The interplay between the intestinal microbiota and the brain

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Abstract | The intestinal microbiota consists of a vast bacterial community that resides primarily in the lower gut and lives in a symbiotic relationship with the host. A bidirectional neurohumoral communication system, known as the gut–brain axis, integrates the host gut and brain activities. Here, we describe the recent advances in our understanding of how the intestinal microbiota communicates with the brain via this axis to influence brain development and behaviour. We also review how this extended communication system might influence a broad spectrum of diseases, including irritable bowel syndrome, psychiatric disorders and demyelinating conditions such as multiple sclerosis.

Humans coexist in a mutualistic relationship with the intestinal microbiota, a complex microbial ecosystem that resides largely in the distal bowel. The lower gastrointestinal tract contains almost 100 trillion micro-organisms, most of which are bacteria. More than 1,000 bacterial species have been identified in this microbiota, primarily using molecular-based approaches because the majority of bacteria are strict anaerobes and have not yet been cultivated. Two bacterial divisions, the genus *Bacteroides* and the phylum Firmicutes, account for over 90% of the known phylogenetic categories in the intestinal microbiota. Although there is considerable intersubject variation in the intestinal microbiome, a core microbiome exists that is shared between individuals[1]. The microbiota collectively encodes more than 3.3 million non-redundant genes[1] — exceeding the number encoded by the human host genome by 150-fold — and many microbial gene products have important effects on metabolism and the health of the host. The advent of massively parallel DNA sequencing has enabled metagenomic and metatranscriptomic analyses that, coupled with proteomic and metabolomic studies, have brought about a resurgence of interest in the intestinal microbiome and its impact on a wide range of host processes in health and disease. In particular, recent studies have hinted that the microbiota can have dramatic effects on the development and function of the host brain.

The notion that the commensal intestinal microbiota can influence brain function has at least one clear clinical origin: the observation that orally administered antibiotics can reverse encephalopathy in patients with decompensated liver disease[2]. Furthermore, psychiatric disorders frequently coexist with common gastrointestinal conditions, such as irritable bowel syndrome (IBS), that are also associated with disturbances of the intestinal microbiota[3]. Emerging animal-based research has extended the idea of microbiota–brain interactions to other psychiatric disorders, as well as to immunologically mediated neurological conditions such as multiple sclerosis (MS) and to the exciting area of early brain development. Thus, this rapidly emerging field has the potential not only to increase our understanding of a broad spectrum of human disease, but also to generate novel therapies for these conditions based on the identification of mechanisms underlying microorganism–host interactions.

Here, we review recent progress in understanding the bidirectional interactions between the intestinal microbiota and the brain, and propose a novel conceptual model of a 'microbiota–gut–brain axis', as illustrated in FIG. 1. We go on to assess the evidence for the microbiota–gut–brain axis in a range of neurological diseases.

The gut–brain axis

The gut–brain axis is a communication system that integrates neural, hormonal and immunological signalling between the gut and the brain[4] (BOX 1), and provides the intestinal microbiota and its metabolites with a potential route through which to access the brain. This communication system is bidirectional, enabling the brain to influence gastrointestinal functions (such as motility, secretion and mucin production) as well as immune functions[4] (including the modulation of cytokine production by cells of the mucosal immune system[5]). Emotional factors such as stress or depression influence the natural history of chronic gastrointestinal illnesses such as inflammatory bowel diseases[6] (the two most common of which are Crohn's disease and ulcerative colitis) and IBS[4] via the gut–brain axis. These conditions are also associated with dysbiosis[7]. Stress has been shown to influence the integrity of the gut epithelium and to alter gut motility, secretions and mucin production, thereby altering the habitat of resident bacteria and promoting changes in microbial composition or activity[8]. In addition, stress-induced release of catecholamines into the gut might influence the microbial community by interfering with interbacterial signalling as well as with the expression of bacterial virulence genes[9,10].

Brain development

Studies using well-established behavioural tests[11] (BOX 2) of young germ-free animals have demonstrated the ability of the intestinal microbiota to influence brain development. The response of the hypothalamic pituitary to mild stress is exaggerated in germ-free mice and is normalized following monocolonization of mice with *Bifidobacterium longum* subsp. *infantis* (strain not identified) at 6 weeks of age but not at 14 weeks[12]. Interestingly, in specific-pathogen-free mice (SPF mice), the response is only partially attenuated, indicating that the microbiota contains bacteria that can either enhance or suppress the hypothalamic pituitary axis[12]. Recent studies[13,14] have shown that germ-free mice exhibit more exploratory and risk-taking behaviours as well as more locomotion than SPF mice, and

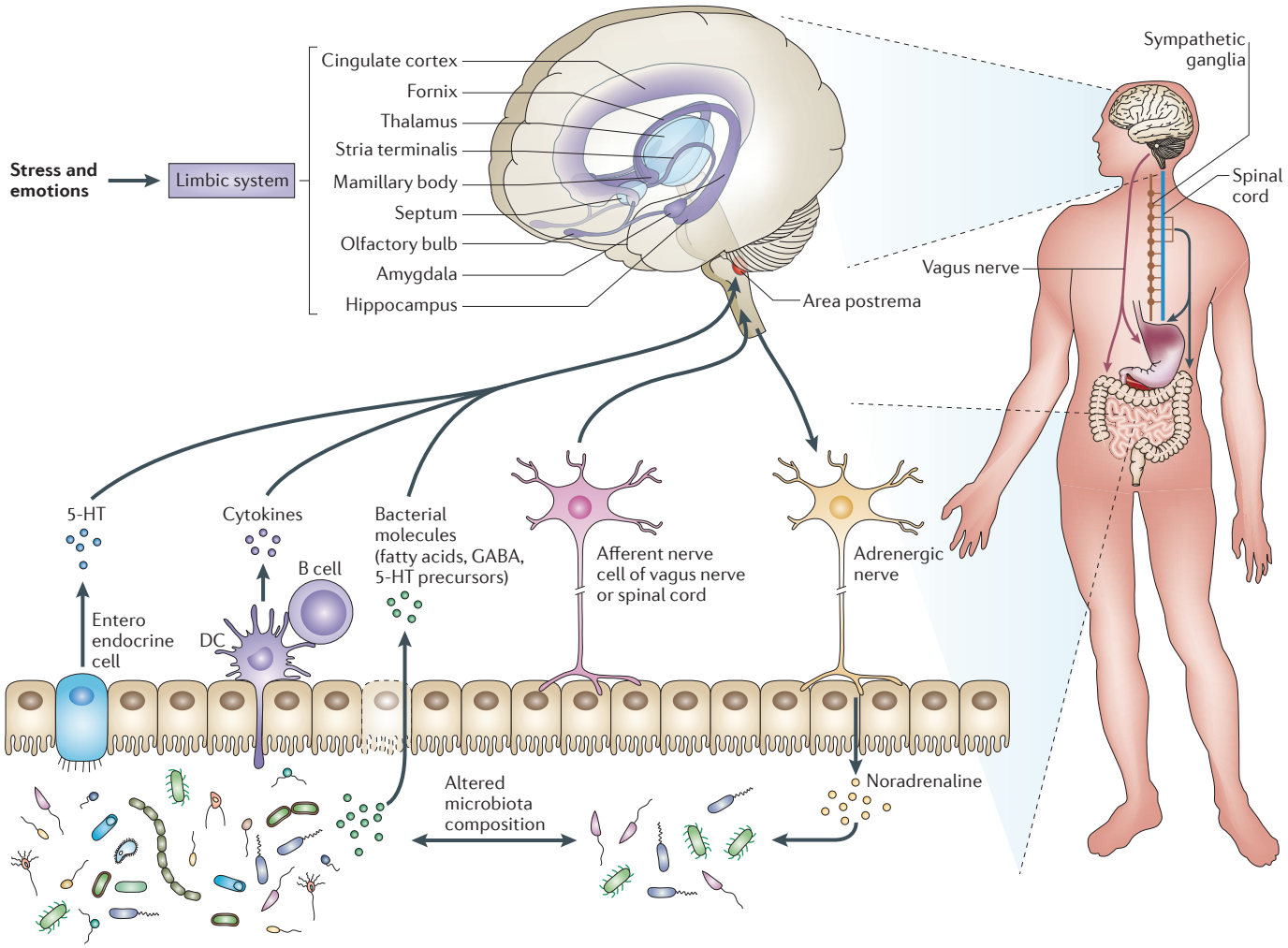


Figure 1 | **The bidirectional microbiota–gut–brain axis.** The neural, immunological, endocrine and metabolic pathways by which the microbiota influences the brain, and the proposed brain-to-microbiota component of this axis. Putative mechanisms by which bacteria access the brain and influence behaviour include bacterial products that gain access to the brain via the bloodstream and the area postrema, via cytokine release from mucosal immune cells, via the release of gut hormones such as 5-hydroxytryptamine (5-HT) from enteroendocrine cells, or via afferent neural pathways, including the vagus nerve. Stress and emotions can influence the microbial composition of the gut through the release of stress hormones or sympathetic neurotransmitters that influence gut physiology and alter the habitat of the microbiota. Alternatively, host stress hormones such as noradrenaline might influence bacterial gene expression or signalling between bacteria, and this might change the microbial composition and activity of the microbiota. DC, dendritic cell; GABA, γ-aminobutyric acid.

suggest that these behaviours are normalized following early, but not late, bacterial colonization[14]. The brain chemistry of germ-free mice differs from that of SPF mice, revealing region-specific changes in the expression of 5-hydroxytryptamine receptor 1 ($5HT_1$) and brain-derived neurotrophic factor (BDNF) in the hippocampus. Furthermore, increased turnover of anxiety-related neurotransmitters such as noradrenaline, dopamine and 5-hydroxytryptamine (5-HT) is seen in germ-free mice, as well as changes in the levels of proteins that regulate the development and function of neural synapses, such as the synaptic vesicle glycoprotein synaptophysin and postsynaptic density protein 95 (PSD95; also known as DLG4) in the striatum. Germ-free mice also exhibit altered spatial working memory and reference memory, which could be indicative of impaired hippocampal development[15]. Taken together, these studies suggest that the presence of intestinal bacteria in mice influences the development of neuronal circuitry that is relevant to a broad spectrum of activities, including anxiety-like behaviour, motor control, memory and learning. BOX 3 describes the relationships between the behavioural phenotype of germ-free mice and the corresponding changes in various brain regions. The regional specificity of some findings either suggests that the mechanisms underlying these changes are selective for certain regions of the brain, or reflects the time point at which experiments were conducted, as the window of opportunity for bacterial influences might differ among brain regions.

The developed brain

In healthy subjects, the intestinal microbiota is generally stable over time[16], in part owing to the presence of a core microbiome[1]. Fluctuations in composition or metabolic activity might occur as a result of dietary change, travel or antibiotic usage, or in response to spontaneous, drug-induced or stress-induced changes in gut transit time[17]. Animal studies have revealed that transient changes in the microbiota influence brain chemistry and behaviour in mice.

For instance, a beef-enriched diet alters the composition of the gut microbiota in mice, and this change is accompanied by improved memory and learning as well as reduced anxiety compared to mice fed a normal diet[18]. Although it is known that a beef diet is rich in amino acids that are known to directly influence learning, such as taurine, these findings suggest that changes in the microbiota also influence behaviour. Another study used a combination of the non-absorbable antimicrobials neomycin, bacitracin and primaricin (also known as nataricin) to induce a transient shift in the intestinal microbiota profile of the mouse[19]. Although the counts for total cultivable bacteria are unchanged in these mice, the antimicrobial treatment reduces the number of gammaproteobacteria (specifically, those from the genera *Shigella and Klebsiella*) and bacteria from the genus *Bacteroides* and increases the lactobacillus and actinobacter populations. The altered microbial profile is accompanied by an increase in exploratory (anxiolytic) behaviour, and corresponding changes in BDNF levels in the hippocampus and amygdala are observed. These changes in brain chemistry and behaviour cannot be attributed to a direct effect of the antimicrobials, as no similar changes are observed when the antimicrobials are administered to SPF mice by intraperitoneal injection or to germ-free mice by gavage. Furthermore, the changes in brain chemistry and behaviour are independent of vagus nerve integrity or sympathetic neurotransmission, and no increase in circulating cytokines is seen[19]. These findings indicate that the behavioural changes induced by destabilization of the microbiota are likely to be mediated by substances of microbial origin acting on the host brain either directly or indirectly (via host metabolism of neuroactive substances). Confirmation that the microbiota influences the brain comes from the observation that behavioural traits of donor mice can be adoptively transferred into adult germ-free mice of a different strain via the intestinal microbiota[19]. This study exploited well-documented differences that exist in the behaviour and the microbiota profiles of strains of laboratory mice. BALB/c mice exhibit anxiety-like behaviour, but when germ-free BALB/c mice are colonized with the microbiota from gregarious SPF US National Institutes of Health (NIH) Swiss mice, they exhibit more exploratory behaviour than SPF BALB/c mice. Conversely, germ-free NIH Swiss mice colonized with the microbiota from SPF BALB/c mice exhibit a reduction in exploratory behaviour compared to SPF NIH Swiss mice. Furthermore, these alterations in behaviour are accompanied by changes in BDNF levels in the hippocampus that parallel the behavioural profiles[19]. Taken together, these findings suggest that in rodents the intestinal microbiota represents a component input to the brain, influencing behaviour on a real-time basis. This may help explain the frequent coexistence of psychiatric illness in patients with IBS[2] — a chronic intestinal disorder in which the microbial composition of the gut exhibits low diversity and is unstable over time[20] — or inflammatory bowel diseases such as ulcerative colitis and Crohn's disease, which have been linked with dysbiosis. In light of the mouse studies described above, shifts in the microbiota might contribute to the behavioural changes that occur in up to 80% of patients with IBS[4]. These studies might also have a bearing on the behavioural changes, ranging from mood alteration and insomnia to mania, that sometimes accompany antibiotic therapy, particularly in the elderly[21].

Box 1 | **Features of the gut–brain axis**

The brain and the gut are intimately connected via the gut–brain axis, which is a bidirectional communication system involving neural and humoral mechanisms. Neural connections involve the central, autonomic and enteric nervous systems. The enteric nervous system receives modulatory input from the brain and provides information to the brain via ascending neural circuits; it can also operate independently of the brain.

The effector limb of the enteric nervous system integrates physiological responses (including gut motility and secretion) and also modulates immune activity, as most immune cells possess receptors for neurotransmitters. The afferent limb comprises sensory nerves that contribute to gut reflexes and convey information to the brain. This information includes signals about noxious stimuli such as gut distension, as well as potentially dangerous signals, including the presence of bacterial endotoxins or pro-inflammatory cytokines. This information is conveyed to the brain and might result in pain, discomfort, or compensatory responses that are aimed at restoring homeostasis; these responses might involve changes in the gut physiology or immune function (for example, cytokine secretion).

The autonomic nervous system links the gut and the brain and consists of sympathetic and parasympathetic nerves. The vagus nerve is a major pathway for signals originating from the foregut and the proximal colon, whereas sacral parasympathetic nerves innervate the distal colon. The sympathetic system primarily exerts an inhibitory influence on the gut, decreasing intestinal motor function and secretion via the release of neurotransmitters such as noradrenaline. Responses to stress are conveyed via the sympathetic system and the hypothalamic–pituitary–adrenal axis.

The autonomic input from the gut is connected to the limbic system of the brain, the most important components of which are the hippocampus, the amygdala and the limbic cortex. The limbic system is responsible for a range of brain processes: the amygdala integrates responses to fear and arousal, whereas the hippocampus is responsible for memory and spatial navigation, and the limbic cortex regulates olfaction and integrates sensory and motor functions. The limbic system receives input from other brain regions that are responsible for a range of behaviours; these regions include the prefrontal cortex, the anterior cingulated gyrus, the temporal lobe and basal ganglia. Communication between the limbic and autonomic systems provides the neural circuitry underlying the strong link between behaviour and gut function in health (such as stomach 'butterflies') and disease (such as irritable bowel syndrome).

The humoral components of the gut–brain axis consist of the hypothalamic–pituitary–adrenal axis, the enteroendocrine system and the mucosal immune system. The hypothalamic–pituitary–adrenal axis is responsible for stress responses, resulting in the release of corticosterone, adrenaline and noradrenaline. Enteroendocrine cells produce hormones such as cholecystokinin and ghrelin, both of which regulate appetite, and 5-hydroxytryptamine, which has a broad range of effects on gut and brain functions.

Probiotics and the gut–brain axis

Studies using defined probiotic bacteria provide further evidence of communication along the microbiota–gut–brain axis and reveal the strain specificity of these interactions. Administration of the probiotic *B. longum* subsp. *infantis* str. 35624 reduces dopamine and 5-HT metabolites in the frontal cortex in rats, but without any discernible change in rat behaviour[22]. However, in a model of depression-like behaviour induced in rat offspring that were separated from their mothers early in life, the same probiotic bacterium normalizes the depression-like behaviour and also induces changes in the levels of noradrenaline and corticotropin-releasing factor (CRF) in the brain[23]. In addition, *B. longum* str. NCC3001 normalizes anxiety-like behaviour and BDNF expression in the hippocampus of mice with mild to moderate colitis (induced by a chronic parasitic infection)[24]. The administration of combinations of probiotic bacteria has also been shown to affect brain function.

Box 2 | **Behavioural assessment in mice**

The choice of behavioural testing in the field of mouse brain research is limited by the risk of bacterial contamination during the experiment, particularly when using germ-free mice. Thus, the apparatus should be simple and amenable to sterilization, and tests involving frequent handling or water immersion are usually avoided. In view of the inherent variability of behavioural responses, large numbers of mice are required (15–20 per test group) to achieve statistical significance. Tests of emotional responsiveness are based on the conflict that is generated by the innate curiosity of mice, causing them to explore unfamiliar objects or environments, and their inherent preference for familiar, dark environments. The light–dark-preference test and the elevated-maze test assess the preference of the mouse for dark, enclosed places over bright, exposed or elevated places. The simpler step-down test measures the latency period for a mouse to leave an elevated platform to explore its surroundings. These tests require simple apparatus, are standardized and are widely used — for example, for the evaluation of anxiolytic drugs. Responses are often described in the context of anxiety-like behaviour. An increase in exploratory behaviour (more time spent in an unfamiliar environment) is described as anxiolytic behaviour. In the tail suspension test, the mouse is suspended from a lever, and the movement in attempting to correct its position is recorded; prolonged immobility is interpreted as a measure of helplessness or depression-like behaviour. Having mice navigate a maze tests their spatial memory, and there are a number of test variations available. Learning is conveniently tested by the ability of a mouse to avoid a noxious stimulus such as a foot shock, and the apparatus of open-field or light–dark-preference tests can be adapted to test learning. A more detailed description of behavioural testing is beyond the scope of this Progress article, and the reader is directed elsewhere[11].

Pretreatment of mice with the combination of *Lactobacillus rhamnosus* str. R0011 and *Lactobacillus helveticus* str. R0052 prevents stress-induced memory dysfunction and normalizes BDNF expression in the CA1 region of the hippocampus[15]. These findings indicate that responses to probiotic bacteria depend in part on the behavioural parameter under study and in part on the model used to perturb behaviour.

The examples given here of probiotic effects on the brain are provided as case studies from a small but rapidly advancing field, rather than as endorsements of any particular probiotics. These studies serve to illustrate the diversity and highly strain-dependent nature of probiotic-induced effects on the brain and their underlying mechanisms, and demonstrate that the specific effects of one strain are not generally shared by other members of the species.

Putative signalling mechanisms

Neural pathways. Two probiotic bacterial strains have been shown to mediate their behavioural effects via the vagus nerve. Chronic administration of *L. rhamnosus* str. JB1 promotes exploratory behaviour in healthy mice, and this is associated with brain region-specific changes in the GABA (γ-aminobutyric acid) system; this effect is dependent on vagal integrity[25]. In a chemical model of low-grade colonic inflammation, *B. longum* str. NCC3001, but not *L. rhamnosus* str. NCC4007, normalizes anxiety-like behaviour in a vagus nerve-dependent manner[26]. Interestingly, *B. longum* str. NCC3001 has no effect on gut inflammation[24,26]. It is possible that the probiotic products alter sensory nerves that link to vagal afferent neurons[27]. The vagal dependence of probiotic effects on the brain contrasts sharply with the vagal independence of the behavioural changes that are induced by destabilization of the microbiota (described above)[19], indicating that gut bacteria communicate with the brain by diverse mechanisms.

Bacterial metabolites and host metabolism. A recent study in mice showed that the microbiota has a substantial impact on the metabolomic profile of the host. Specifically, the microbiota is a major source of both circulating organic acids and tryptophan metabolites[28]. Bacterial fermentation products, including lactic acid and propionic acid, have been shown to influence behaviour in animals. For example, rats that were fed a diet rich in fermentable carbohydrates revealed a strong correlation between D-lactic acid levels in the caecum and displaying anxiety-like behaviour and impaired memory[29]. Human studies also suggest a link between fermentation products and behaviour. For example, high faecal concentrations of propionic acid correlate with anxiety in patients with IBS[30]. In addition, carbohydrate malabsorption, which results in increased substrate availability for bacterial fermentation, has been associated with depression in females[31]. Moreover, bacterial colonization of germ-free mice results in a >2-fold increase in 5-HT and its metabolites (owing to bacterial metabolism of tryptophan), which in turn influence the brain and behaviour[28]. The tryptophan metabolite kynurenic acid acts as an antagonist at excitatory amino acid receptors and has been implicated in major psychiatric illnesses, including schizophrenia[32]. Alterations in the microbial composition of the gut might result in changes in serum kynurenic acid levels and could thus modify central nervous system (CNS) excitation and behaviour. Taken together, these observations suggest that the metabolic products of the intestinal microbiota influence brain function and behaviour in the host. Another putative mechanism by which gut bacteria might influence behaviour is via the production of neurotransmitters. For example, GABA, which has been implicated in anxiety, has recently been shown to be produced by commensal lactobacilli and bifidobacteria in humans[33]. Other neurochemicals that have been isolated from gut bacteria include noradrenaline, 5-HT, dopamine and acetylcholine, and the use of probiotic bacteria that can deliver neurochemicals has been suggested as a novel treatment for neuropsychiatric diseases[34].

Immunological and endocrine mechanisms. The intestinal microbiota imprints and instructs the mucosal immune system throughout the life of the host[35]. The intestinal microbiota also influences immune activation at sites beyond the gastrointestinal tract and might influence host susceptibility to immune-mediated conditions such as diabetes, arthritis and encephalitis[36]. The integrity of the adaptive immune system, and of T lymphocyte responses in particular, are crucial for normal learning and memory in the mouse[37]. Pro-inflammatory cytokines, including interleukin-4 (IL-4) and interferon-γ, have been implicated in a range of psychiatric disorders, including depression[38], and studies in animals[22,23] and humans[39] have shown that manipulation of the gut microbial composition influences systemic cytokine levels. It is possible, therefore, that alterations in the intestinal microbiota influence behaviour by influencing cytokine levels in the systemic circulation and the brain. Under normal conditions, there is a delicate balance between the microbiota and the innate mucosal immune system; changes in the microbial composition that are induced by antibiotics or probiotics disturb this balance, resulting in altered cytokine profiles mediated via activation of Toll-like receptors[40]. For example, *B. longum* subsp. *infantis* str. 35624 induces increased secretion of IL-6 by peripheral blood cells and improves depression-like behaviour in mice that are subjected to maternal separation[23]. In patients with IBS,

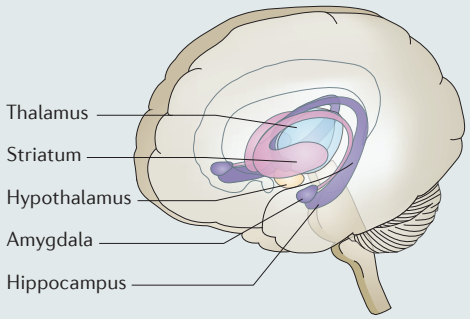
Box 3 | **The microbiota and brain development**

Striatum
The striatum (see the figure) integrates movement and emotional responses and is closely linked to the movement-associated basal ganglia and the limbic system. An increased turnover of dopaminergic and 5-hydroxytryptaminergic neurotransmitters, as well as increases in markers of synaptogenesis, have been observed in the striatum of germ-free mice, contributing to changes in the locomotive and exploratory behaviour of these animals[14].

Hippocampus
The hippocampus (see the figure) is primarily concerned with memory and spatial navigation. A reduction in FOS activity, 5-hydroxytryptamine receptor 1A ($5HT_{1A}$) levels and brain-derived neurotrophic factor (BDNF) expression in the hippocampus contributes to working-memory impairment in germ-free mice[13,15].

Amygdala
The amygdala (see the figure) is part of the limbic system (the 'emotional brain'). In germ-free mice, changes in the levels of NMDARs (*N*-methyl-D-aspartate receptors), $5HT_1$ and BDNF in the amygdala, as well as the changes in the striatum described above, contribute to the increase in risk-taking behaviour that characterizes these mice[12,15]. This brain region provides input to the hypothalamus, which regulates stress responses.

Hypothalamus
The hypothalamus (see the figure) responds to inputs from the limbic system and subsequently initiates stress responses via the pituitary gland and the autonomic nervous system. Germ-free mice exhibit increased expression of corticotropin-releasing factor in the hypothalamus and an exaggerated release of adrenocorticotropic hormone from the pituitary gland in response to a stressful stimulus[12].



administration of the same strain influences the balance between the pro- and counter-inflammatory cytokines released from peripheral blood monocytes[39]. Thus, the intestinal microbiota might influence behaviour by modulating the release of cytokines by the host.

The gastrointestinal tract is the largest endocrine organ in the body; this organ produces hormones such as gastrin and cholecystokinin, which influence appetite control, and 5-HT. Specialized endocrine cells located in the epithelial lining of the gut secrete these molecules and possess specialized microvilli that project into the lumen. As a result, these cells are in close proximity to gut microorganisms, raising the possibility of a functional communication between gut microorganisms and enteroendocrine cells. Indeed, studies comparing germ-free and conventional rats showed that the microbiota influences the number of gut endocrine cells and the release of biologically active peptides[41], providing a further mechanism by which the microbiota might influence behaviour.

The brain alters microbiota composition

The integration of the microbiota into the bidirectional gut–brain axis means that we can also consider the notion that the brain influences the microbial composition of the gut. Along these lines, a recent study using a model of social disruption among adult mice showed that exposure to stress results in substantial changes to the structure of the gut microbial community[42]: *Bacteroides* spp. have a decreased abundance compared to their levels in control mice, whereas the relative abundance of *Clostridium* spp. increases. This stress-induced dysbiosis results in an increase in circulating IL-6 and monocyte chemoattractant protein 1 (MCP1; also known as CCL2)[42]. The mechanisms by which stress influences the gut microbial composition are unclear but might include alterations to the microbial habitat following stress-induced changes in intestinal motility and mucin secretion[8]. Stress also increases the concentration of noradrenaline in the gut lumen, and this might contribute to the changes in microbial composition that are observed in stress models[8]. Catecholamines, including noradrenaline, are known to alter gene expression in some bacteria, resulting in preferential growth of certain communities[9,10,43]. Thus, on the basis of results from animal studies, stress might influence the microbial composition of the gut through several mechanisms that alter the physicochemical properties of the intestinal habitat. This means that the resulting microbiota profile is likely to be nonspecific, as it would depend on the nature, intensity and duration of the stressor and on the stress response of the host.

The intestinal microbiota and CNS disorders

Hepatic encephalopathy. Hepatic encephalopathy is a complication of severe liver disease[2]. In the early stages, it is characterized by an inability to concentrate, forgetfulness, altered sleep patterns and mood changes. This may be followed by abnormal movement (such as tremors), disorientation and, in later stages, deep coma[2]. A recent pyrosequencing-based study showed that patients with cirrhosis exhibit shifts in their gut microbial communities compared to those of healthy controls[44]. In particular, members of the families Alcaligenaceae and Porphyromonadaceae are positively correlated with cognitive dysfunction among encephalopathic patients with cirrhosis. Patients with hepatic encephalopathy also have elevated levels of serum cytokines and endoxins. Although the pathophysiology of hepatic encephalopathy remains incompletely understood, it is likely to be multifactorial and to involve an altered relative abundance of bacteria from certain taxa along with exposure of inflammatory mediators to these bacteria via a leaky intestinal barrier[44]. Nevertheless, these results raise the possibility that faecal transplantation would be beneficial in those patients for whom oral antibiotics either fail to improve cognitive function or produce side effects such as colitis.

Immune-mediated CNS diseases. The microbiota might have a role in immune-mediated CNS diseases such as MS. A well-established model of MS is a spontaneously relapsing experimental autoimmune encephalomyelitis (EAE) in transgenic SJL/J mice that are sensitized to myelin oligodendrocyte glycoprotein (MOG). Germ-free MOG-sensitized transgenic SJL/J mice are resistant to the development of EAE, indicating that there is a role for the microbiota in disease manifestation. Recent studies have identified a crucial role for segmented filamentous bacteria (SFB) in the manifestation of EAE via an IL-17-dependent process[45] and have shown that the commensal microbiota cooperates with the myelin autoantigen to trigger autoimmune-mediated demyelination[46]. These studies provide further evidence that the intestinal microbiota can influence the brain through the activation of mucosal

immune pathways, in this case resulting in severe injury.

Psychiatric disorders. A hypothesis has been put forward that the microbiota is involved in the late-onset (or regressive) forms of autism spectrum disorders, which typically present in individuals older than 18 months. These late-onset forms are associated with a combination of behavioural and gastrointestinal manifestations that include bloating, abdominal discomfort and altered bowel habits. Initially, interest focused on *Clostridium* spp. because of the association of the genus with neurotoxin-mediated tetanus (caused by *Clostridium tetani*) and because autism onset sometimes follows prolonged antibiotic usage (which, for some antibiotics, is known to result in the emergence of *Clostridium* strains), thus implying that there is a postnatal influence on brain development and/or function. Initial culture-based approaches identified *Clostridium clostridioforme* as a candidate organism with a role in autism spectrum disorders, but subsequent molecular-based approaches found a broader range of microorganisms that are more common in children with these disorders than in healthy controls. These organisms include *Desulfovibrio* spp. in addition to several clostridial groups (for a review, see REF. 47). Subsequent studies examined intestinal biopsy material and found that, compared with controls, samples from individuals with late-onset autism spectrum disorders have reductions in *Bifidobacterium* spp. and the mucolytic bacterium *Akkermansia muciniphila* (a species that might predispose to bacterial translocation and promote gut dysfunction)[48]. A metagenomic analysis of intestinal bacteria in patients with late-onset autism found a marked reduction in bacteria from the phylum Bacteroidetes (resulting in an increase in the ratio of Firmicutes members to Bacteroidetes members) as well as an increase in bacteria from the genus *Sutterella* compared to their abundance in controls[49]. This microbial profile is associated with a reduction in ileal transcripts encoding disaccharidases and hexose transporters, implicating carbohydrate malabsorption in these conditions[50]. These findings raise several possibilities regarding dysbiosis and both the gastrointestinal and behavioural manifestations of late-onset autism. Carbohydrate malabsorption results in a different nutritional substrate being available for luminal microorganisms, perhaps promoting a shift in the microbial profile and, consequently, dysbiosis. Given the experimental data presented above, it is possible that the dysbiosis contributes to both the intestinal and behavioural manifestations of these autism spectrum disorders in susceptible individuals. The intestinal-dysbiosis theory of autism is supported by a study demonstrating an apparent benefit of oral vancomycin administration in the treatment of some patients with late-onset autism[51], but this observation requires extensive confirmation in well-controlled trials. To date, little is known about the mechanisms underlying this putative link, but one particularly relevant study showed that intra-cerebroventricular administration of propionic acid, a microbial metabolite, produces autism-like behaviour in rats[52]. It is also possible that microorganisms have a role in autism by influencing the development of the fetal brain. The mouse studies cited in this Progress article indicate that brain development is altered in the offspring of germ-free progenitors, and it is therefore possible that the maternal urogenital and intestinal microbiota influences brain development, not only during and shortly after birth, but also *in utero* (via soluble factors crossing the placental barrier). To date, there is no information correlating maternal microbial profiles with autism or other behavioural conditions in the offspring.

Although altered intestinal fermentation profiles[31] and increases in cytokine levels[39] have been implicated in major depressive disorder, we are unaware of studies comparing the microbiota of untreated patients suffering from this disorder with that of appropriate controls. There has also been speculation about the role of inflammation and the intestinal microbiota in schizophrenia, including the potential role of tryptophan metabolites[32], but no formal studies have been conducted.

Limitations of the field

Although the recent literature provides robust evidence, derived from animal-based studies, that the intestinal microbiota influences brain development and function, there is little information regarding the mechanisms underlying this link. The use of germ-free mice has been crucial in establishing the importance of the microbiota in brain development, but their use in exploring the underlying mechanisms is limited by the fact that host systems are immature in the absence of bacteria. Studies linking the microbiota to behaviour in adult mice have been restricted by the use of strategies (such as antibiotic administration or dietary manipulation) that might influence host behaviour in ways that are independent of their effects on the microbiota[21,53]. The reliance on DNA sequencing alone to characterize the microbiota will hamper mechanistic studies, and such sequencing efforts will need to be combined with transcriptomic and metabolomic approaches to gain mechanistic insights. Our ability to link these studies to human disease states is limited by the fact that few mouse models are congruous with human disease, the fact that tests of

Glossary

5-hydroxytryptamine
(5-HT). A neurotransmitter that is produced mainly by the enteroendocrine cells of the gut, where it is an important regulator of gut physiology (particularly motility); however, 10% of 5-HT is found in the central nervous system, where it contributes to mood. 5-HT, also called serotonin, is derived from tryptophan via the formation of 5-hydroxytryptophan.

Brain-derived neurotrophic factor
A protein that is widely distributed in the nervous system. In the brain, it is found in the hippocampus, amygdala and cortex. It contributes to a range of functions, including memory, mood and learning.

Core microbiome
The 50–100 bacterial species that are common to the microbiomes of many individuals. Outside of these core species, there is considerable diversity in the microbiome components among healthy subjects.

Dysbiosis
A compositional change in the microbiota and/or an abnormality in the interactions between the host and the commensal microbiota.

Encephalopathy
An impairment of brain function, ranging in severity from mild confusion to deep coma. Severe liver disease is a common cause.

GABA
(γ-aminobutyric acid). An inhibitory neurotransmitter that is found throughout the nervous system, including in the central and enteric nervous systems.

Postsynaptic density protein 95
A component of the postsynaptic density, a lattice-like array of proteins that is crucial for synaptic function.

Probiotic
According to the WHO: "Live microorganisms which when administered in adequate amounts confer a health benefit on the host."

Specific-pathogen-free mice
Laboratory mice that are free of defined pathogens.

Synaptophysin
An integral membrane protein of small synaptic vesicles. Initially considered a synaptic marker, it is now thought to have several roles in synaptic function throughout the nervous system.

Toll-like receptors
Transmembrane proteins that recognize highly conserved molecules of microbial origin and subsequently trigger activation of the innate immune system.

behavioural responses in mice are limited by concerns about bacterial contamination, and the fact that substantial difference exist between the human and mouse microbiomes. These reservations notwithstanding, this emerging field of research offers exciting opportunities.

Future directions

Current approaches have laid the groundwork for the use of mice with specific gene deletions in order to identify the mechanisms underlying the ability of the microbiota to influence host behaviour. This approach is applicable primarily to genes that regulate metabolic pathways (for example, tryptophan metabolism) and immunological pathways (for example, Toll-like receptor signalling) in the host. A similar strategy can be applied to microbial gene deletions, provided of course that future research demonstrates the ability of monocolonization or limited-colonization strategies to influence brain development and behaviour. The finding that the behavioural effects of certain probiotics are dependent on the integrity of the vagus nerve warrants a closer examination of the interactions of commensal and probiotic bacteria with enteric nerves.

Future research should exploit the ability of the microbiota to influence repair and remodelling in the CNS, given the evidence supporting a microbial influence on the expression of crucially important proteins such as synaptophysin and PSD95 in the brain[14].

Brain development occurs *in utero* and continues after birth. During vaginal delivery, the gastrointestinal tract of the newborn is colonized by the bacteria in the lower birth canal and perineum of the mother; therefore, the microbiota of infants delivered by caesarian section differs from that of infants delivered through the genital tract. Studies in rats indicate that rats delivered at term by caesarian section exhibit alterations (compared with rats delivered vaginally) in the prepubertal development of the prefrontal cortex and hippocampus[54], regardless of whether the rats suffer anoxia during delivery. A study on human neonates showed that the pattern of electrical activity in the brain is less complex in neonates born by caesarian section than in age-matched neonates born by vaginal delivery[55]. These results raise the possibility that different colonization patterns influence early postnatal brain development and also have longer-term consequences. Additional longitudinal studies are essential to evaluate cognitive function and the microbiome in children born via caesarian section or vaginal delivery.

The data reviewed here will hopefully prompt studies aimed at taxonomic and functional profiling of the microbiota (using 16s rRNA gene sequencing or shotgun DNA sequencing, respectively) in patients with primary psychiatric disorders such as depression, anxiety and schizophrenia. These investigations should be complemented by a broad array of approaches, including metatranscriptomics, proteomics and metabolomics, that will lead to the identification of the mediators and mechanisms underlying microbiota–brain communication. This research should also be extended to immunologically mediated disorders such as MS.

The interpretation of a causal relationship between microbiota profiles and the human (and even mouse) behavioural phenotype is inherently limited in association studies. However, attempts should be made to demonstrate the ability of the identified microbiota to induce behavioural or neurochemical changes in another host. This has been achieved in animal work, which has seen the successful transfer of at least components of a behavioural phenotype between different mouse strains[19]. Previous studies have obtained microbiota-mediated transfer of a phenotype in the context of obesity: the body mass phenotype is exhibited in germ-free mice following colonization with bacteria from obese human donors. The current interest in the therapeutic use of faecal transplantation for conditions such as refractory or recurrent *Clostridium difficile* infection provides an opportunity to poach the pre- and post-transplant behavioural assessments of transplant recipients in future studies.

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Competing interests statement
The authors declare competing financial interests: see Web version for details.

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