Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour

John F. Cryan[1,2] and Timothy G. Dinan[1,3]

Abstract | Recent years have witnessed the rise of the gut microbiota as a major topic of research interest in biology. Studies are revealing how variations and changes in the composition of the gut microbiota influence normal physiology and contribute to diseases ranging from inflammation to obesity. Accumulating data now indicate that the gut microbiota also communicates with the CNS — possibly through neural, endocrine and immune pathways — and thereby influences brain function and behaviour. Studies in germ-free animals and in animals exposed to pathogenic bacterial infections, probiotic bacteria or antibiotic drugs suggest a role for the gut microbiota in the regulation of anxiety, mood, cognition and pain. Thus, the emerging concept of a microbiota–gut–brain axis suggests that modulation of the gut microbiota may be a tractable strategy for developing novel therapeutics for complex CNS disorders.

Microbiota
The collection of microorganisms in a particular habitat, such as the microbiota of the skin or gut.

[1]*Laboratory of Neurogastroenterology, Alimentary Pharmabiotic Centre, University College Cork, Cork, Ireland.*
[2]*Department of Anatomy and Neuroscience, University College Cork, Cork, Ireland.*
[3]*Department of Psychiatry, University College Cork, Cork, Ireland.*
Correspondence to J.F.C.
e-mail: j.cryan@ucc.ie
doi:10.1038/nrn3346
Published online
12 September 2012

The fields of microbiology and neuroscience in modern medicine have largely developed in distinct trajectories, with the exception of studies focused on the direct impact of infectious agents on brain function, including early investigations of syphilis and, more recently, studies of the neurological complications of AIDS. However, it has recently become evident that microbiota, especially microbiota within the gut, can greatly influence all aspects of physiology[1,2], including gut–brain communication, brain function and even behaviour. Indeed, the initiation of large-scale metagenomic projects such as the Human Microbiome Project has allowed the role of the microbiota in health and disease to take centre stage[3,4].

In this Review we discuss recent studies showing that the gut microbiota can influence brain function. We highlight the different methods that have enabled us to increase our understanding of how the microbiota is integrated into the gut–brain axis and how it modulates behaviour. We then summarize the burgeoning knowledge of the contribution of the gut microbiota to a range of CNS disorders. Harnessing such pathways may provide a novel approach to treat various disorders of the gut–brain axis.

The gut–brain axis: from satiety to stress

The reciprocal impact of the gastrointestinal tract on brain function has been recognized since the middle of the nineteenth century through the pioneering work of Claude Bernard, Ivan Pavlov, William Beaumont, William James and Carl Lange. Even Charles Darwin recognized the importance of this interaction in his classic *The Expression of the Emotions in Man and Animals* (1872), in which he wrote: "The manner in which the secretions of the alimentary canal and of certain other organs … are affected by strong emotions, is another excellent instance of the direct action of the sensorium on these organs, independently of the will or of any serviceable associated habit." In the late 1920s, Walter Cannon, the founding father of the study of gastrointestinal motility, emphasized the primacy of brain processing in the modulation of gut function (see REFS 5–7 for historical perspectives). It is now increasingly being recognized that the gut–brain axis provides a bidirectional homeostatic route of communication that uses neural, hormonal and immunological routes, and that dysfunction of this axis can have pathophysiological consequences[6].

Although much research on the gut–brain axis has focused on its contribution to the central regulation of digestive function and satiety[8,9], there has been an increasing emphasis on its role in other aspects of physiology[7]. The role of the enteric nervous system in gut–brain signalling has been well delineated, as has our understanding of how the brain modulates the enteric

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nervous system and therefore gastrointestinal functions. It is now clear that alterations in brain–gut interactions are associated with gut inflammation, chronic abdominal pain syndromes and eating disorders[6], and that modulation of gut–brain axis function is associated with alterations in the stress response and behaviour[10]. The high co-morbidity between stress-related psychiatric symptoms — such as anxiety — and gastrointestinal disorders — including irritable bowel syndrome (IBS) and inflammatory bowel disorder[11] — is further evidence of the importance of this axis in pathophysiology. Thus, modulation of the gut–brain axis is viewed as an attractive target for the development of novel treatments for a wide variety of disorders ranging from obesity, mood and anxiety disorders to gastrointestinal disorders such as IBS[6]. Moreover, the gut microbiota has emerged as a new player that can have marked effects on this axis.

The gut microbiota

The human gastrointestinal tract is inhabited by 1×10^{13} to 1×10^{14} microorganisms — more than 10 times that of the number of human cells in our bodies and containing 150 times as many genes as our genome[12,13] — and the gut microbiota is therefore often referred to as the forgotten organ[14]. Our appreciation of the relationship between the microbiota, the microbiome and the host is changing rapidly and it is now viewed as being mutualistic (with both partners experiencing increased fitness)[15]. In addition, gut microbiota are now known to have a crucial role in the development and functionality of innate and adaptive immune responses[16,17], and in regulating gut motility, intestinal barrier homeostasis, nutrient absorption and fat distribution[18,19]. Over the past 5 years substantial advances have been made in the development of technologies for assessing microbiota composition at the genetic level[13,20], and this has had, and continues to have, an immense impact on our understanding of host–microorganism interactions.

The estimated number of species in the gut microbiota varies greatly, but it is generally accepted that the adult microbiota consists of more than 1,000 species[13] and more than 7,000 strains[21]. Bacteroidetes and Firmicutes are the two predominant bacterial phylotypes in the human microbiota, with Proteobacteria, Actinobacteria, Fusobacteria and Verrucomicrobia phyla present in relatively low abundance[22]. This colonization is a postnatal event; it commences at birth, when vaginal delivery exposes the infant to a complex microbiota. The initial microbiota has a maternal signature and after 1 year of age a complex adult-like microbiota is evident[23–25].

Although bacterial communities vary greatly between individuals and their precise composition is thought to be at least partially genetically determined[26], they have been proposed to fall into just three distinct types (enterotypes) that are defined by their bacterial composition. Each enterotype is characterized by relatively high levels of a single microbial genus: *Bacteroides* spp., *Prevotella* spp. or *Ruminococcus* spp.[27]. It is becoming clear that the microbiota normally has a balanced compositional signature that confers health benefits and that a disruption of this balance confers disease susceptibility[28]. Diet is one of the key factors that can substantially affect microbiota composition. For example, the *Bacteroides* spp. enterotype has been associated with diets that are high in fat or protein, whereas the *Prevotella* spp. enterotype has been associated with high-carbohydrate diets[29]. Other factors, including infection, disease and antibiotics, may transiently alter the stability of the natural composition of the gut microbiota and thereby have a deleterious effect on the well-being of the host[30]. Interestingly, the core microbiota in the elderly has been reported to be different from that of younger adults[31], and its composition is directly correlated with health outcomes[32].

Given the overarching influence of gut bacteria on health it is perhaps not surprising that a growing body of literature focuses on the impact of enteric microbiota on brain and behaviour and that, as a result, the concept of the microbiota–gut–brain axis has emerged[10,28,33] (FIG. 1). It is worth noting, however, that it is still debated in the field whether the role of the microbiota is sufficiently predominant to warrant its nomenclature being included in an axis independent from the well-described gut–brain axis or whether it should simply be recognized as an important node within the gut–brain axis. What is clear is that there is communication between the gut microbiota and the CNS. The neuroendocrine, neuroimmune, the sympathetic and parasympathetic arms of the autonomic nervous system and the enteric nervous system are the key pathways through which they communicate with each other (FIG. 1), and the gastrointestinal tract provides the scaffold for these pathways. These components converge to form a complex reflex network, with afferents that project to integrative cortical CNS structures and efferents that innervate smooth muscle in the intestinal wall[6]. Crucially, there is a growing appreciation that this communication functions bidirectionally[6]: microbiota influence CNS function, and the CNS influences the microbiota composition through its effects on the gastrointestinal tract. How such communication occurs is not fully understood and probably involves multiple mechanisms (BOX 1).

Microbiota and stress

Although the vast majority of research to date has focused on the impact of the microbiota on CNS function and stress perception (see below), it has long been known that stress and the associated activity of the hypothalamus–pituitary–adrenal (HPA) axis can influence the composition of the gut microbiota[34]. However, the functional consequences of this influence are only now being unravelled[35]. Maternal separation is an early life stressor that can result in long-term increases in HPA axis activity[36]. Maternal separation (between 6–9 months of age) in rhesus monkeys resulted in a substantial decrease in faecal lactobacilli (as assessed by enumeration of total and Gram-negative aerobic and facultative anaerobic bacterial species) 3 days after the initiation of the separation procedure, which returned to baseline by day seven[37]. Stress early in life can also have long-term effects on the composition of the gut microbiota. Analysis of the 16S rRNA diversity in the faeces of adult rats that had

Stress response
The name given to the hormonal and metabolic changes that follow exposure to a threat. It involves the activation of the hypothalamus–pituitary–adrenal axis.

Microbiome
The collective genomes of all of the microorganisms in a microbiota.

Hypothalamus–pituitary–adrenal (HPA) axis
The HPA axis is the endocrine core of the stress system. Its activation results in the release of corticotropin-releasing factor from the hypothalamus, adrenocorticotropic hormone from the pituitary and cortisol (corticosterone in rats and mice) from the adrenal glands.

Maternal separation
A model of stress in early life. Isolation of pups from their mother in early life alters maternal behaviour upon being reunited and results in permanent changes in brain and behaviour in the offspring.

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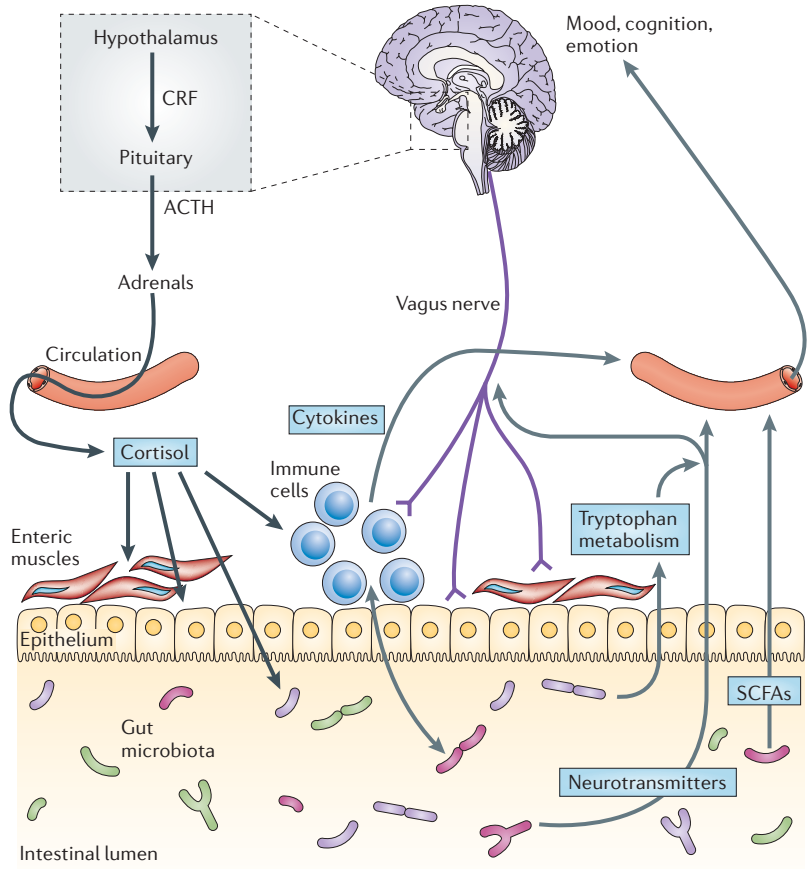


Figure 1 | **Pathways involved in bidirectional communication between the gut microbiota and the brain.** Multiple potential direct and indirect pathways exist through which the gut microbiota can modulate the gut–brain axis. They include endocrine (cortisol), immune (cytokines) and neural (vagus and enteric nervous system) pathways. The brain recruits these same mechanisms to influence the composition of the gut microbiota, for example, under conditions of stress. The hypothalamus–pituitary–adrenal axis regulates cortisol secretion, and cortisol can affect immune cells (including cytokine secretion) both locally in the gut and systemically. Cortisol can also alter gut permeability and barrier function, and change gut microbiota composition. Conversely, the gut microbiota and probiotic agents can alter the levels of circulating cytokines, and this can have a marked effect on brain function. Both the vagus nerve and modulation of systemic tryptophan levels are strongly implicated in relaying the influence of the gut microbiota to the brain. In addition, short-chain fatty acids (SCFAs) are neuroactive bacterial metabolites of dietary fibres that can also modulate brain and behaviour. Other potential mechanisms by which microbiota affect the brain are described in BOX 1. ACTH, adrenocorticotropic hormone; CRF, corticotropin-releasing factor. Figure is modified from REF. 23.

Probiotic
A living microorganism that, when ingested by humans or animals, can beneficially influence health.

Inflamm-ageing
A neologism to reflect the concept that ageing is accompanied by a global reduction in the capacity to cope with various stressors and a concomitant progressive increase in pro-inflammatory status.

undergone maternal separation for 3 hours per day from postnatal days 2–12 revealed an altered faecal microbiota composition when compared with the non-separated control animals[38].

Chronic stress in adulthood also affects the gut microbiota composition. For example, a study using deep-sequencing methods demonstrated that the composition of microbiota from mice exposed to chronic restraint stress (a physical stressor) differed from that in non-stressed control mice[39]. Specifically, exposure to chronic psychosocial stress decreased and increased the relative abundance of *Bacteroides* spp. and *Clostridium* spp., respectively, in the caecum. It also increased circulating levels of interleukin-6 (IL-6) and the chemokine CCL2 (also known as MCP1), which is indicative of immune activation. IL-6 and CCL2 levels correlated with stressor-induced changes in the levels of three other bacterial genera: *Coprococcus* spp., *Pseudobutyrivibrio* spp. and *Dorea* spp. As these genera have only recently been described in humans, the functional importance of these findings to host physiology is unknown. Nevertheless, these data show that exposure to repeated stress affects gut bacterial populations in a manner that correlates with alterations in levels of pro-inflammatory cytokines[39].

In addition to altering the gut microbiota composition, it is important to note that chronic stress also disrupts the intestinal barrier, making it leaky and increasing the circulating levels of immunomodulatory bacterial cell wall components such as lipopolysaccharide[40,41]. These effects can be reversed by probiotic agents[42,43]. In line with these findings, human studies show increased bacterial translocation in stress-related psychiatric disorders such as depression[44]. Recent studies have shown that the potential probiotic *Lactibacillus farciminis* can prevent barrier leakiness, and this underlies its capacity to reverse psychological stress-induced HPA axis activation[43], further confirming the importance of the gut–brain axis in modulating the stress response.

It is worth noting that not all aspects of stress have a negative effect on an animal[45], and the relative contribution of microbiota to the positive stress response and vice versa remains unexplored. Given that we now appreciate that there is a distinct microbiota in the elderly[31,32] and that age is accompanied by a marked diminution in the capacity to cope with a variety of stressors and by a progressive increase in pro-inflammatory status[46], future studies should also focus on the relative contribution of the gut microbiota to this 'inflamm-ageing' process.

Effects on behaviour and cognition

Approaches that have been used to elucidate the role of the gut microbiota on behaviour and cognition include the use of germ-free animals, animals with pathogenic bacterial infections, and animals exposed to probiotic agents or to antibiotics[28] (FIG. 2). Most of these studies highlight a role for the microbiota in modulating the stress response and in modulating stress-related behaviours that are relevant to psychiatric disorders such as anxiety and depression.

Germ-free animals. The use of germ-free animals enables the direct assessment of the role of the microbiota on all aspects of physiology. This approach takes advantage of the fact that the uterine environment is sterile and that colonization of the gastrointestinal tract occurs postnatally in normal rodents and humans. Germ-free animals are maintained in a sterile environment in gnotobiotic units, thus eliminating the opportunity for postnatal colonization of their gastrointestinal tract and allowing for direct comparison with the conventionally colonized gut of their counterparts (FIG. 2).

In a landmark study, Sudo and colleagues[47] provided evidence that intestinal microbiota have a role in the

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development of the HPA axis. In adult germ-free mice, exposure to a mild restraint stress induced an exaggerated release of adrenocorticotropic hormone and corticosterone compared with control mice with a normal composition of microbiota and no specific pathogens (known as specific-pathogen-free mice). The stress response in the germ-free mice could be partially reversed by colonization with faecal matter from control animals and was fully reversed by mono-association with *Bifidobacterium infantis*. Interestingly, the earlier the colonization, the greater the reversal of the effects, and full reversal occurred in the adult offspring when germ-free mothers were inoculated with specific bacterial strains before giving birth[47].

These data clearly demonstrated that the microbial content of the gastrointestinal tract influences the

Box 1 | **Potential mechanisms by which microbiota affect CNS function**

Altering microbial composition. Exogenously administered potential probiotic bacteria or infectious agents can affect the composition of the gut microbiota in multiple ways[121]. For example, they can compete for dietary ingredients as growth substrates, bioconvert sugars into fermentation products with inhibitory properties, produce growth substrates (for example, exocellular polysaccharide or vitamins) for other bacteria, produce bacteriocins, compete for binding sites on the enteric wall, improve gut barrier function, reduce inflammation (thereby altering intestinal properties for colonization and persistence), and stimulate innate immune responses[121]. All of these can have marked effects on gut–brain signalling.

Immune activation. Microbiota and probiotic agents can have direct effects on the immune system[122,123]. Indeed, the innate and adaptive immune system collaborate to maintain homeostasis at the luminal surface of the intestinal host–microbial interface, which is crucial for maintaining health[123]. The immune system also exerts a bidirectional communication with the CNS[124,125], making it a prime target for transducing the effects of bacteria on the CNS. In addition, indirect effects of the gut microbiota and probiotics on the innate immune system can result in alterations in the circulating levels of pro-inflammatory and anti-inflammatory cytokines that directly affect brain function.

Vagus nerve. The vagus nerve (cranial nerve X) has both efferent and afferent roles. It is the major nerve of the parasympathetic division of the autonomic nervous system and regulates several organ functions, including bronchial constriction, heart rate and gut motility. Moreover, activation of the vagus nerve has been shown to have a marked anti-inflammatory capacity, protecting against microbial-induced sepsis in a nicotinic acetylcholine receptor α7 subunit-dependent manner[126]. Approximately 80% of nerve fibres are sensory, conveying information about the state of the body's organs to the CNS[127]. Many of the effects of the gut microbiota or potential probiotics on brain function have shown to be dependent on vagal activation[66,75,76,128]. However, vagus-independent mechanisms are also at play in microbiota–brain interactions, as vagotomy failed to affect the effect of antimicrobial treatments on brain or behaviour[60]. Moreover, the mechanisms through which vagal afferents become activated by the gut microbiota are currently unclear.

Tryptophan metabolism. Tryptophan is an essential amino acid and is a precursor to many biologically active agents, including the neurotransmitter serotonin[129]. A growing body of evidence points to dysregulation of the often-overlooked kynurenine arm of the tryptophan metabolic pathway — which accounts for over 95% of the available peripheral tryptophan in mammals[130] — in many disorders of both the brain and gastrointestinal tract. This initial rate-limiting step in the kynurenine metabolic cascade is catalysed by either indoleamine-2,3-dioxygenase or the largely hepatic-based tryptophan 2,3-dioxygenase. The activity of both enzymes can be induced by inflammatory mediators and by corticosteroids[129]. There is some evidence to suggest that a probiotic bacterium (*Bifidobacterium infantis*) can alter concentrations of kynurenine[82]. However, this is not a universal property of all *Bifidobacterium* strains, as *Bifidobacterium longum* administration had no effect on kynurenine levels[61].

Microbial metabolites. Gut bacteria modulate various host metabolic reactions, resulting in the production of metabolites such as bile acids, choline and short-chain fatty acids that are essential for host health[131]. Indeed, complex carbohydrates such as dietary fibre can be digested and subsequently fermented in the colon by gut microorganisms into short-chain fatty acids such as n-butyrate, acetate and propionate, which are known to have neuroactive properties[110,111,132].

Microbial neurometabolites. Bacteria have the capacity to generate many neurotransmitters and neuromodulators. It has been determined that *Lactobacillus* spp. and *Bifidobacterium* spp. produce GABA; *Escherichia* spp., *Bacillus* spp. and *Saccharomyces* spp. produce noradrenalin; *Candida* spp., *Streptococcus* spp., *Escherichia* spp. and *Enterococcus* spp. produce serotonin; *Bacillus* spp. produce dopamine; and *Lactobacillus* spp. produce acetylcholine[133–135].

Probiotics modulate the concentrations of opioid and cannabinoid receptors in the gut epithelium. However, how this local effect occurs or translates to the anti-nociceptive effects seen in animal models of visceral pain is currently unclear. It is conceivable that secreted neurotransmitters of microorganisms in the intestinal lumen may induce epithelial cells to release molecules that in turn modulate neural signalling within the enteric nervous system, or act directly on primary afferent axons[136].

Bacterial cell wall sugars. The outer exocellular polysaccharide coating of probiotic bacteria is largely responsible for many of their health-promoting effects. Indeed, the exocellular polysaccharide of the probiotic *Bifidobacterium breve* UCC2003 protects the bacteria from acid and bile in the gut and shields the bacteria from the host immune response[137]. Such studies open up the possibility of non-viable bacterial components as microbial-based therapeutic alternatives to probiotics. Indeed, as with neuroactive metabolites, cell wall components of microorganisms in the intestinal lumen or attached to epithelial cells are poised to induce epithelial cells to release molecules that in turn modulate neural signalling or that act directly on primary afferent axons[136].

Mono-association
The inoculation of germ-free animals with a specific bacterium.

Bacteriocins
Proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s).

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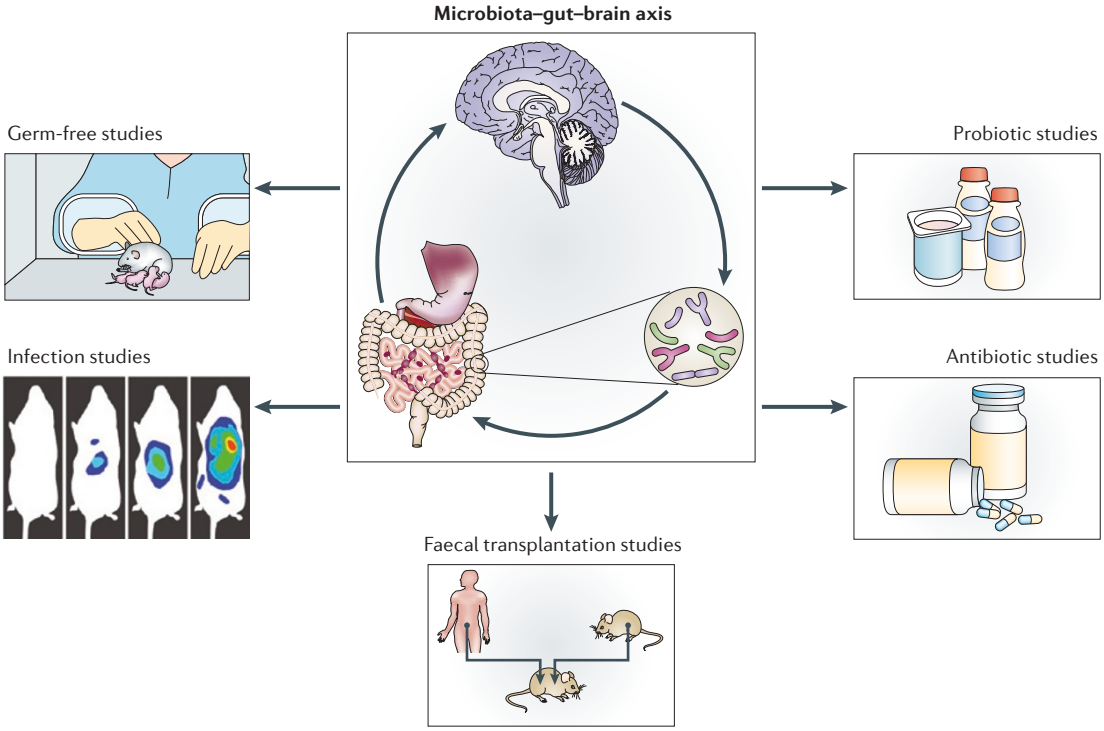


Figure 2 | **Strategies used to investigate the role of the microbiota–gut–brain axis in health and disease.** Although the microbiota–gut–brain axis is a relatively new concept, information about communication along this axis has been delineated through different, converging means. Germ-free mice can be used to assess how loss of microbiota during development affects CNS function. It is worth noting that the clinical translation of such analyses is limited, as no equivalent obliteration of the microbiota can be said to exist in humans. However, germ-free mice also enable the study of the impact of a particular entity (for example, a probiotic) on the microbiota–gut–brain axis in isolation. Moreover, studies in germ-free mice can be expanded to enable research on the 'humanization' of the gut microbiota; that is, transplanting faecal microbiota from specific human conditions or from animal models of disease. Administration of various potential probiotic strains in adult animals or humans can be used to assess the effects of these bacterial 'tourists' on the host. Major strain and species differences occur in terms of their effects on the gut–brain axis. Infection studies have been used to assess the effects of pathogenic bacteria on brain and behaviour, which are mediated largely (although not exclusively) through activation of the immune system. Finally, administration of antimicrobial (that is, antibiotic) drugs can perturb microbiota composition in a temporally controlled and clinically realistic manner and is therefore a powerful tool to assess the role of the gut microbiota on behaviour. However, many antimicrobials are also systemically toxic and this needs to be taken into account when interpreting their effects.

development of an appropriate stress response later in life. Moreover, it seems that there is a critical window in early life during which colonization must occur to ensure normal development of the HPA axis. At the neuronal level, germ-free animals had decreased levels of brain-derived neurotrophic factor (BDNF), a key neurotrophin involved in neuronal growth and survival, and decreased expression of the NMDA receptor subunit 2A (NR2A) in the cortex and hippocampus compared with controls[47].

It took a further 7 years for these findings to be followed up at a behavioural level. Three independent groups have now shown that germ-free animals (of different strains and sex) show reduced anxiety in the elevated plus maze or light–dark box tests[48–50] (but see REF. 51, which failed to show a clear anxiety phenotype); these tests are widely used to assess anxiety-related behaviour[52]. These findings are somewhat puzzling, as an exaggerated HPA axis response to stress is often accompanied by increased anxiety-like behaviour. Interestingly, one study[50] also reported changes in hippocampal *Bdnf* mRNA and 5-hydroxytryptamine (serotonin) 1A (5-HT_{1A}) receptor mRNA expression, as well as *Nr2b* mRNA levels in the amygdala in germ-free mice, but the direction of these changes was not in agreement with data reported in another study[47]. The reasons for these discrepancies are currently unclear. Moreover, although alterations in BDNF, serotonin and glutamate receptor levels have all been implicated in anxiety[53–55], further studies are required to establish how these changes at the molecular level contribute to the manifestation in reduced anxiety-like behaviour observed in germ-free animals.

At the cognitive level, germ-free mice displayed deficits in simple non-spatial and working memory tasks (novel object recognition and spontaneous alternation in the T-maze)[51]. Future studies should focus on enhancing the repertoire of behavioural cognitive assays used. However, maintaining animals in a germ-free environment and conducting complex behavioural studies is not a trivial logistical hurdle.

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It is becoming clear that different mouse strains differ in many aspects of physiology and behaviour[56], including microbiota composition[57–59]. One recent study[60] took advantage of this fact. They reared mice from two strains, BALB/c mice and NIH Swiss mice, under germ-free conditions. When these mice were subsequently colonized with microbiota from their own strain, they exhibited similar exploratory behaviour as their specific-pathogen-free counterparts. However, germ-free mice that were colonized with microbiota from the other strain had a behavioural profile similar to that of the donor strain.

A recent study showed that germ-free animals have elevated hippocampal concentrations of 5-HT and its main metabolite 5-hydroxyindoleacetic acid, compared with conventionally colonized control animals[48]. Plasma concentrations of tryptophan, the precursor of serotonin, were also increased in germ-free animals, suggesting a humoral route through which microbiota can influence serotonergic transmission in the CNS. Interestingly, colonization of the germ-free animals post-weaning restored peripheral tryptophan levels to control values but failed to reverse the changes in serotonin levels in the CNS in adulthood that were induced by an absent microbiota in early life[48]. Importantly, there are sex differences in these effects. Indeed, many of the neurochemical, but not endocrine or immune, effects of growing up in a germ-free environment are only evident in male animals[48].

Taken together, these studies show the utility of germ-free animals in elucidating the mechanisms of communication along the microbiota–gut–brain axis. A growing body of evidence indicates that microbiota have a role in the normal regulation of behaviour and brain chemistry that are relevant to mood and anxiety. Moreover, they intriguingly suggest that an individual's microbiota composition may influence their susceptibility to anxiety and depression. Further behavioural studies in germ-free animals, including the use of other species, such as rats, will greatly expand our knowledge of the role of microbiota in stress-related disorders.

Bacterial infections. Investigating the impact of infections caused by enteric pathogens on brain and behaviour has been an important strategy to interrogate the function of the microbiota–gut–brain axis. A recent set of experiments[61] sought to examine how chronic inflammation of the gut alters behaviour. Here, the authors infected mice with *Trichuris muris*, which is very closely related to the human parasite *Trichuris trichiura*. These mice showed increased anxiety-like behaviour, decreased hippocampal levels of *Bdnf* mRNA, an increased plasma kynurenine:tryptophan ratio (which is indicative of alterations in tryptophan metabolism (BOX 1)), and increased plasma levels of the pro-inflammatory cytokines tumour necrosis factor-α and interferon-γ. Vagotomy before infection with *T. muris* did not prevent anxiety-like behaviour in the infected mice, indicating that the vagus nerve did not mediate the behavioural effects of the infection. Treatment with the anti-inflammatory agents etanercept and budesonide normalized behaviour, reduced circulating cytokine levels and increased tryptophan metabolism, but did not alter *T. muris*-induced changes in hippocampal *Bdnf* mRNA expression. Administration of the probiotic *Bifidobacterium longum* also normalized behaviour. In addition, it restored hippocampal *Bdnf* mRNA levels, but did not affect plasma cytokine or kynurenine levels. Clearly, the mechanism of action of these pharmacological and probiotic interventions differ, nevertheless, all three reversed infection-induced behavioural changes, indicating that the gut microbiota may signal to the brain through multiple routes (BOX 1).

An increasing number of studies have used *Citrobacter rodentium* as an infectious agent to investigate gut–brain axis function. Although infection with this bacterium does not affect baseline behaviour in mice tested 14 days and 30 days after infection[51], an increase in anxiety-like behaviour has been reported a short time following infection[62]. In addition, the animals showed cognitive dysfunction following the resolution of the infection ~30 days post-inoculation (although this only became evident after exposure to an acute stressor protocol) and this effect could be prevented by a pretreatment regimen with a combination of probiotics initiated 7 days before infection[51]. This pretreatment regimen also reduced the increase in serum corticosterone levels and prevented the alterations in hippocampal BDNF and central FOS expression (a marker for neural activity) induced by *C. rodentium* infection. Interestingly, similar cognitive deficits were observed in germ-free mice, regardless of whether they were exposed to acute stress[51].

Together, these data suggest that the effects of infection and stress can converge and synergize to alter CNS function and behaviour and, particularly, cognitive function. Indeed, there is a growing appreciation of the effect of gut–brain signalling on cognitive function in both animals and patients with functional gastrointestinal disorders such as IBS[63]. Similarly, there is a growing body of research aimed at increasing our understanding, at a molecular, cellular and *in vivo* level, of the relationship between dysregulated stress responses and immune system alterations (either individually or in combination) in the aetiology of IBS and the occurrence of symptoms[64].

The vagus nerve is the most probable route for gut-to-brain signalling following infection with *C. rodentium*[62]. Other bacteria also use this route. Studies have taken advantage of FOS immunocytochemistry to map the temporality of the neuronal activation patterns induced by *Campylobacter jejuni*, a food-borne pathogen, in mice[65]. FOS levels were increased in visceral sensory nuclei in the brainstem (1 and 2 days after inoculation) — including the nucleus tractus solitarius, the site of primary afferent termination of the vagus nerve — before areas involved in the stress response such as the paraventricular nucleus of the hypothalamus (2 days after inoculation). In addition, the animals showed increased anxiety-like behaviour in the holeboard test, and the level of anxiety was correlated with neuronal activation as assessed by the number

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Colonic AH neurons
The major intrinsic sensory neurons in the colon. They are termed AH owing to their common electrophysiological properties whereby action potentials are followed by prolonged and substantial after-hyperpolarizing (AH) potentials.

of FOS-expressing cells in the bed nucleus of the stria terminalis, a key component of the extended amygdala fear system[66]. Vagotomy studies have confirmed that the vagus nerve is also involved in the transmission of signals from the gastrointestinal tract to the CNS in rats infected with *Salmonella enterica* subsp. enterica serovar Typhimurium[67]. Although such studies with pathogens do not directly address the ability of the microbiota per se to signal to the brain, they offer key insights in elucidating the pathways through which microorganisms can signal to the brain and affect behaviour.

Probiotics. Probiotics are live organisms that, when ingested in adequate quantities, exert a health benefit on the host[68,69]. They have been reported to have a wide range of effects in both human and animal studies[68,69]; for example in the treatment of the gastrointestinal symptoms of disorders such as IBS[70]. Moreover, there is some clinical evidence to support a role of probiotic intervention in reducing anxiety, decreasing stress responses and improving mood in individuals with IBS and with chronic fatigue[71,72]. Recently, a study assessing the effect of a combination of *Lactobacillus helveticus* and *B. longum* demonstrated that this probiotic cocktail reduced anxiety-like behaviour in animals, and had beneficial psychological effects and decreased serum cortisol levels in humans[73]. This same cocktail also reversed the depression-related behavioural effects observed post-myocardial infarction in rats[74]. Although the mechanism underlying these effects is not known, it has been postulated that they may be due to a dampening down of the effects of pro-inflammatory cytokines and oxidative stress, coupled with modifications in nutritional status[28,71].

In a recent study, ingestion of *Lactobacillus rhamnosus* (JB-1) decreased anxiety and despair-like behaviour and reduced the stress-induced increase of plasma corticosterone levels in mice[75]. Moreover, this potential probiotic altered the mRNA expression of both $GABA_A$ and $GABA_B$ receptors in several brain regions (with a complex pattern of region- and receptor-specific increases and decreases) — alterations in these receptors have been associated with anxious and depression-like behaviours in animal models. Interestingly, these effects are vagus-dependent as vagotomy prevented the anxiolytic and antidepressant effects, as well as the effects on central GABA receptor mRNA levels, of this bacterium. This suggests that parasympathetic innervation is necessary for *L. rhamnosus* to participate in the microbiota–brain interaction. Although some studies have shown that potential probiotics can reverse the behavioural effects of colitis, infection or stress[61], these data are, to our knowledge, the first to show beneficial effects of a probiotic per se in animal assays used to assess anxiolytic or antidepressant activity[52].

Previous studies have shown that the probiotic *B. longum* NCC3001 but not *L. rhamnosus* NCC4007 reversed inflammation and colitis-induced anxiety and alterations in hippocampal *Bdnf* mRNA levels in mice, without affecting gut inflammation or circulating cytokine levels[61,76]. The anxiolytic effect of *B. longum* NCC3001 was absent in mice that had undergone vagotomy, suggesting that a neural mechanism underlies this effect[61,76]. To confirm a neuronal route of action for this potential probiotic, myenteric neurons were treated *in situ* with *B. longum*-fermented medium to determine whether bacterial products generated during fermentation can directly alter the excitatory properties of enteric nerves. Indeed, the firing of action potentials in response to electrical stimulation was greatly decreased in enteric nerves perfused with *B. longum*-fermented medium, indicating that their excitability was directly modulated by probiotic fermentation products[76]. In line with a route of communication through the enteric nervous system, studies have shown that other potential probiotics, such as *L. rhamnosus* (JB-1) (formerly misidentified as a *Lactobacillus reuteri)*, prevented hyperexcitability of colonic dorsal root ganglion neurons induced by noxious stimuli[77] and altered baseline excitability of colonic AH neurons by inhibiting calcium-dependent potassium channels[78]. Other studies have shown that acute administration of *Lactobacillus johnsonii* intraduodenally influenced renal sympathetic and gastric vagal nerve activity through histaminergic pathways[79].

Further evidence of positive effects of probiotics on behaviour arises from studies which demonstrate that the probiotic agent *B. infantis* had antidepressant-like effects and normalized peripheral pro-inflammatory cytokine and tryptophan concentrations, both of which have been implicated in depression[80] and in a maternal separation model of depression[81,82]. Finally, recent studies have shown that fatty acid concentrations in the brain (including arachidonic acid and docosahexaenoic acid) are elevated in mice whose diets were supplemented with the *Bifidobacterium breve* strain NCIMB 702258 (REF. 83). Interestingly, this effect was bacterial strain-dependent as it was not induced by the *B. breve* strain DPC 6330. Arachadonic acid and docosahexaenoic acid are known to play important roles in neurodevelopmental processes, including neurogenesis, can alter neurotransmission and protect against oxidative stress[84,85]. Moreover, their concentrations in the brain influence anxiety, depression and learning and memory[85,86].

Taken together, these data show that certain probiotic strains can modulate various aspects of brain function and behaviour, some of which are vagus dependent. However, caution needs to be exercised when generalizing such effects from one bacterial strain to another, and efforts need to be directed at identifying the mechanism by which each strain induces its effects. Moreover, clinical validation is required to fully investigate the translatability of the encouraging results seen in animal studies to humans. In this vein, it is of interest to note the preliminary report that a probiotic mixture (containing *Bifidobacterium lactis* CNCM I-2494, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, as well as *Lactobacillus lactis*) can substantially alter brain activity in the mid and posterior insula during an emotional reactivity test in healthy volunteers[87]. This finding is particularly interesting as the insula is a key brain region involved in modulating interoceptive signalling from the viscera[88] and has a role in anxiety disorders[89].

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Antibiotics. The use of antimicrobial drugs is one of the most commonly used artificial methods to induce intestinal dysbiosis in experimental animals. Verdu and colleagues[90] have shown that perturbation of the microbiota by oral administration of the non-absorbable antimicrobials neomycin and bacitracin along with the antifungal agent pimaricin (also known as natamycin) in adult mice increased visceral hypersensitivity in response to colorectal distension — an effect that could be reversed by subsequent administration of *Lactobacillus paracasei*. A similar antimicrobial regimen induced an increase in exploratory behaviour and altered BDNF levels in hippocampus and amygdala in mice[60]. These effects were not due to any off-target, systemic effects of these medications as they failed to affect behaviour in germ-free conditions or affect gut inflammation per se. Interestingly, neither vagotomy nor sympathectomy affected the ability of the antimicrobials to induce their effects on behaviour. This suggests that other, as yet unidentified mechanisms, are involved in gut–brain communication in this model of dysbiosis-induced behavioural change[19].

These data highlight the utility of antimicrobial-based strategies in examining the role of microbiota in gut–brain function. Moreover, they demonstrate that assessing the impact on the brain of widespread use of antibiotics in humans is warranted. Future studies with antibiotics could further explore the role of the gut microbiota on brain function and physiology.

The gut microbiota in CNS-related conditions

To date, studies investigating the effects of microbiota composition on brain function predominantly involved animal models of behavioural disorders such as anxiety, depression and cognitive dysfunction, as detailed above. However, accumulating evidence suggests that the composition of the gut microbiota may also have a role in several other conditions that involve the CNS.

Pain. Some of the most convincing data on the importance of the microbiota–gut–brain axis has emerged from the field of pain research, especially visceral pain. Visceral pain is a pronounced and, at times, dominant feature of various gastrointestinal disorders, including IBS. Recurrent, episodic but often unpredictable painful events can have a disabling impact on daily life and result in a reduced quality of life.

The perception of visceral pain involves complex mechanisms. These include peripheral sensitization of sensory nerves and, on a central level, cortical and subcortical pathways. Of interest, there is substantial overlap in the brain areas underlying visceral pain and those that are involved in the processing of psychological stress, a key predisposing factor for visceral hypersensitivity[91]. Imaging studies in humans with IBS[92,93] and in animal models[94–96] have shown increased activation of the anterior cingulate and in the prelimbic and infralimbic cortices in response to viscerally painful and stressful stimuli, indicating that the prefrontal cortex has a key role in IBS.

There is also growing evidence suggesting that both the central and peripheral mechanisms involved in visceral pain perception can be affected by intestinal microbiota. For example, animal studies have shown that probiotics, in particular those of the species *Lactobacilli* and *Bifidobacteria*, can alleviate visceral pain induced by stress and IBS[90,97–101], and many different probiotics have been shown to have beneficial effects in humans with abdominal pain[19,70]. The mechanisms of action of such effects currently remain unclear and may involve a combination of neural, immune and endocrine effects.

A recent study demonstrated that ingestion of the probiotic *B. infantis* 35624 increases the pain threshold and reduces the number of pain behaviours following colorectal distension, which induces visceral pain both in a rat strain that is hypersensitive to visceral pain and in a normal rat strain[98]. Administration of the probiotic *Lactobacillus acidophilus* reduced visceral hypersensitivity in rats by inducing cannabinoid 2 receptor and μ-opioid 1 receptor expression in the colonic epithelium[99]. Furthermore, evidence of a neural mechanism for these effects emerges from studies demonstrating that *Lactobacilli* spp. affected the excitability of rat enteric neurons and nerves innervating the gut, which in turn has effects on colonic motility[77,78,102].

Autism. Autism spectrum disorders (ASD) are neurodevelopmental disorders that are characterized by impairments in social interaction and communication, as well as by the presence of limited, repetitive and stereotyped interests and behaviour. Gastrointestinal symptoms are frequently reported in children with ASD, and this has led to the suggestion that gastrointestinal disturbances, perhaps linked to an abnormal composition of the gut microbiota, may have a role in ASD[103].

Several, albeit relatively small, studies have demonstrated altered intestinal microbiota composition in children with ASD compared with neurotypical children[104–108]. However, such data should be interpreted with caution, as individuals with ASD have a higher incidence of antibiotic usage and often have different diets compared with neurotypical individuals, either of which can influence the composition of the gut microbiota (as discussed above). Interestingly, a recent study also highlights alterations in the faecal concentrations of the short-chain fatty acids in children with ASD[109], suggesting that altered production of such microbial metabolites, which have shown to have neuroactivity, may be a mechanism by which bacteria may alter brain function (BOX 1).

Notably, intracerebroventricular administration of relatively high doses of the short-chain fatty acid propionic acid to animals results in some autistic-like behaviours[110,111]. It is currently unclear whether the doses of propionic acid used in animal studies reflect the potential alterations in short-chain fatty acids observed in individuals with ASD. Interestingly, there has been some transient success in using the antibiotic vancomycin in treating some of the symptoms of regressive-onset autism[112]. Although promising, such studies need replication in a greater numbers of patients. Together, it is clear that larger controlled clinical studies using more sophisticated bacterial analyses are warranted to assess

Dysbiosis
A microbial imbalance on or within the body, often localized to the gut.

Colorectal distension
A method for assessing visceral hypersensitivity. It is a noxious visceral stimulus that can be used in studies performed in animals and humans.

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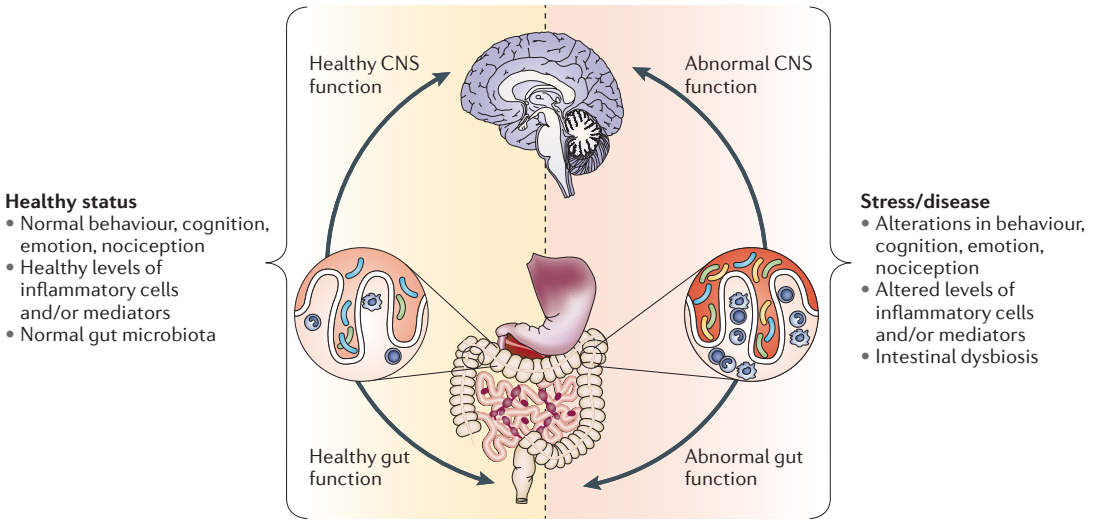


Figure 3 | **Impact of the gut microbiota on the gut–brain axis in health and disease.** It is now generally accepted that a stable gut microbiota is essential for normal gut physiology and contributes to appropriate signalling along the gut–brain axis and, thereby, to the healthy status of the individual, as shown on the left-hand side of the figure. As shown on the right-hand side of the figure, intestinal dysbiosis can adversely influence gut physiology, leading to inappropriate gut–brain axis signalling and associated consequences for CNS functions and resulting in disease states. Conversely, stress at the level of the CNS can affect gut function and lead to perturbations of the microbiota. Figure is modified from REF. 23.

whether ASD is associated with alterations in the gut microbiota and, if so, whether such alterations play a part in the gastrointestinal, behavioural and cognitive symptoms seen in children with ASD.

Obesity. The role of the gut microbiota in the regulation of body weight and metabolism has received much attention over the past 5 years[113]. Gordon and colleagues[114] demonstrated that germ-free mice have less total body fat than conventionally reared mice and are resistant to diet-induced obesity. Moreover, several studies in humans have found a causal link between the composition of the gut microbiota and obesity[113].

Food intake (and, by extension, obesity) is a complex process that involves both peripheral and central mechanisms[115,116]. Most studies investigating the potential role of the gut microbiota on obesity have focused on the peripheral control of food intake, and it is currently unclear whether the gut microbiota can also influence the central regulation of food intake; such studies are now warranted[117]. Obesity can also be a side effect of centrally acting psychotropic drugs, such as atypical antipsychotics, and it is currently being investigated whether gut microbiota mediate these effects. Such studies are based on the finding that gut microbiota composition was altered following treatment with olanzapine in rats[118].

Multiple sclerosis. Multiple sclerosis is a devastating autoimmune disease that is characterized by the progressive deterioration of neurological function. It has been suggested that the gut microbiota may have a role in multiple sclerosis[119]. One study[120] recently showed that the induction of experimental autoimmune encephalomyelitis (EAE), an animal model for the disease, by myelin oligodendrocyte glycoprotein (MOG) peptide in complete Freund's adjuvant (CFA) was greatly attenuated in germ-free mice. This relative resistance is probably due to the reduced immune responses to MOG-CFA in the germ-free animals[120], further exemplifying the extent of the effects of the gut microbiota on CNS function via the immune system.

Similar effects were shown in another study[119], in which mice that were genetically predisposed to spontaneously develop EAE were housed under germ-free or specific-pathogen-free conditions and, as a result, remained fully protected from EAE throughout their life. This protection dissipated upon colonization with conventional microbiota in adulthood. These data illustrate a key role for the gut microbiota in immunomodulatory mechanisms underlying multiple sclerosis, and further studies should also investigate whether other aspects of multiple sclerosis pathophysiology, especially at the spinal-cord level, are affected by the gut microbiota.

Conclusions and perspectives

A growing body of experimental data and clinical observations support the existence of the microbiota–gut–brain axis and suggest that it is poised to control canonical aspects of brain and behaviour in health and disease (FIG. 3). Future research should focus on delineating the relative contributions of immune, neural and endocrine pathways through which the gut microbiota communicates with the brain (BOX 1). A better understanding of these pathways will inform our understanding of the role of the gut microbiota in a range of gastrointestinal and other disorders, including neuropsychiatric diseases such as depression and anxiety, as well as in normal brain function.

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Further work is also needed to tease apart the various factors at play in this complex communication network. Importantly, it is not clear how the various microbial strains can differentially affect CNS functioning, but differences in metabolite production by gut bacteria, the presence of polysaccharides on the bacterial cell wall, direct structural and physical interactions and activation of the immune system are likely contributors. For example, the metabolism of dietary fibre to short-chain fatty acids by some gut bacteria is an important energy source for humans and these metabolites are of importance for gut motility, have a trophic effect on epithelial cells, influence immune system development and modulate enteroendocrine hormone secretion[23]. In addition, certain microorganisms, including *Lactobacillus* spp., are able to convert nitrate to nitric oxide, which is a potent regulator of both the immune and nervous systems, whereas other microorganisms can produce neuroactive amino acids such as GABA[30]. Elucidating the mechanisms by which microbiota communicate with the gut–brain axis will be crucially important for the development of any microbiota-based and microbiota-specific therapeutic strategies for CNS diseases.

As the impact of the gut microbiota in complex conditions such as anxiety and depression, and in cognition, is increasingly being recognized, it is clear that the clinical translation of animal data is now warranted. However, it is important that such studies should be carried out with the same rigour as in pharmaceutical drug development to avoid the emergence of any spurious claims that could affect the perception of the entire field. An additional issue that requires closer examination is the long-term consequences of perturbations in gut microbiota composition in early life by antibiotic treatment or caesarian delivery on brain and behaviour in adulthood. Overall, it is becoming increasingly apparent that behaviour, neurophysiology and neurochemistry can be affected in many ways through modulation of the gut microbiota. Whether this translates to microbial-based CNS therapeutics remains a tempting possibility and one that is worthy of much further investigation.

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Acknowledgements
The authors thank M. Julio-Pieper at Imágenes Ciencia for assistance with figures, and G. Clarke and L. Desbonnet for helpful comments on the paper. The Alimentary Pharmabiotic Centre is a research centre funded by Science Foundation Ireland (SFI), through the Irish Government's National Development Plan. The authors and their work were supported by SFI (grant numbers 02/CE/B124 and 07/CE/B1368).

Competing interests statement
The authors declare no competing financial interests.

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