Cell. Mol. Life Sci.
DOI 10.1007/s00018-012-1028-z

Cellular and Molecular Life Sciences

REVIEW

Voices from within: gut microbes and the CNS

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Received: 8 March 2012 / Revised: 3 May 2012 / Accepted: 7 May 2012
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Abstract Recent advances in research have greatly increased our understanding of the importance of the gut microbiota. Bacterial colonization of the intestine is critical to the normal development of many aspects of physiology such as the immune and endocrine systems. It is emerging that the influence of the gut microbiota also extends to modulation of host neural development. Furthermore, the overall balance in composition of the microbiota, together with the influence of pivotal species that induce specific responses, can modulate adult neural function, peripherally and centrally. Effects of commensal gut bacteria in adult animals include protection from the central effects of infection and inflammation as well as modulation of normal behavioral responses. There is now robust evidence that gut bacteria influence the enteric nervous system, an effect that may contribute to afferent signaling to the brain. The vagus nerve has also emerged as an important means of communicating signals from gut bacteria to the CNS. Further understanding of the mechanisms underlying microbiome–gut–brain communication will provide us with new insight into the symbiotic relationship between gut microbiota and their mammalian hosts and help us identify the potential for microbial-based therapeutic strategies to aid in the treatment of mood disorders.

Keywords Microbiota · Commensal bacteria · Probiotic · Brain · Behavior · Vagus

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Microbiota–gut–brain axis

It is now well established that the brain and the gut are engaged in constant bi-directional communication. Most individuals are made aware of such communication when alteration in gastrointestinal function is communicated to the brain bringing about the perception of visceral events such as nausea, satiety, and pain or when, in turn, stressful experiences lead to altered gastrointestinal secretions and motility [1].

The mechanisms underlying gut–brain axis communication involve neural pathways as well as immune and endocrine mechanisms. The gastrointestinal tract is a point of interaction between the body's largest concentration of immune cells, a vast network of 500 million neurons and the gut microbiota. With an estimated mass of 1–2 kg, the approximately 100 trillion bacteria that constitute the human gut microbiota consist of at least 1,800 genera and up to 40,000 species of bacteria [2] and together possess 100 times the number of genes in the human genome [3]. Given the scale of the metabolic and genetic coding capacity of this "virtual organ", it is not surprising that the gut microbiota impacts various aspects of host physiology [4–7]. It is now clear that these influences include modulation of gut–brain communication. Indeed, it is emerging

Published online: 27 May 2012

that the gut microbiota can modulate host neural development and adult function, both peripherally, in the enteric nervous system, and centrally. Perhaps, most remarkably, evidence suggests a hitherto unrealised dimension to the integration of host and microbiome; that the overall balance in composition of the microbiota, together with the influence of pivotal species that induce specific responses, can influence the CNS leading to the modulation of brain function and consequently mood and behavior.

This review will highlight existing evidence that changes in the gut microbiota or intestinal exposure to specific commensal bacteria can modulate the peripheral and central nervous systems to consequently alter brain functions. There will also be a discussion of the potential mechanisms through which signals from gut bacteria are communicated to the brain.

The immunomodulatory effects of the gut microbiota and commensal bacteria have been extensively discussed elsewhere [8, 9], and it is clear that cytokine production and other immune changes can modulate the peripheral and central nervous system and are associated with altered mood and behavior [10, 11]. Thus, while acknowledging that the immune system may play an important role in many of the phenomena described below [12, 13], here we will focus specifically on non-immune aspects of communication between gut bacteria and the CNS.

Flies, pheromones, and neuropeptides

The study of insects has provided some clear examples of the potentially profound effect of the gut microbiota on behavior. The congregation of locusts into the vast swarms that result in crop devastation is dependent on pheromones, the major components of which are phenol and guaiacol [14]. Dillon et al. [14] identified that locust gut microbiota were critical in the production of aggregation pheromones. Specifically, it was determined that guaiacol was absent and phenol present at a reduced level in fecal pellets from germ-free insects [14]. Furthermore, the introduction and establishment of the bacterium *Pantoea agglomerans* in the gut of axenic locusts resulted in the re-appearance of the two phenolics in the feces. These investigators went on to determine that a number of bacterial species that commonly comprise the locust gut microbiota are capable of converting plant derived vanillic acid to guaiacol [15], indicating a closer degree of integration between the locust and its microbial community had previously been suspected.

In a recent study by Sharon et al. [16], a population of fruit flies was divided with one half fed on molasses medium and the other on a starch medium. When the isolated populations were mixed, molasses fed flies preferred to mate with other molasses fed flies while starch-fed flies preferred to mate with other starch-fed flies. These differences in mating preference occurred after only one generation on different growth media and could be maintained for at least 37 generations [16]. Antibiotic treatment abolished mating preference, suggesting that the fly microbiota was responsible for the phenomenon. The mating preference could be re-established in antibiotic-treated flies by infecting them with microbiota obtained from fly media. Starch-fed flies had markedly higher levels of *Lactobacillus* species in the microbiota than malt-fed. Significantly, mating preferences of starch-fed antibiotic-treated flies could be reestablished by infecting with a mixed culture of *Lactobacillus* species and a pure culture of *Lactobacillus plantarum.* Importantly, parallel experiments using other bacterial species isolated from starch-bred flies had no effect on mating preference [16]. Thus, these experiments demonstrated that a single bacterial species could induce mating preferences in fruit flies. Again, this study served to identify a highly integrated relationship between microbiota and host. Indeed, it is proposed that these findings provide support for the hologenome theory of evolution [17]. The hologenome is defined as the sum of the genetic material of the host and its microbiota. It is posited that the holobiont (host plus its associated microorganisms) acts as a unit of selection in evolutionary change, and that variation, an important factor in evolution, can occur through modification in either the host or the microbiota genomes [17].

While Sharon et al. [16] did not identify a specific mechanism by which bacteria induce mating preference, they suggest that, as with aggregation pheromone in locusts [14], the bacterially-induced mating signal could be a volatile compound emitted by the fly or a detectable compound on its surface. In support of this, the study identified five cuticular hydrocarbon sex pheromones, which play a major role in fly mating [18], were produced at significantly different levels between starch- and malt-raised flies [16]. These differences were reduced with antibiotic treatment [18], suggesting that specific symbiotic bacteria can influence the levels of fly sex pheromones and, by doing so, modify fly behavior.

While gut bacteria producing mating and aggregation pheromones in insects may appear far removed from mammalian systems, there may be clear analogies in the underappreciated fact that bacteria can act as a source of various biologically active peptides and mediators normally associated with mammalian neurotransmission. Molecules such as GABA, serotonin, melatonin, histamine, and acetylcholine have been identified as being produced by bacteria [19].

Bacteria can also produce gaseous neurotransmitters. Lactobacilli have been demonstrated to convert nitrate to

nitric oxide (NO), a potent regulator of both the immune and nervous systems [20]. NO levels in the small intestine and the cecum were 3–8 fold higher in rats that had been fed live lactobacilli and nitrate compared to controls. In addition, H_2S that is produced by constituents of the gut microflora has been shown to modulate gut motility through action at the vanilloid receptor TRPV1 on capsaicin-sensitive nerve fibers [21].

It has been proposed that late horizontal gene transfer can explain the existence of genes encoding many of the enzymes involved in the synthetic and metabolic pathways of catecholamines, histamine, acetylcholine, and GABA from bacteria. This concept is concordant with increasing evidence that signaling molecules of quorum-sensing systems, used by bacteria to communicate and coordinate their actions [22], can also bind to mammalian receptors and directly influence the host [23, 24]. This concept of shared signaling pathways is further supported by evidence that neurotransmitters produced by the host can influence the function of components of the microbiota. For example, in *Escherichia coli* O157:H7, the QseC sensor kinase is a bacterial receptor for host-derived epinephrine/norepinephrine which activates transcription of virulence genes in the bacteria; a response that can be blocked specifically by adrenergic antagonists [25].

Visceral perception and interoception

While the concept that the brain can alter gut function is widely acknowledged, and the relationship between stress and disorders such as irritable bowel syndrome has been the focus of extensive research, it is less readily accepted that signals from the gut might influence the CNS with associated consequences for mood and behavior. Such gut-driven changes to brain function are more readily understood when considered within the context of interoception.

The term interoception refers to sensing the physiological condition of the body [26], as well as the representation of the internal state [27] within the context of ongoing activities. Interoception is closely associated with emotional awareness [28] and motivated actions to homeostatically regulate the internal state [27]. Interoceptive signals include sensations such as pain, temperature, itch, tickle, sensual touch, muscle tension, air hunger, stomach discomfort related to low pH, and intestinal tension [26]. These sensations are transmitted to the brain by vagal and glossopharyngeal afferents synapsing with the nucleus of the solitary tract (NTS) and via small diameter primary sympathetic afferent fibers to a specific thalamocortical relay nucleus, and are integrated to provide a sense of the body's physiological condition [26].

In the early 1970s, Cabanac [29] proposed that a given external stimulus can be perceived as either pleasant or unpleasant, depending upon interoceptive signals. However, the role of visceral sensory input in physiological or pathological modulation of perception was only recently recognized. While early studies concentrated on the modulation of responses directly relevant to a given sensory input (e.g., hunger to feeding, stomach movements to nausea), there is now experimental data to suggest that changes in visceral sensation can affect the perception and interpretation of external inputs [30]. This has led to the suggestion that altered interoceptive signals can influence our attitude to the outside world and that pathological changes in visceral sensory inputs may increase the risk of affective behavioral disorders [31]. If beneficial bacteria could alter interoceptive signaling in an appropriate way, they may have a future potential as adjuncts in the treatment of these disorders.

Microbiota and the enteric nervous system

Gut bacteria may modulate gut motility by action on the enteric nervous system (ENS), which consists of ganglionated plexuses in the gut wall and whose presence is essential to life. The myenteric plexus component of the ENS controls peristalsis. Hence, enteric aganglionosis due to Hirschsprung [32] or Chagas [33] diseases, or chemical ablation using benzalkonium chloride [34], severely reduces peristalsis and produces pseudo-obstruction in the affected region.

Myenteric Dogiel type II AH cells innervate the mucosa and are chemosensitive intrinsic primary afferent neurons (IPANs) in guinea pig [35], rat [36], and mouse [37]. IPANs project directly to motor- and interneurons (S cells), though which they modulate the intensity and timing of muscle motor complexes and co-ordinate peristalsis [38]. In fact, selective silencing of only AH cells causes aperistalsis similar to total aganglionosis [39].

By far the richest innervation of mucosal epithelial layer cells derives from the myenteric plexus, which provides more than 90 % of sensory neuropeptide-containing fibers to the mucosal layer [40, 41]. Each enteric IPAN innervates 80–120 villi [38], and there are about 500,000 neuropeptide (calcitonin gene-related peptide, CGRP)-containing IPANs in the mouse [42]. Thus, IPANs are ideally placed to respond to luminal commensal and probiotic microorganisms, and are plausible targets though which the microbes could influence gastrointestinal physiology, perhaps independent of commensal bacteria to immune system signaling (Fig. 1).

That IPANs are indeed a cellular target of neuroactive bacteria has been demonstrated by whole cell patch

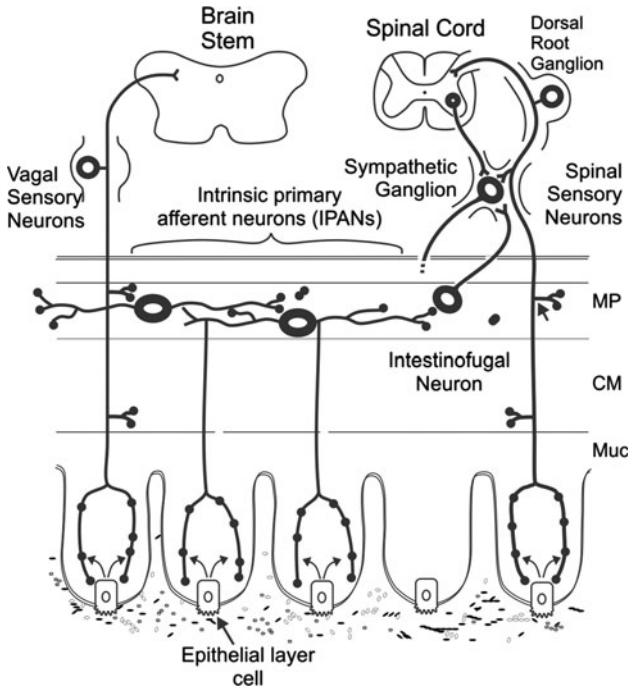

Fig. 1 Potential neural pathways from gut bacteria to the CNS. Sensory neurons include intrinsic primary afferent neurons (IPANs) in the myenteric plexus (MP) of the enteric nervous system, and vagal and spinal extrinsic primary afferent neurons. IPANs are multipolar with their somata and all neurites confined to the intestine. Vagal and spinal primary afferent neurons are pseudounipolar with somata extrinsic to the intestine; they have collaterals that enter enteric ganglia and form synapses with enteric neurons. Sympathetic and myenteric ganglia reciprocally innervate each other. Some 90 % of sensory neuropeptide containing axons that innervate the mucosal layers derive from intrinsic rather than extrinsic primary afferent neurons. Chemicals including hormones released from epithelial cells act on adjacent primary afferent neuron axons (*curved arrows*). Cell wall components or secreted products, including neurotransmitters, of microorganisms in the lumen or attached to epithelial cells may induce epithelial cells to release transmitter molecules that in turn modulate neural signaling, or act directly on primary afferent axons. *MP* myenteric plexus, *CM* circular muscle, *Muc* mucosa

clamp recording experiments using rats that were fed *L. rhamnosus* JB-1. Myenteric IPANs, but not motor- or interneurons, within colon segments taken from fed animals were more excitable than were those from controls. *JB-1* reduced the action potential firing threshold and discharge accommodation during injections of excitatory current pulses [36]. This increase in excitability was accompanied by a reduction in the post-action potential slow after hyperpolarization, which mediates discharge accommodation in IPANs [36]. It was proposed that the underlying molecular mechanism involved an intermediate conductance calcium-dependent potassium (IK_{Ca}) (Gardos type [43]) channel, because application of the IK_{Ca} channel blocker TRAM-34 mimicked the effects of JB-1, namely reducing the IPAN slow after hyperpolarization [36, 44].

Certain commensal or probiotic bacteria may have an analgesic action on the host. *L. rhamnosus* [45], *L. acidophilus* [46], or *L. paracasei* [47], as they have been shown to moderate pseudo-affective responses to nociceptive colorectal distension and to inhibit spinal neuron cellular memory of the distension.

The presence of anatomical synapses between extrinsic primary afferent (vagal [48] or spinal [49] axons and myenteric neurons suggest the possibility that the analgesia may have resulted from IPAN to extrinsic primary afferent transmission. However, while extrinsic fibres activate enteric neurons via slow excitatory postsynaptic potentials [50], intramural synaptic transmission does not appear to go in the opposite direction; that is, from enteric neurons to extrinsic primary afferent fibers [51]. Thus, the mechanisms underlying probiotic analgesia may involve alterations of the intensity of gut contractions [52] or modification of the excitability of extrinsic spinal primary afferent terminals within the mucosa.

Some of the anti-nociceptive effects accorded to a *Lactobacillus rhamnosus* were also seen with heat-killed or gamma-irradiated bacteria and even with conditioned medium obtained after culture of these bacteria [45]. Such experiments clearly suggest that components of bacteria and/or secreted products can mimic the effects of the live organisms. Ingestion by rats of a mutant bacterium, *L. plantarum*, in which D-alanine was markedly reduced within a cell wall constituent, lipoteichoic acid, was more effective than treatment with the parent wild strain in terms of immuno-regulatory effects [53] as well as inhibition of perception of visceral pain [54]. Thus, in this case, a bacterial cell wall component must, in part, have been a determinant of the immune as well as the neuronal effects.

In contrast to pain transmission, there are few chemical correlates of the functional effects that probiotics have on enteric neurons. Ingestion of *Saccharomyces boulardii* has been shown to decrease the number of pig myenteric AH cells that express the vitamin D-dependent cytosolic calcium binding protein calbindin-D28k [55]. A change in calcium intracellular buffering, as is suggested by this result, might be expected to alter the opening probability of IK_{Ca}. Yet, it is not clear, without further experiments, precisely how changes in calbindin correlate with the slow after hyperpolarization and neuronal excitability. The expression of μ-opioid and cannabinoid receptors in gut mucosal epithelial cells has been reported as being increased by feeding an analgesic stain of *L. acidophilus* [46]. Receptor tolerance that such receptors exhibit [56, 57] suggests that the increased expression may have resulted from a reduction in receptor activation by endogenous or microbial-produced agonists. However, it is not clear how epithelial opioid or cannabinoid receptors could gate

afferent signals in enteric nociceptive neurons. Clearly, further research is needed.

Evidence of gut microbiota influences on the CNS and behavior

Brain and behavior in the absence of gut microbiota

A number of important insights into the impact of the gut microbiota on host physiology have come from the study of germ-free animals. These key studies have indicated a role for gut bacteria in the normal development of behavior, and in particular in the stress response. Some of the earliest indications of a critical role of the gut microbiota in stress responses come from studies by Sudo and colleagues [7]. Germ-free animals were identified as having exaggerated hypothalamic–pituitary–adrenal (HPA) axis activation in response to stress. This hyperresponsiveness was reversed by reconstitution with feces from animals kept in a pathogen-free environment or with a single bacterial strain, *Bifidobacterium infantis* [7]. In contrast, mono-association with an enteropathogenic *E. coli* further enhanced the response to stress.

More recently, two studies have indicated that the absence of a microbiota results in decreased anxiety-like behavior compared to conventional animals [58, 59]. In one of these studies, Neufeld and colleagues [59] also demonstrated an increase in baseline plasma corticosterone of the germ-free mice. While seemingly incongruent with reduced anxiety, this finding is in keeping with the previous reports of an increased stress response in germ-free animals [7].

Interestingly, Heijtz et al. [58] demonstrated that early colonization of germ-free mice could normalize several germ-free behavioral patterns while conventionalization of adult mice failed to normalize the behavior. This indicates, as suggested by the earlier work of Sudo et al. [7], that the gut microbiota contributes to developmental programming; a process whereby an environmental factor acting during a developmental "window of vulnerability" can have a potentially life-long impact on physiological function [60].

Addressing neural correlates of reduced anxiety in germ-free animals, Heijtz et al. [58] demonstrated that NGFI-A mRNA expression was significantly lower in various subregions of the prefrontal cortex, including the orbital frontal cortex and the striatum, hippocampus dentate gyrus, and amygdala, compared with specific pathogen-free mice. Germ-free mice also had significantly lower BDNF mRNA expression in the hippocampus, amygdala, and cingulate cortex, which are important components of the neural circuitry underlying anxiety and fear [61, 62]. Such a reduction in BDNF expression levels in the cortex and hippocampus relative to conventional mice was also noted by Sudo et al. [7]. Brain-derived neurotrophic factor is involved in the regulation of multiple aspects of cognitive and emotional behaviors, being a key promoter of neuronal survival and growth as well as differentiation of new neurons and synapses [63–65]. Serum levels of BDNF are significantly decreased in the plasma of depressed patients [66, 67], and in post-mortem hippocampal tissue from depressed suicide patients [68, 69]. The association between anxiety and BDNF is less clear, and studies have identified positive, negative, or no correlation between hippocampal levels and anxiety [70–73]. Perhaps reflecting this, Neufeld et al. [59] identified that reduced anxiety in germ-free mice was associated with an upregulation, rather than a decrease, in the expression of BDNF mRNA in the dentate gyrus of the hippocampus. The reasons underlying the conflicting findings regarding hippocampal BDNF in germ-free mice is unclear; however, both studies describing decreased BDNF expression were conducted in male mice [7] while Neufeld et al. [59] exclusively used female animals. This may be significant given existing evidence that the neurochemical and behavioral consequences of stress are sex-dependent [74]. Furthermore, the influence of BDNF on behavior appears to be sex-specific with increased anxiety-like behaviors observed in male but not female mice with joint serotonin transporter (SERT) and BDNF deficiency [75].

In addition to altered neurotrophin levels, changes have been reported in NMDA receptor subunit expression with decreased NR1 and NR2A in the hippocampus, decreased NR2A in the cortex, and decreased NR2B in the amygdala, but not in the hippocampus [7, 59]. Enhanced turnover rate of noradrenaline, dopamine, and 5-HT has also been demonstrated in the striatum of germ-free mice compared with specific pathogen-free mice [58].

It should be noted that at least one study has found no reduction in anxiety of germ-free mice when compared to controls, but instead identified impaired memory as assessed in the T maze [76]. The reasons for these distinct findings are unclear; however, taken together, existing data suggest that gut microbiota can influence a number of aspects of brain chemistry, stress responses, and behavior.

Modulation of the microbiota

In addition to the study of germ-free animals, the effects of changes in the composition of the conventional gut microbiota on behavior and brain chemistry have also been explored. Alterations in diet can lead to marked shifts in gut microbial populations [77, 78]. In a study by Li et al. [79], mice fed a diet containing 50 % lean ground beef were found to have a greater diversity of gut bacteria than those receiving standard rodent chow. The increase in

bacterial diversity was associated with an increase in working and reference memory as assessed in a hole-board open field test [79]. Furthermore, mice receiving the beef diet exhibited less anxiety-like behavior in response to the novelty of the testing environment. While no causal relationship was established, this study did provide early support for the suggestion that, in addition to any direct effects of dietary components, diet-induced changes in bacterial diversity may influence behavior.

More recent studies have involved the induction of experimental dysbiosis through the use of antimicrobial drugs. Bercik et al. [80] demonstrated that, in adult BALB/c mice, oral administration of neomycin and bacitracin along with the antifungal agent primaricin led to a transient change in the composition of the gut microbiota. Interestingly, antibiotic treatment did not lead to quantitative changes in culturable bacteria but induced a significant change in composition; specifically, an increase in *Actinobacteria* and *Lactobacilli* species and decrease in *γ-proteobacteria* and *bacteroidetes*. The antibiotics also induced changes in behavior, with treated animals demonstrating evidence of increased exploratory drive and decreased apprehension in both the step-down and light/dark preference tests. As was demonstrated in comparisons between germ-free and conventional animals, behavioral changes in antibiotic-treated animals were associated with altered BDNF levels in the brain, being decreased in the amygdala while increased in the hippocampus [80].

The effects of antibiotic treatment on the composition of the intestinal microbiota and on behavior were transient with treated mice resembling controls after a 2-week washout period. In these studies, a causal relationship between microbiota changes and behavioral effects is supported by the demonstration that, in contrast to oral antibiotic treatment, i.p. treatment did not influence behavior. Furthermore, antibiotic treatment had no effect on the behavior of germ-free animals [80]. Whether the behavioral changes can be attributed to specific alterations in the microbiota, e.g., increased *lactobacilli* and *acintobacteria* or decreased *γ-proteobacteria* and *bacteroidetes*, was not investigated. However, this is an intriguing idea especially given subsequent studies demonstrating anxiolytic effects of feeding certain lactobacilli and bifidobacterium strains [81, 82], and as such it would be interesting to assess the effects of agents that promote the growth of bifidobacteria and lactobacilli (often termed prebiotics [83]) on behavior.

Inflammatory models

Studies in animal models using chemical colitis or infection with pathogens have demonstrated that inflammation of the gastrointestinal tract can alter brain chemistry with accompanying changes in behavior that include increased anxiety-like responses and anorexia. There is now evidence that these central responses to intentional infection and inflammation may be modulated by commensal bacteria.

Citrobacter rodentium is being used increasingly as an infectious agent to investigate gut–brain axis function. In one such study of *C. Rodentium*-infected mice [76], no behavioral abnormalities were observed, either at the height of infection or following bacterial clearance. However when infected mice were exposed to acute stress, demonstrated to increase intestinal permeability [84, 85] as well as influence gut bacterial function[25], memory impairment was apparent both during infection and following clearance [76]. The dysfunction of non-spatial and working memory, assessed by the novel object and T maze tests, respectively, could be prevented by daily treatment of infected mice with a commercially available, mixed strain, probiotic preparation [76]. This probiotic pretreatment also ameliorated stress-induced serum corticosterone levels as well as preventing *Citrobacter rodentium*-induced reductions in hippocampal BDNF and c-fos expression [76].

In a recent series of studies, Bercik and colleagues [86], examined behavior and brain chemistry in mice following chronic mild gut inflammation induced by infection with *Trichuris muris*. They observed increased anxiety-like behavior as assessed by step-down and light/dark preference tests together with an associated decrease in mRNA message for hippocampal BDNF [86]. Feeding mice with a probiotic strain of *B. longum* normalized behavior and BDNF mRNA, and while many probiotic bacteria have been demonstrated to have anti-inflammatory actions [8], this particular *B. longum* strain did not alter intestinal levels of inflammatory cytokines TNF or IFNγ [86]. In the same model, treatment with the anti-inflammatory agents etanercept and budesonide, not surprisingly, did reduce TNF and IFNγ in the intestine, but also normalized behavior. However this normalized behavior was not accompanied by a corresponding increase in central BDNF expression [86]**.** These results suggest different modes of action in normalizing behavior between *B. longum* and anti-inflammatory agents, but may also argue against a direct relationship between BDNF levels and anxiety-like behavior. While direct interaction between the probiotic and infectious agent may have contributed to the efficacy of *B. longum* in this model, it is important to note that the same strain of bacteria was demonstrated to normalize behavior in mice with non-infectious, chemically-induced colitis [82], again without altering markers of intestinal inflammation, in this case histological score and MPO levels.

These studies provide clear evidence for gut–brain communication being altered by changes in gut microbiota and more specifically following exposure to commensal or

probiotic strains. From a clinical perspective, they may be particularly relevant to certain inflammatory conditions, such as rheumatoid arthritis, chronic obstructive pulmonary disease, asthma, and inflammatory bowel disease, that are strongly associated with mood disorders or depression. However, there is also evidence that gut exposure to specific bacteria can alter constitutive brain chemistry and behavior in normal animals.

Conventional animals

Early evidence that bacteria in the gut can directly modulate central neural pathways even in the absence of an immune response came from the study of pathogen exposure [87]. Orally administered *Camphylobacter jejuni*, in subclinical doses, too low to elicit immune activation, can have anxiety-provoking effects in mice. In addition, at these same low doses, *C. jejuni* can activate visceral sensory nuclei in the brainstem. The areas of brainstem activation, the NTS and lateral parabrachial nucleus, participate in neural information processing that ultimately lead to autonomic neuroendocrine and behavioral responses [87].

It is also clear that non-pathogenic bacteria can activate central neural pathways. Tanida et al. [88] demonstrated that intraduodenal injection of the bacterial strain *Lactobacillus johnsonii* La1 reduced renal sympathetic nerve activity and blood pressure while enhancing gastric vagal nerve activity. All these effects could be abolished by pretreatment with a histaminergic H3-receptor antagonist. Similarly, the effects were absent in animals that had bilateral lesions of the hypothalamic suprachiasmatic nucleus, a major regulator of circadian rhythm. These findings suggest that the influence of the bacteria on autonomic neurotransmission and subsequently blood pressure is mediated centrally, likely through histaminergic nerves and the suprachiasmatic nucleus [88].

Early evidence that chronic treatment with a specific bacterial strain could affect, beneficially, neuronal systems and behaviors relevant to depression was obtained in a study using the rat maternal separation model [89]. This study compared the impact of chronic probiotic treatment with those of the antidepressant drug, citalopram, on behavior and biochemical changes in adult maternally separated offspring. Maternal separation induced a decrease in swim behavior and a concomitant increase in immobility in the forced swim test, features considered to indicate a state of behavioral despair [90]. The behavioral changes were associated with decreases in noradrenaline content in the brain, elevated CRF mRNA levels in the amygdaloid cortex, and enhanced release of the cytokine IL-6 following immune stimulation [89]. While the effects were not as marked as treatment with citalopram, treatment with the probiotic bacteria *B. infantis* resulted in reversal of behavioral deficits, restoration of basal noradrenaline concentrations in the brainstem, and normalization of the immune response [89]. Similarly, Gareau et al. [91] demonstrated that treatment of rat pups with a mixture of two lactobacillus strains attenuated the increase in serum corticosterone levels induced by maternal separation, suggesting an normalization of the HPA response in these animals.

More recently it was demonstrated that long-term (28-day) oral administration of a *L. rhamnosus* strain (JB1) could alter the normal behavior of adult balb/c mice [81]. Chronic treatment with the bacteria reduced anxiety-like behavior as assessed in an elevated plus maze and decreased the time spent immobile in a forced swim test. In addition, stress-induced plasma corticosterone levels were lower in treated mice, a similar effect to subchronic or chronic treatment with antidepressants that can prevent forced swim stress-induced increases in plasma corticosterone in both mice and rats. Overall, changes induced with *L. rhamnosus* were indicative of reduced anxiety and decreased depression-like behavior [81].

Experiments also indicated that the lactobacillus-treated mice had increased cue- and context-dependent freezing responses in the recall phase of a fear conditioning paradigm. While this may be suggestive of increased fear memory, this type of increased emotional learning may also be interpreted as enhanced anxiety behavior; under this interpretation, it may be that the bacteria have differential effects on conditioned compared with unconditioned aspects of anxiety [81].

Mice that received *L. rhamnosus* also demonstrated alterations in central GABA receptor subunit mRNA expression. Long-term *L. rhamnosus* administration decreased expression of GABA type B (GABAB) subunit 1 isoform b (GABAB1b) mRNA in the amygdala and hippocampus, while increased expression was detected in cortical areas. Furthermore, expression of GABAAα2 receptor mRNA was reduced in the amygdala and cortical areas, whereas levels were increased in the hippocampus [81]. As with many of the studies described here, it is difficult to attribute a causal relationship between behavioral effects observed and changes in brain chemistry. However, it is relevant to note that reduced expression of GABAB1b mRNA, in the amygdala, hippocampus, and locus coeruleus is consistent with the antidepressant-like effect of GABAB receptor antagonists [92]. The enhanced memory to an aversive cue and context is also suggestive of changes at the level of the amygdala and hippocampus [93, 94]. The changes in behavior and GABA receptor expression following *L. rhamnousus* treatment were also in keeping with studies of GABAB1b-deficient animals, indicating an important role for this subunit in the development of

cognitive processes, including those relevant to fear [95, 96]. However, no extensive investigation of the cognitive effects of bacteria treatment was pursed in this study. Indeed, with the exception of evidence of some memory dysfunction in germ-free mice [76], little is known of the potential for altered gut microbiota or exposure to specific gut bacteria to modulate cognitive functions.

Mechanisms underlying gut bacteria effects on the CNS

The vagus nerve

Information from the heart, lungs, pancreas, liver, stomach, and intestines are delivered tonically to the brain via sensory fibers in the vagus nerve [97]. Sensory vagal inputs arrive in the nucleus of the solitary tract (NTS), and are thence transmitted to widespread areas of the CNS, including the cerebral cortex and medulla oblongata. Neurones of the rostral ventrolateral medulla oblongata (RVLM) provide one of two major sources of afferent inputs to the locus coeruleus [98], which in turn projects to areas of the cortex that are associated with stress-related behavior and affective disorders. The locus coeruleus is also considered a major site for integrating stress responses [99]. Following repeated activations, a feed-forward system between noradrenergic locus coeruleus neurones and areas of the forebrain that produce corticotropin-releasing factor (CRF) can lead to altered behavioral responses [100]. Chronic activation of this system induces changes in neuronal activity that underlies anxiety, panic disorders, and depression [101].

The concept of interoception and experimental data suggesting that changes in visceral sensation can affect the perception and interpretation of external inputs [30] has led to the suggestion that altered sensory vagal inputs can influence our attitude to the outside world and that pathological changes in sensory vagal inputs may increase the risk of affective behavioral disorders. It has been proposed that chronic sensory vagal inputs could act as 'natural' breaks for augmentation of stress-related behavioral responses via tonic modulation of the neuronal activity in the locus coeruleus and in turn the forebrain [31]. In keeping with this, vagal stimulation is an FDA-accepted alternative treatment for intractable depression, and has also been used successfully in the treatment of refractory epilepsy, demonstrating clear behavioral effects of modulating vagal afferent signals [102].

Thus, given the key role of the vagus in communicating visceral signals to brain, and particularly to neural circuitry associated with mood and anxiety, it is perhaps not surprising that many investigations of communication between gut bacteria and the CNS have examined the role of the vagus. There is now strong evidence from animal studies that gut microorganisms can activate the vagus nerve, and that such activation plays a critical role in mediating effects on the brain and, subsequently, behavior.

Such evidence came early from the study of animals infected with pathogens. Subdiaphragmatic vagotomy attenuated c-fos expression in the PVN of rats inoculated with *Salmonella typhimurium* [103]. Although *S. typhimurium* infection was accompanied by intestinal inflammation, subsequent studies have indicated that microorganisms in the gastrointestinal tract can directly activate neural pathways even in the absence of an identified immune response [87]. The anxiogenic effect of orally administered subclinical doses of *Camphylobacter jejuni* in mice was associated with a significant increase in c-fos expression in neurons bilaterally in the vagal ganglia and activated visceral sensory nuclei in the brainstem. The areas of brainstem activation, the NTS and lateral parabrachial nucleus, participate in neural information processing that ultimately lead to autonomic neuroendocrine and behavioral responses [87]. Similarly, the effect of a combination of *C. rodentium* infection and stress on the central nervous system of mice was accompanied by increased neuronal activation in vagal ganglia, leading the authors to propose that the gut to brain signaling in this instance was mediated through the vagus nerve [76].

Non-pathogenic bacteria also appear to activate vagal signaling from gut to brain. Intraduodenal injection of *L. lactis La1* was demonstrated to activate the gastric vagal nerve [88]. Consequently, infradiaphragmatic denervation of vagal nerve fibers surrounding the esophagus eliminated the ability of *L. lactis La1* to reduce renal sympathetic nerve activity and blood pressure, indicating that at least some of the effects of this bacteria on autonomic nerve responses were elicited by interaction with afferent vagal nerve fibers [88].

Subdiaphragmatic vagotomy blocked the anxiolytic and antidepressant effects of chronic *L. rhamnosus* ingestion in normal adult Balb/c mice, while also preventing the associated alterations in GABAAα2 mRNA expression in the amygdala [81]. Similarly, the ability of *B. longum* to attenuate DSS colitis-induced anxiety was abolished by vagotomy [82].

Overall, studies indicate that vagal pathways mediate signals that can induce both anxiogenic and anxiolytic effects depending on the nature of the stimulus, and, interestingly, the vagus appears to differentiate between non-pathogenic and potentially pathogenic bacteria even in the absence of overt inflammation. Certainly, important advances in our understanding of the microbiome–gut–brain axis will come from studies of how distinct microbial stimuli activate the vagus and the nature of the signals transmitted to the brain that lead to differential changes in

the neurochemistry of the brain and behavior. However, while it appears that the vagus in critical to mediating gut–brain communication by specific bacteria in some model systems, it is by no means the only potential signaling method. Indeed, largely due to technical difficulties, few studies have investigated the role of spinal afferents in mediating bacteria-induced changes in behavior and brain chemistry. It is certainly possible that the observed changes in brain chemistry behavior induced by gut bacteria require parallel input from both the vagal and spinal afferents.

Furthermore, behavioral changes induced through disruption of the microbiota by antibiotic treatment have been demonstrated to be independent of vagal signaling [80], with some additional evidence that neither sympathetic afferents nor immune modulation is required. This clearly suggests that the bacteria in the gut can communicate to the brain through multiple pathways. A potential means of communication, that has been somewhat neglected in existing studies, involves hormonal signaling pathways.

The gut hormonal response

In addition to direct neural pathways, the gut also communicates to the brain utilizing hormonal signaling pathways that involve the release of gut peptides from enteroendocrine cells which can act directly on the brain at the area postrema (which lies outside the blood–brain barrier). These gut peptides include orexin, galanin, ghrelin, gastrin, and leptin. Primarily identified for their role in modulating feeding behavior and energy homeostasis, the gut hormonal response has also be linked with changes in sleep wake cycle, sexual behavior, arousal, and anxiety [104, 105].

Galanin stimulates the activity of the central branch of the HPA axis (i.e. the release of corticotropin-releasing hormone and ACTH), thereby enhancing glucocorticoid secretion from the adrenal cortex. This peptide can also directly stimulate glucocorticoid secretion from adrenocortical cells and norepinephrine release from adrenal medulla [106, 107]. Galanin appears to play a role in modulating the HPA axis response to stress and, given the established deleterious effects of galanin on cognitive function, the hormone may act as a link between stress, anxiety, and memory [108, 109]**.** In this regard, it has been suggested that galaninergic drugs could provide a novel therapeutic option for psychopathologies, such as post-traumatic stress syndrome [107]. Similarly, ghrelin possesses a marked ACTH/cortisol-releasing effect in humans, and is probably involved in the modulation of the HPA response to stress or changes in nutritional/metabolic status [110, 111]. Ghrelin acts in the brain to mediate anxiogenesis and increase memory retention [112]. Studies in gastrin-deficient mice indicate increased anxiety-like behavior compared to wild-type animals, suggesting normal circulating levels of gastrins may play a direct or indirect role in the regulation of locomotor activity and anxiety-like behavior [113, 114].

Neurotensin is an endogenous brain–gut peptide, with a close functional relationship with the mesocorticolimbic and neostriatal dopamine system. Dysregulation of neurotensin neurotransmission in this system has been hypothesized to be involved in the pathogenesis of schizophrenia. Additionally, neurotensin-containing circuits have been demonstrated to mediate some of the mechanisms of action of antipsychotic drugs, as well as the rewarding and/or sensitizing properties of addictive drugs [115].

The pancreatic polypeptide-fold (PP-fold) family includes pancreatic polypeptide (PP) and peptide YY (PYY), and neuropeptide Y (NPY). These peptides have broad peripheral actions on a number of organs. Both NPY and PYY have anxiolytic effects in rats, and NPY has been implicated in feeding and obesity, neuronal excitability, memory retention, anxiety, and depression [116]. Moreover, intracerebroventricular injection of NPY to rats has anti-depressive effects that are antagonized by NPY receptor blockers [117].

Leptin, a hormone secreted from adipose tissue, was originally discovered to regulate body weight. Leptin receptors can be found in limbic structures suggesting a potential role for this hormone in emotional processes. Indeed, Lu et al. [118] demonstrated that rats exposed to chronic unpredictable stress and chronic social defeat exhibit low leptin levels in plasma, and that systemic treatment with leptin reversed behavioral changes induced by chronic unpredictable stress. The behavioral effects of leptin were accompanied by neuronal activation in limbic structures, particularly in the hippocampus. Similar antidepressant-like effects of leptin have also been observed in diabetic mice [119].

While studies in germ-free animals suggest that the gut microflora influences the release of biologically active peptides and participates in the regulation of gastrointestinal endocrine cells [120], little is known about the effect of changes in gut microbiota or probiotic treatment on the expression and release of the hormonal components of gut–brain communication. However, given the ability of gut microbiota to alter nutrient availability [121], and the close relationship between nutrient sensing and peptide secretion by enteroendocrine cells [122], it seems plausible that probiotic treatment may modulate hormonal signaling by the gut. In support of this, piglets treated with the probiotic *Pediococcus acidilactici* were demonstrated to have a greater number of galanin- and calcitonin gene-related peptide (CGRP)-immunoreactive neurons than controls in the submucosal plexus ganglia of the ileum [123].

Furthermore, Lesniewska et al. [124] demonstrated that treatment with a mixture of *Lactobacillus rhamnosus* GG, *Bifidobacterium lactis*, and inulin, in addition to altering gut microflora, increased the portal plasma levels concentrations of NPY and PYY in adult rats, while, in elderly animals, the PYY concentration was unchanged and NPY levels were decreased by treatment. This study not only supports the idea that changes in composition of gut microflora can alter gut hormone release but it also suggests that the effects are dependant on the age and presumably the initial gut physiology and microbiome of the host.

A potentially novel means of gut bacterial influence over hormonal communication to the brain has been proposed by Fetissov and colleagues [125, 126]. These researchers detected IgG and IgA autoantibodies directed against leptin, ghrelin, peptide YY, neuropeptide Y, and other gut regulatory peptides are present in normal human and rat sera, suggesting that the immune system may interfere with peptidergic systems involved in appetite and emotional control [125]. This concept is supported by the demonstration of autoantibodies directed against two melanocortin peptides, α-MSH and adrenocorticotropic hormone (ACTH), in subjects with eating disorders, with correlation between autoantibody levels and the core psychopathologic traits in these patients [127, 128].

Decreased levels of IgA autoantibodies directed against several gut humoral peptides and increased levels of antighrelin IgG were found in germ-free rats compared with specific pathogen-free rats [125]. It was thus identified that, while the commensal microbiota is not required for the presence of IgA or IgG autoantibodies directed against regulatory peptides, it can selectively influence the levels of at least some of these autoantibodies [125].

The potential ability of the microbiota to selectively modulate regulatory peptide autoantibodies may be related to the concept of molecular mimicry. Fetissov et al. [125] identified numerous cases of sequence homology with these peptides among commensal and pathogenic microorganisms, including *Lactobacilli*, *bacteroides*, *Helicobacter pylori*, *Escherichia coli*, and *Candida* species.

The presence of fragments with identical sequences between microbial proteins and regulatory peptides suggests that such microbial proteins, presenting these sequences in the Peyer's patches or other lymphoid organs, may stimulate the production of immunoglobulins capable of binding to the identical region present in endogenous regulatory peptides, and thus modulate the corresponding hormonal signaling pathways.

The role of the gut hormonal response in mediating effects of gut microbiota changes on the CNS is clearly an area of research that demands more attention.

Conclusion

While the field is still in its infancy, study of the microbiome–gut–brain axis has already provided us with strong evidence to support the influence of gut bacteria on the nervous system and brain function. The emerging picture (Table 1) suggests that the gut microbiota plays a role in normal CNS development and, in particular, influences systems associated with stress response and anxiety [7, 58, 59], but may also affect memory function [76]. Exposure to certain key strains of bacteria can also mitigate the effects of early life stress on CNS development [89, 91].

It is also clear that disruption of the microbiota or exposure to specific gut bacteria can modulate brain chemistry and behavior in adult mammals. Effects of gut bacteria in adults include protection from the central effects of infection and inflammation [86, [82, 76], as well as modulation of normal behavioral responses of the animals [81]. While behavioral effects described by gut bacteria in adult animals are again largely related to stress responses and anxiety, these are the behaviors that investigators have focused on to date, and we await future studies that provide a more detailed analysis of gut microbiota influences on additional aspects of brain function, particularly memory and cognition.

An altered HPA axis response to stress is a common effect of gut bacteria in many model systems [7, 59, 81, 91, 89]. This may have important implications when considering the therapeutic potential of gut microbiota modulation. Psychological stress is a common risk factor for the development of major depression, and an identifiable stressor precedes most initial episodes of major depression [129]. Furthermore, hyperactivity of the HPA axis has been found in some psychiatric disorders, especially in older patients with severe depression [130]. Such studies suggest that the relationship between the state of the HPA axis and depression may at least in part be causal. There is therefore the potential that changes in gut microbiota or exposure to specific commensal bacteria may alter the HPA axis or other stress response systems, and in turn modulate stress related mood or behavioral disorders.

There is now robust evidence that gut bacteria influence the enteric nervous system, effects that may, in addition to regulating gut motility, contribute to afferent signaling to the brain [36, 44, 131]. The vagus nerve, which closely monitors gut contractions, has emerged as an important [81, 82], but clearly not exclusive [80], means of communicating signals from gut bacteria to the CNS. The central neural circuits influenced by the gut microbiota are reported to include the GABAergic [81], glutaminergic [7, 59] serotonergic [58], dopaminergic [58], histaminergic [88], and adrenergic [89] systems. Similarly, a number of studies have demonstrated that gut bacteria influence

Table 1 Studies of the microbiome-gut-brain axis

Study	Model	Bacterial treatment	Behavior	HPA stress response	Hippocampal BDNF	Other neural correlates	Vagus
Sudo et al. [7]	Germ-free (mice)	na	–	↑CORT ↑ACTH[a]	↓	NR1, NR2A↓ (hippocampus)	–
Neufeld et al. [59]	Germ-free (mice)	na	Anxiety↓[b]	↑CORT (baseline)	↑	NR2B↓ (amygdala) 5HT1A↓ (hippocampus)	–
Heijtz et al. [58]	Germ-free (mice)	na	Anxiety↓[b]	–	↓	NA, DA, and 5-HT turnover↑ (striatum)	–
Gareau et al. [76]	Germ-free (mice)	na	Memory ↓ Anxiety ○	–	–	–	–
Bercik et al. [80]	Antibiotic (mice)	na	Anxiety↑	–	↓	–	Vagus independent
Gareau et al. [76]	*C. rodentium* infection (mice)	*L. rhamnosus/ L helveticus*	Anxiety↓	↓CORT	↑	–	Vagus activated
Bercik et al. [86]	*T .murius* infection (mice)	*B. longum*	Anxiety↓	–	↑	–	–
Bercik et al. [82]	DSS colitis (mice)	*B. longum*	Anxiety↓	–	–	–	Vagus dependent
Tanida et al. [88]	Mice	*L. johnsonii*[c]	–	–	–	Suprachiasmatic nucleus activation	Vagus dependent
Gareau et al. [91]	Maternal deprivation (rats)	*L. rhamnosus/ L helveticus*	–	↓CORT	–	–	–
Desbonnet et al. [89]	Maternal deprivation (rats)	*B. infantis*	Despair↓	–	–	↑5-HIAA (frontal cortex) ↓DOPAC (amygdala)	–
Bravo et al. [81]	Mice	*L .rhamnosus*	Anxiety↓	↓CORT	–	↓GABAR(Bb1)R (amygdala,hippocampus) ↓GABAR (Aα2) (amygdala) ↑GABAR (Aα2) hippocampus	Vagus dependent

↑ Increase, ↓ decreased, ○ unchanged

5-HT 5-hydroxytryptamine, *5HT1A* 5-hydroxytryptamine receptor 1A, *ACTH* adrenocorticotropic hormone, *CORT* corticosterone, *DA* dopamine; *DOPAC* 3,4-dihydroxyphenylacetic acid, *GABAR* gamma-aminobutyric acid receptor, *NA* noradrenaline, *NR N*-methyl-D-aspartate receptor, – no data provided, *na* not applicable

[a] Effects are mitigated by early colonization with SPF microbiota or *B. infantis* and exacerbated by pathogenic *E. coli*

[b] Effects mitigated by early colonization

[c] Intraduodenal injection

BDNF levels, particularly in the hippocampus [7, 59, 86]. How any of these alterations in brain chemistry are related to specific behavioral changes is unclear, but will likely be a focus of future research efforts.

Some of the major questions remaining concern what the relationship between gut bacteria and the brain means for human health. Is the composition of the gut microbiota associated with psychiatric conditions, as has been proposed for conditions such as obesity [132]? Can the hygiene [133] or microbiota [134] hypothesis for allergic disease also be applied to depression? And can we develop microbial-based therapeutic strategies for mood disorders? [135]. In this regard, human studies have been limited; however, there have been reports of reduced fatigue and anxiety in subjects with chronic fatigue syndrome [136, 137], and a study with a small number of subjects suggested beneficial psychological effects of treatment with a combination of *Lactobacillus helveticus* and *Bifidobacterium longum* in healthy adults [138].

We are at the very early stages of understanding the complex communication systems between gut bacteria and the brain. However, there is already strong supporting evidence for what was, only a few years ago, a largely hypothetical relationship between the gut microbiota, mood, and behavior [13, 135]. The rising interest in this area of research will no doubt lead to greater insights into the mechanisms underlying microbiome–gut–brain communication, and provide us with new understanding of the symbiotic relationship between the gut microbiota and their human host. Future studies will also help us identify the potential for microbial-based therapeutic strategies that may aid in the treatment of mood disorders.

References

1. Drossman DA (1998) Presidential address: gastrointestinal illness and the biopsychosocial model. Psychosom Med 60:258–267
2. Frank DN, Pace NR (2008) Gastrointestinal microbiology enters the metagenomics era. Curr Opin Gastroenterol 24:4–10
3. Kurokawa K, Itoh T, Kuwahara T, Oshima K, Toh H et al (2007) Comparative metagenomics revealed commonly enriched gene sets in human gut microbiomes. DNA Res 14:169–181
4. Marchesi J, Shanahan F (2007) The normal intestinal microbiota. Curr Opin Infect Dis 20:508–513
5. O'Hara AM, Shanahan F (2007) Gut microbiota: mining for therapeutic potential. Clin Gastroenterol Hepatol 5:274–284
6. Sudo N, Sawamura S, Tanaka K, Aiba Y, Kubo C et al (1997) The requirement of intestinal bacterial flora for the development of an IgE production system fully susceptible to oral tolerance induction. J Immunol 159:1739–1745
7. Sudo N, Chida Y, Aiba Y, Sonoda J, Oyama N et al (2004) Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. J Physiol 558:263–275
8. Forsythe P, Bienenstock J Immunomodulation by commensal and probiotic bacteria. Immunol Invest 39: 429–448
9. Lomax AR, Calder PC (2009) Probiotics, immune function, infection and inflammation: a review of the evidence from studies conducted in humans. Curr Pharm Des 15:1428–1518
10. Dantzer R, Konsman JP, Bluthe RM, Kelley KW (2000) Neural and humoral pathways of communication from the immune system to the brain: parallel or convergent? Auton Neurosci 85:60–65
11. Vitkovic L, Konsman JP, Bockaert J, Dantzer R, Homburger V et al (2000) Cytokine signals propagate through the brain. Mol Psychiatry 5:604–615
12. Fagundes CT, Amaral FA, Teixeira AL, Souza DG, Teixeira MM (2012) Adapting to environmental stresses: the role of the microbiota in controlling innate immunity and behavioral responses. Immunol Rev 245:250–264
13. Forsythe P, Sudo N, Dinan T, Taylor VH, Bienenstock J (2010) Mood and gut feelings. Brain Behav Immun 24:9–16
14. Dillon RJ, Vennard CT, Charnley AK (2000) Exploitation of gut bacteria in the locust. Nature 403:851
15. Dillon RJ, Vennard CT, Charnley AK (2002) A note: gut bacteria produce components of a locust cohesion pheromone. J Appl Microbiol 92:759–763
16. Sharon G, Segal D, Zilber-Rosenberg I, Rosenberg E (2011) Symbiotic bacteria are responsible for diet-induced mating preference in *Drosophila melanogaster*, providing support for the hologenome concept of evolution. Gut Microbes 2:190–192
17. Rosenberg E, Koren O, Reshef L, Efrony R, Zilber-Rosenberg I (2007) The role of microorganisms in coral health, disease and evolution. Nat Rev Microbiol 5:355–362
18. Ferveur JF (1997) The pheromonal role of cuticular hydrocarbons in *Drosophila melanogaster*. BioEssays 19:353–358
19. Iyer LM, Aravind L, Coon SL, Klein DC, Koonin EV (2004) Evolution of cell–cell signaling in animals: did late horizontal gene transfer from bacteria have a role? Trends Genet 20:292–299
20. Sobko T, Huang L, Midtvedt T, Norin E, Gustafsson LE et al (2006) Generation of NO by probiotic bacteria in the gastrointestinal tract. Free Radic Biol Med 41:985–991
21. Schicho R, Krueger D, Zeller F, Von Weyhern CW, Frieling T et al (2006) Hydrogen sulfide is a novel prosecretory neuromodulator in the Guinea-pig and human colon. Gastroenterology 131:1542–1552
22. Hughes DT, Sperandio V (2008) Inter-kingdom signalling: communication between bacteria and their hosts. Nat Rev Microbiol 6:111–120
23. Boontham P, Robins A, Chandran P, Pritchard D, Camara M et al (2008) Significant immunomodulatory effects of *Pseudomonas aeruginosa* quorum-sensing signal molecules: possible link in human sepsis. Clin Sci (Lond) 115:343–351
24. Telford G, Wheeler D, Williams P, Tomkins PT, Appleby P et al (1998) The *Pseudomonas aeruginosa* quorum-sensing signal molecule *N*-(3-oxododecanoyl)-L-homoserine lactone has immunomodulatory activity. Infect Immun 66:36–42
25. Clarke MB, Hughes DT, Zhu C, Boedeker EC, Sperandio V (2006) The QseC sensor kinase: a bacterial adrenergic receptor. Proc Natl Acad Sci USA 103:10420–10425
26. Craig AD (2002) How do you feel? Interoception: the sense of the physiological condition of the body. Nat Rev Neurosci 3:655–666
27. Craig AD (2009) How do you feel—now? The anterior insula and human awareness. Nat Rev Neurosci 10:59–70
28. Craig AD (2003) Interoception: the sense of the physiological condition of the body. Curr Opin Neurobiol 13:500–505
29. Cabanac M (1971) Physiological role of pleasure. Science 173:1103–1107

30. Crucian GP, Hughes JD, Barrett AM, Williamson DJ, Bauer RM et al (2000) Emotional and physiological responses to false feedback. Cortex 36:623–647
31. Zagon A (2001) Does the vagus nerve mediate the sixth sense? Trends Neurosci 24:671–673
32. Amiel J, Sproat-Emison E, Garcia-Barcelo M, Lantieri F, Burzynski G et al (2008) Hirschsprung disease, associated syndromes and genetics: a review. J Med Genet 45:1–14
33. Matsuda NM, Miller SM, Evora PR (2009) The chronic gastrointestinal manifestations of Chagas disease. Clinics (Sao Paulo) 64:1219–1224
34. Sato A, Yamamoto M, Imamura K, Kashiki Y, Kunieda T et al (1978) Pathophysiology of aganglionic colon and anorectum: an experimental study on aganglionosis produced by a new method in the rat. J Pediatr Surg 13:399–435
35. Kunze WA, Bornstein JC, Furness JB (1995) Identification of sensory nerve cells in a peripheral organ (the intestine) of a mammal. Neuroscience 66:1–4
36. Kunze WA, Mao YK, Wang B, Huizinga JD, Ma X et al. (2009) *Lactobacillus reuteri* enhances excitability of colonic AH neurons by inhibiting calcium dependent potassium channel opening. J Cell Mol Med 13:2261–2270
37. Mao Y, Wang B, Kunze W (2006) Characterization of myenteric sensory neurons in the mouse small intestine. J Neurophysiol 96:998–1010
38. Kunze WA, Furness JB (1999) The enteric nervous system and regulation of intestinal motility. Annu Rev Physiol 61:117–142
39. Howe DG, Clarke CM, Yan H, Willis BS, Schneider DA et al (2006) Inhibition of protein kinase A in murine enteric neurons causes lethal intestinal pseudo-obstruction. J Neurobiol 66:256–272
40. Keast JR, Furness JB, Costa M (1984) Somatostatin in human enteric nerves. Distribution and characterization. Cell Tissue Res 237:299–308
41. Ekblad E, Winther C, Ekman R, Hakanson R, Sundler F (1987) Projections of peptide-containing neurons in rat small intestine. Neuroscience 20:169–188
42. Furness JB (2006) The enteric nervous system. Blackwell, Oxford
43. Ishii TM, Silvia C, Hirschberg B, Bond CT, Adelman JP et al (1997) A human intermediate conductance calcium-activated potassium channel. Proc Natl Acad Sci USA 94:11651–11656
44. Wang B, Mao YK, Diorio C, Pasyk M, Wu RY et al (2010) Luminal administration ex vivo of a live *Lactobacillus* species moderates mouse jejunal motility within minutes. FASEB J 24:4078–4088
45. Kamiya T, Wang L, Forsythe P, Goettsche G, Mao Y et al (2006) Inhibitory effects of *Lactobacillus reuteri* on visceral pain induced by colorectal distension in Sprague–Dawley rats. Gut 55:191–196
46. Rousseaux C, Thuru X, Gelot A, Barnich N, Neut C et al (2007) *Lactobacillus acidophilus* modulates intestinal pain and induces opioid and cannabinoid receptors. Nat Med 13:35–37
47. Verdu EF, Bercik P, Verma-Gandhu M, Huang XX, Blennerhassett P et al (2006) Specific probiotic therapy attenuates antibiotic induced visceral hypersensitivity in mice. Gut 55:182–190
48. Phillips RJ, Walter GC, Powley TL (2010) Age-related changes in vagal afferents innervating the gastrointestinal tract. Auton Neurosci 153:90–98
49. Mazzia C, Clerc N (1997) Ultrastructural relationships of spinal primary afferent fibres with neuronal and non-neuronal cells in the myenteric plexus of the cat oesophago-gastric junction. Neuroscience 80:925–937
50. Takaki M, Nakayama S (1988) Effects of mesenteric nerve stimulation on the electrical activity of myenteric neurons in the guinea pig ileum. Brain Res 442:351–353
51. Mueller MH, Xue B, Glatzle J, Hahn J, Grundy D et al (2009) Extrinsic afferent nerve sensitivity and enteric neurotransmission in murine jejunum in vitro. Am J Physiol Gastrointest Liver Physiol 297:G655–G662
52. Sarna SK (2007) Enteric descending and afferent neural signaling stimulated by giant migrating contractions: essential contributing factors to visceral pain. Am J Physiol Gastrointest Liver Physiol 292:G572–G581
53. Grangette C, Nutten S, Palumbo E, Morath S, Hermann C et al (2005) Enhanced antiinflammatory capacity of a *Lactobacillus plantarum* mutant synthesizing modified teichoic acids. Proc Natl Acad Sci USA 102:10321–10326
54. Duncker SC, Wang L, Hols P, Bienenstock J (2008) The D-alanine content of lipoteichoic acid is crucial for *Lactobacillus plantarum*-mediated protection from visceral pain perception in a rat colorectal distension model. Neurogastroenterol Motil 20:843–850
55. Kamm K, Hoppe S, Breves G, Schroder B, Schemann M (2004) Effects of the probiotic yeast *Saccharomyces boulardii* on the neurochemistry of myenteric neurones in pig jejunum. Neurogastroenterol Motil 16:53–60
56. Nestler EJ (2005) The neurobiology of cocaine addiction. Sci Pract Perspect 3:4–10
57. Guagnini F, Cogliati P, Mukenge S, Ferla G, Croci T (2006) Tolerance to cannabinoid response on the myenteric plexus of Guinea-pig ileum and human small intestinal strips. Br J Pharmacol 148:1165–1173
58. Heijtz RD, Wang S, Anuar F, Qian Y, Bjorkholm B et al (2011) Normal gut microbiota modulates brain development and behavior. Proc Natl Acad Sci USA 108:3047–3052
59. Neufeld KM, Kang N, Bienenstock J, Foster JA (2011) Reduced anxiety-like behavior and central neurochemical change in germ-free mice. Neurogastroenterol Motil 23(255–64):e119
60. Lucas A (1991) Programming by early nutrition in man. Ciba Found Symp 156: 38–50 (discussion 50–5)
61. Cannistraro PA, Rauch SL (2003) Neural circuitry of anxiety: evidence from structural and functional neuroimaging studies. Psychopharmacol Bull 37:8–25
62. Sah P, Faber ES, Lopez De Armentia M, Power J (2003) The amygdaloid complex: anatomy and physiology. Physiol Rev 83:803–834
63. Deng YS, Zhong JH, Zhou XF (2000) Effects of endogenous neurotrophins on sympathetic sprouting in the dorsal root ganglia and allodynia following spinal nerve injury. Exp Neurol 164:344–350
64. Garraway SM, Petruska JC, Mendell LM (2003) BDNF sensitizes the response of lamina II neurons to high threshold primary afferent inputs. Eur J Neurosci 18:2467–2476
65. Nguyen N, Lee SB, Lee YS, Lee KH, Ahn JY (2009) Neuroprotection by NGF and BDNF against neurotoxin-exerted apoptotic death in neural stem cells are mediated through Trk receptors, activating PI3-kinase and MAPK pathways. Neurochem Res 34:942–951
66. Karege F, Perret G, Bondolfi G, Schwald M, Bertschy G et al (2002) Decreased serum brain-derived neurotrophic factor levels in major depressed patients. Psychiatry Res 109:143–148
67. Shimizu E, Hashimoto K, Okamura N, Koike K, Komatsu N et al (2003) Alterations of serum levels of brain-derived neurotrophic factor (BDNF) in depressed patients with or without antidepressants. Biol Psychiatry 54:70–75
68. Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT (2001) Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry 50:260–265
69. Karege F, Vaudan G, Schwald M, Perroud N, La Harpe R (2005) Neurotrophin levels in postmortem brains of suicide victims and

the effects of antemortem diagnosis and psychotropic drugs. Brain Res Mol Brain Res 136:29–37
70. Fuss J, Ben Abdallah NM, Hensley FW, Weber KJ, Hellweg R et al. (2010) Deletion of running-induced hippocampal neurogenesis by irradiation prevents development of an anxious phenotype in mice. PLoS One 5:e12769
71. Martinowich K, Manji H, Lu B (2007) New insights into BDNF function in depression and anxiety. Nat Neurosci 10:1089–1093
72. Yee BK, Zhu SW, Mohammed AH, Feldon J (2007) Levels of neurotrophic factors in the hippocampus and amygdala correlate with anxiety- and fear-related behaviour in C57BL6 mice. J Neural Transm 114:431–444
73. Bergami M, Rimondini R, Santi S, Blum R, Gotz M et al (2008) Deletion of TrkB in adult progenitors alters newborn neuron integration into hippocampal circuits and increases anxiety-like behavior. Proc Natl Acad Sci USA 105:15570–15575
74. Zuena AR, Mairesse J, Casolini P, Cinque C, Alema GS et al (2008) Prenatal restraint stress generates two distinct behavioral and neurochemical profiles in male and female rats. PLoS ONE 3:e2170
75. Ren-Patterson RF, Cochran LW, Holmes A, Lesch KP, Lu B et al (2006) Gender-dependent modulation of brain monoamines and anxiety-like behaviors in mice with genetic serotonin transporter and BDNF deficiencies. Cell Mol Neurobiol 26:755–780
76. Gareau MG, Wine E, Rodrigues DM, Cho JH, Whary MT et al (2011) Bacterial infection causes stress-induced memory dysfunction in mice. Gut 60:307–317
77. Crowther JS, Drasar BS, Goddard P, Hill MJ, Johnson K (1973) The effect of a chemically defined diet on the faecal flora and faecal steroid concentration. Gut 14:790–793
78. Zentek J, Marquart B, Pietrzak T, Ballevre O, Rochat F (2003) Dietary effects on bifidobacteria and *Clostridium perfringens* in the canine intestinal tract. J Anim Physiol Anim Nutr (Berl) 87:397–407
79. Li W, Dowd SE, Scurlock B, Acosta-Martinez V, Lyte M (2009) Memory and learning behavior in mice is temporally associated with diet-induced alterations in gut bacteria. Physiol Behav 96:557–567
80. Bercik P, Denou E, Collins J, Jackson W, Lu J et al. (2011) The intestinal microbiota affect central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterology 141: 599–609 (609.e1-3)
81. Bravo JA, Forsythe P, Chew MV, Escaravage E, Savignac HM et al (2011) Ingestion of *Lactobacillus* strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci USA 108:16050–16055
82. Bercik P, Park AJ, Sinclair D, Khoshdel A, Lu J et al (2011) The anxiolytic effect of *Bifidobacterium longum* NCC3001 involves vagal pathways for gut-brain communication. Neurogastroenterol Motil 23:1132–1139
83. Bruzzese E, Volpicelli M, Squaglia M, Tartaglione A, Guarino A (2006) Impact of prebiotics on human health. Dig Liver Dis 38(Suppl 2):S283–S287
84. Groot J, Bijlsma P, Van Kalkeren A, Kiliaan A, Saunders P et al (2000) Stress-induced decrease of the intestinal barrier function. The role of muscarinic receptor activation. Ann NY Acad Sci 915:237–246
85. Saunders PR, Kosecka U, McKay DM, Perdue MH (1994) Acute stressors stimulate ion secretion and increase epithelial permeability in rat intestine. Am J Physiol 267:G794–G799
86. Bercik P, Verdu EF, Foster JA, Macri J, Potter M et al (2010) Chronic gastrointestinal inflammation induces anxiety-like behavior and alters central nervous system biochemistry in mice. Gastroenterology 139(2102–2112):e1
87. Goehler LE, Gaykema RP, Opitz N, Reddaway R, Badr N et al (2005) Activation in vagal afferents and central autonomic pathways: early responses to intestinal infection with *Campylobacter jejuni*. Brain Behav Immun 19:334–344
88. Tanida M, Yamano T, Maeda K, Okumura N, Fukushima Y et al (2005) Effects of intraduodenal injection of *Lactobacillus johnsonii* La1 on renal sympathetic nerve activity and blood pressure in urethane-anesthetized rats. Neurosci Lett 389:109–114
89. Desbonnet L, Garrett L, Clarke G, Kiely B, Cryan JF et al (2010) Effects of the probiotic *Bifidobacterium infantis* in the maternal separation model of depression. Neuroscience 170: 1179–1188
90. Porsolt RD, Anton G, Blavet N, Jalfre M (1978) Behavioural despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 47:379–391
91. Gareau MG, Jury J, Macqueen G, Sherman PM, Perdue MH (2007) Probiotic treatment of rat pups normalizes corticosterone release and ameliorates colonic dysfunction induced by maternal separation. Gut 56:1522–1528
92. Cryan JF, Slattery DA (2010) GABAB receptors and depression. Current status. Adv Pharmacol 58:427–451
93. Kesner RP, Hardy JD (1983) Long-term memory for contextual attributes: dissociation of amygdala and hippocampus. Behav Brain Res 8:139–149
94. Marschner A, Kalisch R, Vervliet B, Vansteenwegen D, Buchel C (2008) Dissociable roles for the hippocampus and the amygdala in human cued versus context fear conditioning. J Neurosci 28:9030–9036
95. Jacobson LH, Bettler B, Kaupmann K, Cryan JF (2007) Behavioral evaluation of mice deficient in GABA(B(1)) receptor isoforms in tests of unconditioned anxiety. Psychopharmacology 190:541–553
96. Jacobson LH, Kelly PH, Bettler B, Kaupmann K, Cryan JF (2007) Specific roles of GABA(B(1)) receptor isoforms in cognition. Behav Brain Res 181:158–162
97. Browning KN, Mendelowitz D (2003) Musings on the wanderer: what's new in our understanding of vago-vagal reflexes? II. Integration of afferent signaling from the viscera by the nodose ganglia. Am J Physiol Gastrointest Liver Physiol 284:G8–G14
98. Aston-Jones G, Ennis M, Pieribone VA, Nickell WT, Shipley MT (1986) The brain nucleus locus coeruleus: restricted afferent control of a broad efferent network. Science 234:734–737
99. Aston-Jones G, Rajkowski J, Kubiak P, Valentino RJ, Shipley MT (1996) Role of the locus coeruleus in emotional activation. Prog Brain Res 107:379–402
100. Ziegler DR, Cass WA, Herman JP (1999) Excitatory influence of the locus coeruleus in hypothalamic–pituitary–adrenocortical axis responses to stress. J Neuroendocrinol 11:361–369
101. Arborelius L, Owens MJ, Plotsky PM, Nemeroff CB (1999) The role of corticotropin-releasing factor in depression and anxiety disorders. J Endocrinol 160:1–12
102. Walsh SP, Kling MA (2004) VNS and depression: current status and future directions. Expert Rev Med Devices 1:155–160
103. Wang X, Wang BR, Zhang XJ, Xu Z, Ding YQ et al (2002) Evidences for vagus nerve in maintenance of immune balance and transmission of immune information from gut to brain in STM-infected rats. World J Gastroenterol 8:540–545
104. Cameron J, Doucet E (2007) Getting to the bottom of feeding behaviour: who's on top? Appl Physiol Nutr Metab 32:177–189
105. Wren AM, Bloom SR (2007) Gut hormones and appetite control. Gastroenterology 132:2116–2130
106. Tortorella C, Neri G, Nussdorfer GG (2007) Galanin in the regulation of the hypothalamic-pituitary-adrenal axis (review). Int J Mol Med 19:639–647

107. Wrenn CC, Holmes A (2006) The role of galanin in modulating stress-related neural pathways. Drug News Perspect 19:461–467
108. Rustay NR, Wrenn CC, Kinney JW, Holmes A, Bailey KR et al (2005) Galanin impairs performance on learning and memory tasks: findings from galanin transgenic and GAL-R1 knockout mice. Neuropeptides 39:239–243
109. Wrenn CC, Kinney JW, Marriott LK, Holmes A, Harris AP et al (2004) Learning and memory performance in mice lacking the GAL-R1 subtype of galanin receptor. Eur J Neurosci 19:1384–1396
110. Giordano R, Pellegrino M, Picu A, Bonelli L, Balbo M et al (2006) Neuroregulation of the hypothalamus–pituitary–adrenal (HPA) axis in humans: effects of GABA-, mineralocorticoid-, and GH-Secretagogue-receptor modulation. Sci World J 6:1–11
111. Jaszberenyi M, Bujdoso E, Bagosi Z, Telegdy G (2006) Mediation of the behavioral, endocrine and thermoregulatory actions of ghrelin. Horm Behav 50:266–273
112. Carlini VP, Perez MF, Salde E, Schioth HB, Ramirez OA et al (2010) Ghrelin induced memory facilitation implicates nitric oxide synthase activation and decrease in the threshold to promote LTP in hippocampal dentate gyrus. Physiol Behav 101:117–123
113. Yamada K, Wada E, Wada K (2000) Male mice lacking the gastrin-releasing peptide receptor (GRP-R) display elevated preference for conspecific odors and increased social investigatory behaviors. Brain Res 870:20–26
114. Yamada K, Wada E, Wada K (2001) Female gastrin-releasing peptide receptor (GRP-R)-deficient mice exhibit altered social preference for male conspecifics: implications for GRP/GRP-R modulation of GABAergic function. Brain Res 894:281–287
115. Holsboer F (2003) The role of peptides in treatment of psychiatric disorders. J Neural Transm Suppl 17–34
116. Berglund MM, Hipskind PA, Gehlert DR (2003) Recent developments in our understanding of the physiological role of PP-fold peptide receptor subtypes. Exp Biol Med (Maywood) 228:217–244
117. Ishida H, Shirayama Y, Iwata M, Katayama S, Yamamoto A et al (2007) Infusion of neuropeptide Y into CA3 region of hippocampus produces antidepressant-like effect via Y1 receptor. Hippocampus 17:271–280
118. Lu XY, Kim CS, Frazer A, Zhang W (2006) Leptin: a potential novel antidepressant. Proc Natl Acad Sci USA 103:1593–1598
119. Hirano S, Miyata S, Kamei J (2007) Antidepressant-like effect of leptin in streptozotocin-induced diabetic mice. Pharmacol Biochem Behav 86:27–31
120. Uribe A, Alam M, Johansson O, Midtvedt T, Theodorsson E (1994) Microflora modulates endocrine cells in the gastrointestinal mucosa of the rat. Gastroenterology 107:1259–1269
121. Hsiao WW, Metz C, Singh DP, Roth J (2008) The microbes of the intestine: an introduction to their metabolic and signaling capabilities. Endocrinol Metab Clin North Am 37:857–871
122. Moran-Ramos S, Tovar AR, Torres N (2012) Diet: friend or foe of enteroendocrine cells-how it interacts with enteroendocrine cells. Adv Nutr 3:8–20
123. Di Giancamillo A, Vitari F, Savoini G, Bontempo V, Bersani C et al (2008) Effects of orally administered probiotic *Pediococcus acidilactici* on the small and large intestine of weaning piglets. A qualitative and quantitative micro-anatomical study. Histol Histopathol 23:651–664
124. Lesniewska V, Rowland I, Cani PD, Neyrinck AM, Delzenne NM et al (2006) Effect on components of the intestinal microflora and plasma neuropeptide levels of feeding *Lactobacillus delbrueckii*, *Bifidobacterium lactis*, and inulin to adult and elderly rats. Appl Environ Microbiol 72:6533–6538
125. Fetissov SO, Hamze Sinno M, Coeffier M, Bole-Feysot C, Ducrotte P et al (2008) Autoantibodies against appetite-regulating peptide hormones and neuropeptides: putative modulation by gut microflora. Nutrition 24:348–359
126. Fetissov SO, Hamze Sinno M, Coquerel Q, Do Rego JC, Coeffier M et al (2008) Emerging role of autoantibodies against appetite-regulating neuropeptides in eating disorders. Nutrition 24:854–859
127. Fetissov SO, Hallman J, Oreland L, Af Klinteberg B, Grenback E et al (2002) Autoantibodies against alpha-MSH, ACTH, and LHRH in anorexia and bulimia nervosa patients. Proc Natl Acad Sci USA 99:17155–17160
128. Fetissov SO, Harro J, Jaanisk M, Jarv A, Podar I et al (2005) Autoantibodies against neuropeptides are associated with psychological traits in eating disorders. Proc Natl Acad Sci USA 102:14865–14870
129. Kendler KS, Thornton LM, Gardner CO (2000) Stressful life events and previous episodes in the etiology of major depression in women: an evaluation of the "kindling" hypothesis. Am J Psychiatry 157:1243–1251
130. Leonard BE (2005) The HPA and immune axes in stress: the involvement of the serotonergic system. Eur Psychiatry 20(Suppl 3):S302–S306
131. Wang B, Mao YK, Diorio C, Wang L, Huizinga JD et al (2010) *Lactobacillus reuteri* ingestion and IK(Ca) channel blockade have similar effects on rat colon motility and myenteric neurones. Neurogastroenterol Motil 22(98–107):e33
132. Tehrani AB, Nezami BG, Gewirtz A, Srinivasan S (2012) Obesity and its associated disease: a role for microbiota? Neurogastroenterol Motil 24:305–311
133. Bufford JD, Gern JE (2005) The hygiene hypothesis revisited. Immunol Allergy Clin North Am 25: 247–62
134. Noverr MC, Huffnagle GB (2005) The 'microflora hypothesis' of allergic diseases. Clin Exp Allergy 35:1511–1520
135. Logan AC, Katzman M (2005) Major depressive disorder: probiotics may be an adjuvant therapy. Med Hypotheses 64:533–538
136. Rao AV, Bested AC, Beaulne TM, Katzman MA, Iorio C et al (2009) A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. Gut Pathog 1:6
137. Sullivan A, Nord CE, Evengard B (2009) Effect of supplement with lactic-acid producing bacteria on fatigue and physical activity in patients with chronic fatigue syndrome. Nutr J 8:4
138. Messaoudi M, Violle N, Bisson JF, Desor D, Javelot H et al (2011) Beneficial psychological effects of a probiotic formulation (*Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175) in healthy human volunteers. Gut Microbes 2:256–261