suggesting that immune signaling is important to gut-brain modulation of behaviour [37*].

Yet another study utilizing the *L. helveticus* and *B. longum* combination was shown to cause no changes in intestinal permeability in non-stressed conditions, while stress alone significantly increased intestinal permeability [40]. The addition of two-week pretreatment with the probiotic significantly increased expression of neurotrophic factor (BDNF, brain-derived neurotrophic factor) and decreased the expression of cytoskeleton organization and microglial activation markers, synaptogenesis and cell adhesion markers. The intestinal permeability caused by stress was also prevented by administration of the probiotic, leading the researchers to conclude that the treatment decreased activity of the HPA axis and the autonomic nervous system activity in response to chronic stress, which was supported by a decrease in plasmatic levels of corticosterone, adrenaline, and noradrenaline in stressed mice [40]. An additional study evaluating the effect of *Lactobacillus farciminis* also found a decrease in the HPA axis response to stress, evidence by a decrease in plasma adrenocorticotrophic hormone and corticosterone concentration and hypothalamic CRH expression, and prevention of stress-induced colonic paracellular hyperpermeability, LPS upload, and central neuroinflammation [41]. Parallel studies in human subjects confirmed the reduction in anxiety-like behaviours, and subsequent analysis confirmed that even in individuals with lower stress levels (indicated by urinary free cortisol levels) significant improvement in mood, specifically related to anxiety and depression, was observed [42*].

Future considerations

We now know that microbiota influence behaviour, in particular, stress-related behaviours such as anxiety and depression. Attention to the importance of microbiota and behaviour is rapidly expanding. Clinically, the link between obesity and anxiety is suggested [43], however studies have not yet considered the role of microbiota in this overlap. Understanding the link between mood and metabolism is a necessary direction for research studies to pursue. Changes in the microbiome, the metabolome, and behaviour need to be monitored as interventions such as diet modification, probiotic administration, and changes in the environment occur. Beyond microbiome composition,

looking at active metabolites at specific time points of interest and evaluating how interventions related to diet and probiotic supplementation can change metabolomic profiles, and in doing so affect fundamental changes with both gastrointestinal and neurological implications.

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This work demonstrates the beneficial effects of probiotic treatment in healthy adults. Both *Lactobacillus* spp. and *Bifidobacterium* spp. have been shown in this study and in related animal studies to have beneficial effects on stress-related behaviours.