The gut microbiota–brain axis in behaviour and brain disorders

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Abstract | In a striking display of trans-kingdom symbiosis, gut bacteria cooperate with their animal hosts to regulate the development and function of the immune, metabolic and nervous systems through dynamic bidirectional communication along the 'gut–brain axis'. These processes may affect human health, as certain animal behaviours appear to correlate with the composition of gut bacteria, and disruptions in microbial communities have been implicated in several neurological disorders. Most insights about host–microbiota interactions come from animal models, which represent crucial tools for studying the various pathways linking the gut and the brain. However, there are complexities and manifest limitations inherent in translating complex human disease to reductionist animal models. In this Review, we discuss emerging and exciting evidence of intricate and crucial connections between the gut microbiota and the brain involving multiple biological systems, and possible contributions by the gut microbiota to neurological disorders. Continued advances from this frontier of biomedicine may lead to tangible impacts on human health.

At the intersection of microbiology and neuroscience, seminal research, largely over the past decade, is revealing dynamic interactions between animals and their resident bacterial communities that contribute to the formation and function of neurological systems. These conversations are complex, involving languages spoken through immunological, neuronal and chemical signalling, but they have crucial impacts on health and how we understand neurological disease. Historically, mental illness has been viewed as driven solely by defects in brain processes; however, this brain-centric perspective neglects the fact that the development and function of the nervous system (BOX 1) is affected by the metabolic and immune state of the body[1]. Contemporary research is starting to appreciate how microorganisms influence the brain through their ability to produce and modify many metabolic, immunological and neurochemical factors in the gut that ultimately impact the nervous system[2–5]. This new perspective has led to a flood of research correlating microbial communities, and their function, to neuropsychiatric disorders associated with development (for example, autism spectrum disorder (ASD) and schizophrenia), mood (for example, depression and anxiety) and neurodegeneration (for example, Parkinson disease (PD), Alzheimer disease (AD) and multiple sclerosis). Research to decipher these relationships has relied heavily on simplified animal models, which are limited in their ability to recapitulate the complexities of human disease. Although widespread validation of mechanisms underlying connections between the gut microbiota and neuropsychiatric disorders is lacking[6], new technologies are being developed to move beyond correlative studies to the discovery and validation of biological mechanisms of action that offer real potential to treat human disease. In this Review, we describe the state of the art in gut microbiota–brain research, evidence for involvement of the gut microbiota in disorders of the brain and intriguing opportunities for new therapeutic and diagnostic options to augment existing interventions.

The gut microbiota–brain axis

All animals, including humans, evolved in intimate association with microbial communities, comprising bacteria, archaea, fungi and viruses. These collections of microorganisms, termed the microbiota, inhabit nearly every environmentally exposed body surface, with the community in the gastrointestinal tract (that is, the gut microbiota) representing the greatest density and absolute abundance of microorganisms in the human body. Rather than being passive passengers in our bodies, considerable research has revealed the crucial importance of the gut microbiota to the function of our immune systems[7], metabolism[8] and even the development of various organs[9]. Gut bacterial communities are dynamic entities that can change both in composition and activity throughout our lives and in response to host factors, such as age and genetics[10], and to changing environmental factors, chief amongst them being diet[11] and drugs[12]. Although gut microbiota research includes analyses of

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Box 1 | **The mammalian nervous system**

The mammalian nervous system is organized into two subdivisions: the central nervous system (CNS) and the peripheral nervous system. Whereas the CNS is composed of the brain and spinal cord, the peripheral nervous system is formed by ganglia nerve branches that innervate different organs of the body. Mammalian behaviour arises from the activity of a complex network of highly specialized cells (for example, microglia, astrocytes, oligodendrocytes and glial progenitors) that communicate through synapses. Some aspects of brain development depend on signals from the gut microbiota. As an example, germ-free (GF) mice exhibit alterations in neurogenesis[184] and colonization of GF mice with preterm human faecal samples affects early neuron and oligodendrocyte development[185]. Furthermore, appropriate myelin patterns in the brain are disrupted in GF mice[186] and perturbations to the microbiota with antibiotics during the postnatal period alter myelination[187]. These and other studies discussed in this Review support the idea of developmental windows, wherein appropriate brain development depends on input from the gut microbiota during specific prenatal or postnatal periods[188].

The autonomic nervous system is a subdivision of the peripheral nervous system that regulates essential visceral processes through complementary responses coordinated by sympathetic and parasympathetic systems. The discovery of the enteric nervous system (ENS), which is a branch of the autonomic nervous system, marked a major scientific advance in better understanding the bidirectional communication between the CNS and the gastrointestinal tract. Known as the 'second brain of the body', the ENS is crucial for stable gut health and is maintained through a collaborative effort between enteric neurons and connections to the CNS[189]. Communication between the CNS and the ENS travels through different signalling pathways, including both direct neuronal and endocrine pathways[30]. Importantly, this communication can be modulated by the gut microbiota.

fungi, archaea and viruses, the greatest amount of information comes from studies of bacteria, which is the focus of this Review. Bacteria found in the gut microbiota equal the number of human cells in the body[13] and the genetic repertoire found in the collective gut microbiome is estimated to include a staggering 232 million genes[14], which greatly expand the metabolic potential of humans (the microbiota has equivalent metabolic capacity to the human liver)[15]. The gut microbiota acts as a filter and biological rheostat for sensing, modifying and tuning vast amounts of chemical signals from the environment that then circulate throughout the body. As such, gut bacterial communities lie at the intersection of the host and the environment, and may directly influence human health.

The 'gut–microbiota–brain axis' refers to the network of connections involving multiple biological systems that allows bidirectional communication between gut bacteria and the brain (FIG. 1), and is crucial in maintaining homeostasis of the gastrointestinal, central nervous and microbial systems of animals[1,6]. The communication pathways in these biological networks include both direct and indirect signalling via chemical transmitters, neuronal pathways and the immune system, as described below. Given the many biological systems involved, it is likely that multiple mechanisms and pathways act in concert to mediate various aspects of disease pathogenesis and more research is needed to understand the mechanisms involved. The complexity of these connections is explored in more detail throughout the Review, and areas of intersection between the various communication modalities (for example, chemical, neuronal and immunological) are highlighted in the context of human disease.

Chemical signalling between the gut and the brain. The gut microbiota can help modulate homeostasis and behaviour in its animal host through chemical communication with the nervous system, including both 'direct' and 'indirect' signalling (FIG. 2). As an example of direct signalling, short-chain fatty acids (SCFAs) are lipids produced by intestinal microorganisms through fermentation of dietary fibre that can act on the central nervous system (CNS) by regulating neuroplasticity, epigenetic and gene expression, and the immune system in preclinical models (reviewed in REF.[16]). SCFAs may impact disease and behaviour. For example, expression of a neuronal factor associated with depression, brain-derived neurotrophic factor (BDNF), was altered in mice by acute, exogenous administration of the SCFA sodium butyrate[17]. In the same study, chronic administration of exogenous sodium butyrate over 28 days resulted in a significant decrease in depressive-like behaviours in mice. The microbiota also influences the nervous system and behaviour through indirect chemical signalling, as can be seen in microbial regulation of the neuroendocrine system[18]. Gut microorganisms can affect their host's appetite and feeding behaviours by modulating production of endocrine signals from enteroendocrine cells (EECs) in the gut epithelium, including production of the hormone glucagon-like peptide 1 (GLP1)[19,20]. Mice lacking an endogenous microbiota, termed germ-free (GF) mice, eat less than conventional mice with an intact microbiota[21] and both GF and antibiotic-treated mice produce less GLP1 than their conventionally colonized counterparts[22], thus showing that the gut microbiota can affect this endocrine-mediated behaviour. In fact, hunger can be modulated by GLP1 secreted by colonic enteroendocrine L cells in response to the bacterial metabolite indole that stimulates colonic vagal afferent activity in rats[23]. Moreover, the gut microbiota also modulates concentrations of neurotransmitters in model systems, which implicates microorganisms as mediators of classical signalling molecules used by the nervous system[24–27] (FIG. 2). Gut microorganisms are capable of synthesizing neurotransmitters themselves and can induce production of neurotransmitters by their animal hosts. For example, several microorganisms (such as *Bacteroides*, *Bifidobacterium*, *Parabacteroides* and *Escherichia* spp.) are known to produce the neurotransmitter γ-aminobutyric acid (GABA)[24].

Bacteria are also important in the production of the neurotransmitter serotonin (5-hydroxytryptamine (5-HT)), as has been demonstrated in model systems[3]. 5-HT production and secretion by EECs is affected by microbial metabolites including indole, SCFAs, secondary bile acids, α-tocopherol, *p*-aminobenzoate and tyramine[26,28]. GF mice and antibiotic-treated mice show decreased 5-HT biosynthesis, but this phenotype can be rescued by inoculation with spore-forming bacteria that increase tryptophan metabolism by enterochromaffin cells[26]. Notably, spore-forming bacteria from healthy human gut microbiota are also able to induce similar effects when transplanted into GF mice, indicating that the gut microbiota's effects on tryptophan metabolism are common evolutionary features across mammals[26]. The majority of 5-HT is produced in the gut, and enteric

Microglia
The primary resident immune cells in the central nervous system, responsible for pathogen surveillance, immune protection and synaptic pruning. Microglia have been implicated in psychiatric and neurodegenerative disorders, largely in animal models.

Astrocytes
A subtype of glial cells in the central nervous system that play an essential role in blood–brain barrier formation and function, among other activates such as interfacing with microglia and neurons.

Oligodendrocytes
Brain cells that regulate development of neurons and insulate neuronal axons through the formation of the protective myelin sheath.

Homeostasis
The process of maintaining physiological functions necessary for survival of an organism.

Neuroplasticity
The ability of the nervous system to change activity by reorganizing its structure and function.

Epigenetic
DNA modifications that do not alter the sequence but can impact gene expression and biological outcomes.

Brain-derived neurotrophic factor
(BDNF). A protein that has an important role in neuronal survival, growth and synaptic plasticity. Alterations in expression are associated with mood disorders.

γ-Aminobutyric acid
(GABA). The main inhibitory neurotransmitter in the adult brain; crucial for synaptic plasticity and learning.

levels do not directly affect levels of 5-HT in the brain as 5-HT cannot bypass the blood–brain barrier (BBB)[26]. Nevertheless, GF mice have decreased concentrations of 5-HT and its metabolic precursor tryptophan in the hippocampus, suggesting a role for the gut microbiota in modulating 5-HT signalling pathways in the CNS[3]; however, a mechanistic link between the gut microbiota and 5-HT production in the brain remains to be determined. In fact, it is difficult to determine the extent to which microbial metabolism directly influences activity of the CNS, partly because we do not have a clear understanding of the general rate of transport into the brain for many microbial metabolites. As such, direct effects of microbial metabolites on CNS function are difficult to separate from other communication pathways (that is, immunological or neuronal), which confound in vivo experiments[29]. In future research, integrating metabolomic and metagenomic profiles with functional behavioural outcomes will allow a better resolution of the impact of chemical signalling on gut–brain connections.

Neuronal pathways for gut–brain interactions. Neuronal pathways physically link the gut and the brain. Chief amongst these neuronal pathways is the vagus nerve, which extends from the brainstem to innervate the gut and the enteric nervous system (ENS) (BOX 2). Development and function of the ENS is partially mediated by the gut microbiota (reviewed in REF.[30]), although this area of research remains largely unstudied. Neuronal innervation of the colonic epithelium is reduced in GF mice and restored by microbial colonization[31]. In addition, gut microbiota regulate the development of enteric glial cells in mice, which are important for regulating gut homeostasis and maintenance of neuronal networks[32,33]. The gut microbiota can affect the function of enteric neurons through chemical signalling, as shown by recent evidence that activation of aryl hydrocarbon receptors in adult mice can regulate gut motility through effects on the ENS[34]. Microbial products including bacterial

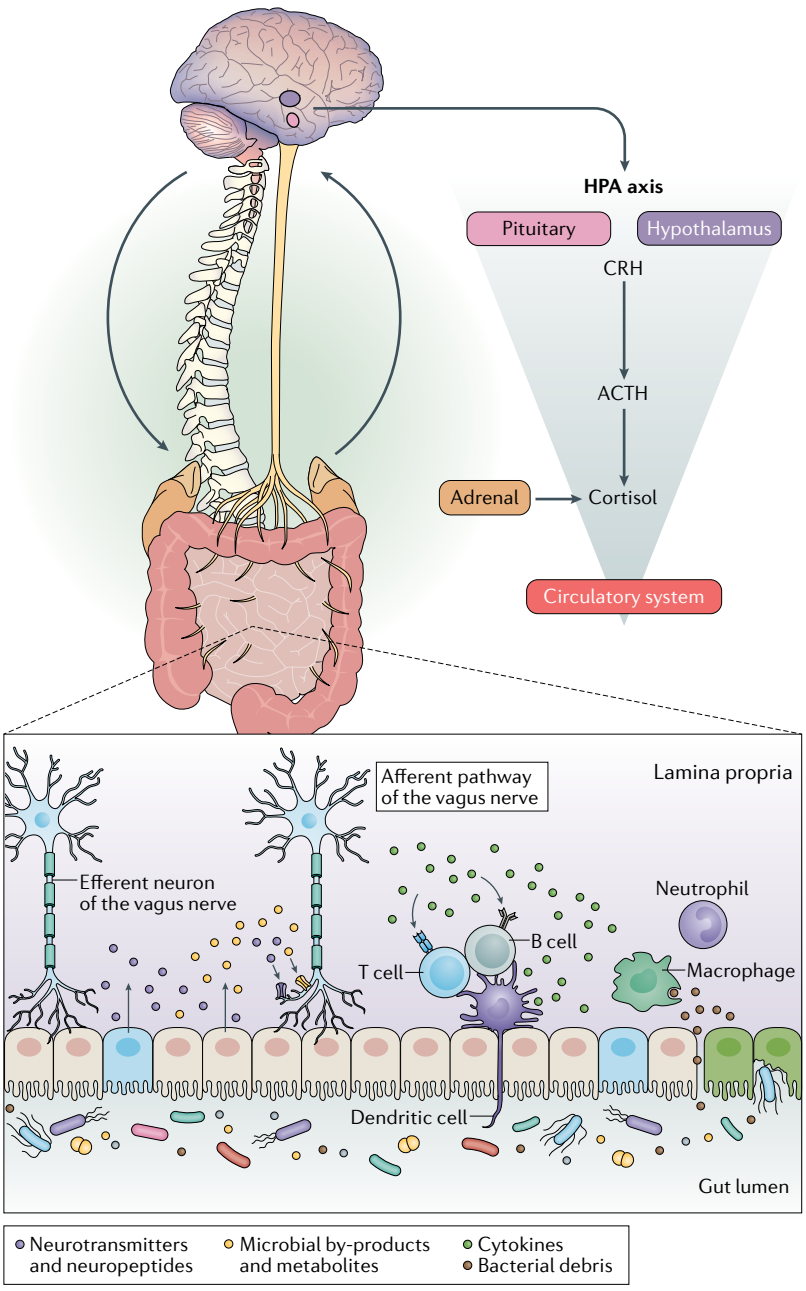

Fig. 1 | **The gut microbiota–brain axis.** Bidirectional communication between the gut microbiota and the central nervous system (CNS) is mediated by several direct and indirect pathways of the gut–brain axis. Most of the information on host–microbiota interactions, and thus the data presented in this figure, is derived from studies in animal models where researchers can effectively control the environment of the test animals. The routes of communication involve the autonomic nervous system (for example, the enteric nervous system (ENS) and the vagus nerve), the neuroendocrine system, the hypothalamic–pituitary–adrenal (HPA) axis, the immune system and metabolic pathways. Within the gut, the microbiota can produce neuroactive compounds such as neurotransmitters (for example, γ-aminobutyric acid (GABA), noradrenaline, dopamine and serotonin (5-hydroxytryptamine (5-HT))), amino acids (for example, tyramine and tryptophan) and microbial metabolites (for example, short-chain fatty acids and 4-ethylphenylsulfate). These metabolites can travel through portal circulation to interact with the host immune system, influence metabolism and/or affect local neuronal cells of the ENS and afferent pathways of the vagus nerve that signal directly to the brain. The gut microbiota can also influence gut barrier integrity that controls the passage of signalling molecules from the gut lumen to the lamina propria, which contain immune cells and terminal ends of ENS neurons, or to portal circulation. Gut barrier integrity can become disrupted in some neuropsychiatric conditions, such as anxiety, autism spectrum disorder and depression. Within the nervous system, stress can activate the HPA axis response that involves neurons of the hypothalamus that secrete hormones such as corticotropin receptor hormone (CRH) into the brain or the portal circulation, triggering the release of adrenocorticotrophic hormone (ACTH), which then initiates the synthesis and release of cortisol. Cortisol regulates neuroimmune signalling responses that, in turn, affect intestinal barrier integrity. Stress hormones, immune-mediators and CNS neurotransmitters can activate neuronal cells of the ENS and afferent pathways of the vagus nerve, which can change the gut environment and alter the microbiota composition.

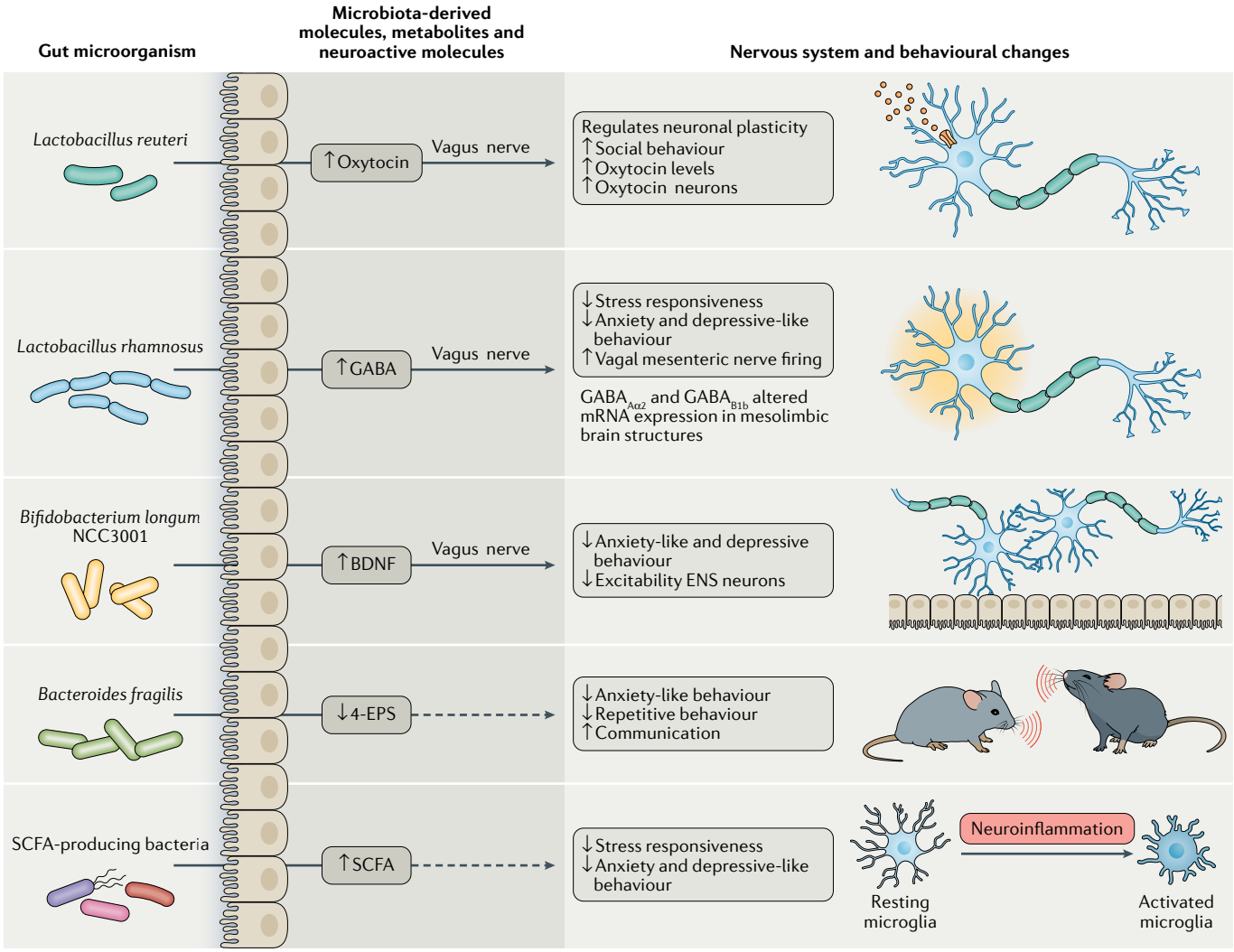

Fig. 2 | **Microbiota and microbial-derived molecules modulate host behaviour and nervous system function.** Microorganisms can induce host production of metabolites and neurotransmitters that mediate gut–brain signalling and can produce some of these neuroactive compounds themselves. Microbial-derived molecules signal to the brain via neuronal pathways of the vagus nerve or modulate the immune system. For example, *Lactobacillus reuteri* has the capacity to upregulate plasma[27] and brain levels of oxytocin in mice[45], a molecule that increases social behaviour. The administration of *L. reuteri* increases social behaviour in mouse models of autism spectrum disorder (ASD)[45,79]. However, the effects of supplementation with *L. reuteri* on humans with ASD remain to be determined. *Lactobacillus rhamnosus* produces γ-aminobutyric acid (GABA) and regulates GABA receptors in the brain (that is, $GABA_{Aα2}$ and $GABA_{B1b}$ receptors), and has been shown to attenuate depression and anxiety-like behaviour in mice[44] but failed to improve stress symptoms in healthy humans[183]. *Bifidobacterium longum* NCC3001, which has been demonstrated to ameliorate mood alterations in individuals with irritable bowel syndrome[155], upregulates brain-derived neurotrophic factor (BDNF)[166], augments neuronal plasticity in the enteric nervous system (ENS) and reduces anxiety and depression-like behaviours in mice. Each of these three phenomena described above requires the presence of intact vagus nerve signalling. In other cases, a direct mechanism remains to be explored. For instance, *Bacteroides fragilis* is known to improve anxiety-like behaviour, repetitive behaviour and communication in mice[52]. The effects of *B. fragilis* are in part due to reduction of 4-ethylphenylsulfate (4-EPS), which modulates anxiety-like behaviour in mice[52]. Short-chain fatty acids (SCFAs) can regulate genes that are involved in microglia maturation and induce morphology changes in mice[2].

cell wall components[35], SCFAs[29] and others[36] have been shown to influence ENS activity and regulate gut motility in rodents, underscoring the intimate connection between the microbiota and neuronal function in the gut. The effects of microbial products on neurons can even extend to the brain via neuronal pathways. Using neuronal tracing techniques, a recent study showed that mice lacking a gut microbiota have increased activation of gut extrinsic neurons connecting the brainstem sensory nuclei and gut sympathetic neurons when compared with mice that have a gut microbiota[29], suggesting that the gut microbiota has a suppressive effect on certain gut-to-brain signalling pathways. Furthermore, activation of this neuronal pathway, which helps mediate gut motility, can be suppressed by administration of SCFA-producing gut microorganisms, supporting a role for the gut microbiota in regulation of gut motility[29]. These findings demonstrate that intestinal bacteria can, through microbial metabolites, modulate neuronal pathways of the gut–brain axis.

The gut microbiota also communicates with the brain via the vagus nerve, which is the most direct and

Serotonin
(5-Hydroxytryptamine (5-HT)). A neurotransmitter involved in controlling mood, social behaviour, gut motility and the sleep cycle.

Blood–brain barrier
(BBB). A physical gatekeeper to separate the brain microenvironment from the rest of the body, formed by mural and microvascular endothelial cells connected by tight-junction proteins.

Internal validity
A measure of the reliability of cause-and-effect relationships determined in a research setting. Internal validity can be improved with an experimental design including blind testing, unbiased analysis and appropriate statistical power.

External validity
A measure of how translatable findings from one experimental setting can be to other experimental settings and to the rest of the world. External validity fails when confounding factors are not considered or controlled in research.

Stress
A physiological and neurological response to demands for change in response to real or perceived threats.

well-studied pathway between the gut and the CNS (reviewed in REF.[37]). Vagus nerve fibres innervate the muscle and mucosa layers of the gastrointestinal tract, detect sensory signals and then relay these signals to the CNS[38]. The transmission of signals from the peripheral ends of the vagus nerve to the CNS occurs though activation of mechanoreceptors that can sense luminal volume or chemoreceptors triggered by chemical stimuli such as hormones, neurotransmitters and metabolites produced by EECs[39–42], which may themselves be influenced by the gut microbiota[29,37]. For example, a study in cultured intestinal organoids demonstrated that EECs can act as chemosensors for SCFAs, resulting in calcium signalling that could be relayed to specialized vagus nerve fibres that innervate the gut epithelium[42]. Vagus nerve efferent fibres propagate information from the brain to the viscera and have an important role in immune function and metabolism[43]. These factors, in turn, may alter the gut microbiota by affecting the environment of the gut, which implicates the vagus nerve as an important mediator of bidirectional communication both to and from the brain.

Microbiota–brain communication via the vagus nerve is also important in modulating host behaviour, as demonstrated in several studies using animal models. For example, administration of *Lactobacillus rhamnosus* JB-1 alters the expression of GABA receptors in brain regions associated with fear and emotions, such as the amygdala and hippocampus, and modulates anxiety-like behaviours in mice[44]. Most effects of *L. rhamnosus* JB-1 on behaviour and changes in GABA receptor expression are abrogated in vagotomized mice where the vagus nerve has been surgically severed, suggesting that the effects of the bacteria depend on neuronal communication to the brain[44] (FIG. 2). Moreover, the vagus nerve is crucial to the beneficial effects of *Lactobacillus reuteri* in promoting social behaviour in animal models of ASD[45] (FIG. 2). These findings point to the possibility of activating the vagus nerve as a method of treating human disease. For example, vagus nerve stimulation, which is performed through surgical implantation of an electrical device that activates the vagus nerve, is an approved therapy for treatment-resistant epilepsy and depression (reviewed in REF.[46]). It may be possible to avoid this surgery if appropriate microbial stimulation of the vagus nerve can be achieved. Although proof of concept and elucidation of mechanism will need to be demonstrated in animal models, owing to the technical challenges of studying the vagus nerve in people, the translatability of these findings to humans remains promising.

Gut microbiota–brain signalling through the immune system. Both the CNS and the gut microbiota directly affect, and are affected by, the immune system. The gut microbiota is a crucial factor in modulating the development and function of the peripheral immune system (reviewed in REF.[7]). The microbiota is also necessary for healthy development, maturation and activation of microglia, innate immune cells of the brain (reviewed in REF.[47]). When compared with conventional mice, GF mice have increased numbers of immature microglia in several brain regions as shown by analysis of cell morphology and transcriptional markers of maturity in microglia cells, a finding that is supported by research in antibiotic-treated mice[2]. Microglia-mediated immune programming seems to depend on signals from microbial metabolism, as treatment of GF mice with bacterial-derived SCFAs restores microglial morphology and function[2] (FIG. 2). Furthermore, a complex microbiota and/or specific bacterial taxa may be necessary for proper microglia function and development, as transfer of a complex microbiota, but not a limited subset of commensal organisms, was able to rescue microglia deficiencies in GF mice[2]. In a separate study, feeding of GF mice with a consortium consisting of four *Bifidobacterium* spp. showed that these bacteria may influence microglia development and activation through transcriptional mechanisms[48]. The impact of gut bacteria on microglia seems to occur in a sex and time-specific manner, as changes in microglia gene expression and morphology are more pronounced in male GF mice during the embryonic phase and in female GF mice during adulthood[49]. Thus, future studies aiming to investigate the effects of the microbiota on brain cells should consider sex an important biological variable. Crucially, alterations in microglia function have been linked to stress, behavioural and neurodegenerative disorders[50,51], which suggests that the gut microbiota may influence human neurological diseases through effects mediated by microglia.

The gut microbiota and the brain also interact through the systemic immune system via circulating cytokines[52]. Changes to systemic immunity drive altered immune signalling within the brain and increased peripheral inflammation is found in many neuropsychiatric diseases, including depression, anxiety and

Box 2 | **Animal models of gut microbiota–brain axis research**

Some central nervous system features are evolutionarily conserved across species, allowing the study of certain characteristics of human behaviour and emotions in animal models. Animal models have proven invaluable for uncovering gut microbiota–brain axis mechanisms; however, it is widely accepted that animal models that are designed to study human behaviour have limitations. Although animal models can be used as tools for studying selective phenotypes that are evolutionarily shared across species, they are not designed to fully recapitulate the human experience in a single model. Merging new tools from microbiology and neuroscience will be essential for improving the application of animal models in gut microbiota–brain research. One promising approach in bridging this gap is the use of animals transplanted with a human microbiota, often referred to as 'humanized animals'. Humanized animals have become increasingly employed for mechanistic studies that aim to investigate the contribution of the gut microbiota to human brain diseases. Yet it should be acknowledged that gut microbiomes are substantially different across species and there remains the need for developing methods that would sustain microbial engraftment when transplanting microbiomes between different species. Furthermore, behavioural and brain disorders are often heterogeneous and multifactorial, with symptoms varying across individuals and over time. Thus, it is important to work with well-characterized and defined populations when doing microbiome transplant studies. Moreover, animal models of brain and behaviour disorders must follow well-established validity standards, with internal validity and external validity being crucial for translating findings from the bench to the bedside[190]. Recently, the presence of segmented filamentous bacteria in the commensal microbiota of mice was demonstrated to affect the reproducibility of the behavioural phenotype in an animal model of neurodevelopmental disorders[59], underscoring the need to integrate multiple animal models with various environmental exposures to fully capture the complexities of human diseases.

ASD (reviewed in REF.[53]). Cytokines and chemokines can either be produced by brain-resident immune cells or access the CNS through direct transport across the BBB. Importantly, there is evidence that the permeability of the BBB is influenced by the gut microbiota, as some reports show that GF mice have increased BBB permeability relative to control mice, partially due to reduced expression of tight-junction proteins such as occludin and claudin 5 (REF.[54]). Infections, autoimmune disease and injury can alter BBB integrity, thus increasing accessibility of the brain to microbial products in the circulatory system and sensitizing the brain to subsequent pathology[55]. In fact, elevated BBB permeability is a defining feature of many neuropathological conditions, further highlighting the potential impact of connections between systemic immunity and outcomes in the brain[56].

The gut microbiota–brain axis in disease

Numerous cross-sectional studies have shown that the composition of the gut microbiota in individuals with various neurological diseases is different relative to healthy individuals[51,57,58]. Furthermore, preclinical models of neurological diseases have been able to recapitulate alterations in gut microbiota composition[51,59] and have shown that human gut bacteria may contribute to behaviour and brain pathology in mice[57,60–62]; however, further work is needed to identify mechanisms underlying these phenotypes[63]. Studies have led to the characterization of putative probiotics that can ameliorate disease symptoms[45,64] as well as the identification of bacteria and bacterial factors that influence disease progression in mice[5,60], providing a template for further investigations in humans. Importantly, communication along the gut microbiota–brain axis occurs throughout life, as can be seen in diseases of neurodevelopment (for example, ASD), neurodegeneration (for example, PD and AD) and behaviour (for example, depression and anxiety). Here, we briefly describe what is currently known about the role of bacteria in neurological diseases and their cognate preclinical models.

Gut microbiota–brain axis in autism spectrum disorder. ASD is a group of neurodevelopmental disorders that manifest early in life, affecting one in every 59 children in the United States and with increased prevalence in males[65]. Symptoms of ASD are heterogeneous but are currently characterized by changes in behavioural domains such as social communication, social interaction and repetitive behaviours[66]. Additionally, gastrointestinal dysfunction is more prevalent in individuals with ASD compared with neurotypical counterparts, including increased susceptibility to intestinal inflammation[67] and altered gut permeability[68]. Intriguingly, there is a positive correlation between the severity of behavioural and gastrointestinal symptoms[69], which suggests a link between the gut and the brain in neurodevelopment. The aetiology of ASD has not been fully elucidated but likely involves interactions between various genetic and environmental factors during crucial developmental windows in early childhood[70]. Importantly, numerous studies report that the composition of the gut microbiota differs between individuals with ASD and neurotypical individuals[58,71–75]. In addition, research in mice has shown that gut microorganisms are capable of influencing behaviours that are core features of ASD. For example, in the absence of a gut microbiota, GF mice prefer to spend more time exploring an empty compartment than a chamber containing a mouse and do not exhibit preference for social novelty, a crucial aspect of sociability. Post-weaning colonization of GF mice with a specific pathogen free gut microbiota increases sociability but not the social novelty phenotype[76], showing that crucial developmental windows for social behaviour that are affected by the gut microbiota can occur early in life. Complementary studies that depleted the gut microbiota using antibiotics also observed reduced sociability in both rats[77] and mice[78]. Addition of specific microorganisms (for example, *L. reuteri*) to the gut can improve sociability in several animal models of ASD-like behaviour[45,79]. Taken together, these and other studies highlight the importance of the gut microbiota in the development of social behaviour, a key domain of ASD pathology.

Recent research has also reported beneficial effects of faecal microbiota transplantation therapy for individuals with ASD. In an open-label clinical study, 18 children diagnosed with ASD received an antibiotic treatment for 2 weeks followed by an initial, high dose of faecal microbiota transplants and subsequent lower maintenance doses administered daily for 7–8 weeks. This treatment reduced gastrointestinal symptoms (for example, constipation and abdominal pain) and, to some degree, improved ASD core symptoms (for example, social skills deficits and repetitive behaviour), with benefits persisting when measured 8 weeks post treatment[71]. Faecal microbiota transplantation also increased overall bacterial diversity and the abundance of beneficial microorganisms in individuals with ASD, and these effects, including improved behavioural measures, were durable over time as they were still observed in the 2-year follow-up study of the same cohort[80]. Nonetheless, it is important to acknowledge that the study was limited to a small number of male participants from the United States, and the lack of control groups within this open-label study design reduces the translatability of this study to a wider population. Although replication and placebo-controlled validation with larger cohorts of demographically diverse populations are needed, these preliminary findings are corroborated by previous clinical studies reporting transient behavioural improvements in individuals with ASD following antibiotic treatment[81,82].

Similar to findings in humans, microbiota differences have been demonstrated in several preclinical models of ASD[45,59,79,83–85]. As the microbiota of laboratory mice is vastly different from that of humans, efforts to 'humanize' gut bacteria in mice offer opportunities to bridge the gap between suboptimal preclinical models and people. Indeed, faecal transplant of microbiota from individuals with ASD into GF mice elicited different behavioural features to transplants from neurotypical controls[5], suggesting that gut bacteria may contribute to altered expression of ASD-like behaviours. Although the experimental design in this study was unable to

Developmental windows
Crucial periods (for example, prenatal, early life and adolescence) in which dynamic changes in development and maturation of multiple physiological systems are susceptible to environmental factors, such as those of the microbiota.

Synapses
Highly specialized contacts between nerve cells that are the connections underlying dynamic and complex neuronal systems networks.

Oxytocin system
A key neuropeptide system that modulates social behaviour, bonding, mating and stress in animals. Known to be associated with symptoms of autism spectrum disorder.

Face and construct validity
Face validity is achieved when a wide range of features present in human disorders, such as behaviour and circuit abnormalities, are reproduced in an animal model. Construct validity refers to mimicking a disease aetiology in animals, such as environmental or genetic risks for human disease.

account for variability between donors, a shortcoming that limits its broader interpretations, this approach has been used by the majority of reports to date employing humanized mouse models of disease[63], and future studies should assign the human donor as the biological variable. Nonetheless, mice colonized with microbiota from human donors with ASD had altered gene expression patterns in the brain that was enriched for the expression of genes previously linked to ASD by genomic studies in large patient populations. Increased sample sizes and rigorous statistical methods will discern whether the human microbiome contributes to ASD-like behaviours in mice, enabling research strategies that are currently unethical or impossible to perform in clinical trials.

Animal models have been used to highlight the multiple pathways of communication between the gut microbiota and the brain, and show how chemical, neuronal and immune-based signalling can influence ASD-like phenotypes. Numerous observational human studies have shown altered metabolism in ASD, with different levels of urinary, blood and faecal metabolites compared with neurotypical controls[86–90]. As an example of chemical signalling, metabolomic analyses of colon contents and serum from mice colonized with a microbiota from individuals with ASD identified reduced levels of 5-aminovaleric acid and taurine in recipient animals, with both metabolites having GABA receptor agonist properties[5]. Indeed, treatment with these two molecules was able to reduce repetitive behaviours and restore sociability in the BTBR $T^{+}Itpr3^{tf}$/J mouse model of ASD (referred to here as BTBR mice)[91], demonstrating a potential functional role for chemical signalling in this mouse model and suggesting a possible functional role for dysregulated metabolism in human ASD.

Mouse models of ASD can also be used to explore neuronal signalling between the gut microbiota and the brain. Disruption of the ASD risk gene *Shank3*, which encodes a scaffolding protein found in synapses and is associated with ASD symptoms in humans and ASD-like behaviour in mice, results in significant changes to bacterial communities in the gut of mutant mice relative to controls[45,85]. In this model, the administration of *L. reuteri* to *Shank3b*-mutant mice reversed deficits in social interaction, social novelty preference and neuronal plasticity in a manner that was dependent on activation of the oxytocin system in the hypothalamus and an intact vagus nerve[45]. Finally, the connection between inflammation and ASD can be modelled through maternal immune activation with polycytidylic acid (poly(I:C)), a double-stranded RNA analogue that acts on Toll-like receptor 3 (TLR3)[92]. This model, which exhibits all core behaviours of human autism, is based on the epidemiological observation that a severe infection with high fever during pregnancy increases the likelihood of an ASD diagnosis in the child[93]. Accordingly in mice, offspring of dams treated with the inflammation-inducing molecule poly(I:C) display alterations in gut microbiota composition and dysregulation of metabolite concentrations in the serum, including increased levels of the microbial metabolite 4-ethylphenylsulfate[52]. Although administration of 4-ethylphenylsulfate did not induce social deficits, it was sufficient to induce anxiety-like behaviour in otherwise untreated mice[52]. Remarkably, treatment at weaning with the human gut commensal *Bacteroides fragilis*[94] was able to improve anxiety-like behaviour, communication deficits, repetitive behaviour and increased gut permeability, and normalized 4-ethylphenylsulfate plasma levels in offspring mothers given poly(I:C)[52] (FIG. 2), thus showing that probiotic administration can alleviate ASD-like symptoms in a mouse model with face and construct validity. The link between the gut microbiota, inflammation and ASD-like behaviour in mice is further supported by the finding that activation of the inflammatory IL-17-producing T helper cell immune pathway drives increased ASD-related phenotypes and is dependent on colonization by specific gut bacteria[59]. Although preliminary, these findings in animals advance understanding of the impact by the gut microbiota on behavioural and brain disorders.

Although ASD is uniquely a human disorder and mice only model features of ASD, the study of animal models to understand interactions between the gut microbiota and behaviour can help identify candidate molecular targets and neurocircuits that may be involved in people. Moreover, the advent of conditional genetic models (Supplementary Table 1) could help tailor cell-specific effects induced by the gut microbiota and help with identification of crucial developmental windows where the brain is more susceptible to influences by the gut microbiota, which may facilitate the development of new treatment options. It is important to note that ASD is heterogeneous in aetiology and manifestations, and involves a wide range of behavioural and non-behavioural changes with diverse and complex contributions for genetic and environmental risk factors. Although animal models of ASD can reproduce specific behavioural and molecular alterations related to the disorder, a single animal model does not replicate the spectrum of behavioural and associated phenotypes. Thus, investigations of the gut microbiota should be conducted in multiple animal models, including non-human primates, to fully recapitulate the diverse presentations of ASD. The relative ease and presumed safety of microbiota-inspired therapies has facilitated recent clinical trials in ASD, including several with interventional arms[95–98]. However, validating a probiotic treatment for ASD currently remains aspirational.

Neurodegenerative disorders and the gut. PD is the second most common neurodegenerative disorder after AD, affecting 0.3% of individuals in the general population and more than 1% of the elderly population worldwide[99]. PD is a progressive neurodegenerative disorder characterized by an inability to control voluntary movements due to profound changes in the functional organization of the substantia nigra and striatum regions of the brain. These changes include degeneration of dopaminergic neurons, aggregation of phosphorylated forms of the neuronal protein α-synuclein (αSyn), mitochondrial dysfunction, excessive reactive oxygen species and an increase in microglia activation[100]. Symptoms of PD include tremors, difficulty walking, hunched

posture and muscle rigidity. Intriguingly, gastrointestinal issues, primarily in the form of constipation, can occur in up to 80% of individuals with PD[101] and can precede PD diagnoses by many years[102]. The presence of gut inflammation, increased intestinal permeability and early accumulation of phosphorylated αSyn in the ENS and in the dorsal motor nucleus of the vagus nerve, a gateway between the gut and the brain, all suggest that PD pathology may start in the gut and reach the brain by navigating through neuronal pathways of the vagus nerve[103–107]. In fact, the earliest report of gut involvement in PD was first recorded in 1817 by James Parkinson, in his essay on shaking palsy[108]. A contemporary view of the association between the gut and PD was postulated by Braak's hypothesis[109], which suggests that pathology in some (perhaps most) cases of PD initiates in the gut, before affecting the brain. In support of this notion, individuals who have undergone vagotomy are at reduced risk of developing PD as they age[110]. Recent studies suggest a link between the gut microbiota and PD, as both the composition of the microbial community and the metabolic profile in the serum of individuals with PD are distinct from those of healthy individuals, with increasing levels of Enterobacteriaceae and a loss of gut microorganisms associated with anti-inflammatory properties[111–113]. Increased abundance in Enterobacteriaceae positively correlates with the severity of certain PD symptoms[114]. Enterobacteriaceae are also associated with gut inflammation in Crohn's disease (a form of inflammatory bowel disease), and individuals with Crohn's disease are at increased risk for developing PD, whereas individuals with Crohn's disease who are treated with anti-inflammatory drugs are partially protected from PD[115], suggesting that inflammation in the gut could be a driver of PD pathology. Interestingly, PD-like symptoms can be exacerbated by gastrointestinal infection in mice[116]. Motor symptoms can be aggravated by intestinal inflammation caused by *Citrobacter rodentium* infection in a mouse model of PD based on knockout of the PTEN-induced kinase 1 (*Pink1*) gene, polymorphisms of which are associated with PD in humans[116]. Together, these studies link gut inflammation to neurodegeneration, although more research is needed to precisely define the mechanisms. The gut microbiota may also drive PD symptomatology through metabolic effects in addition to induction of inflammation. Individuals with PD display metabolic profiles distinct from those of healthy individuals with alterations in metabolites associated with the gut microbiota, including β-glucuronate and tryptophan levels[117] as well as reduced concentrations of SCFAs[118]. The gut microbiota may also affect PD treatments, as the gut microbiota can reduce the efficacy of anti-PD drugs, including the standard levodopa (L-dopa) treatment, either through increased rates of drug inactivation[119] or through a reduction in the rates of drug absorption[120,121]. Taken together, these findings in humans and animal models suggest that the gut microbiota can aggravate PD pathology, possibly through modulating inflammation (and an associated increase in αSyn misfolding), alteration of host metabolism and reductions in therapeutic efficacy of approved PD treatment.

Vagotomy
A surgical procedure that severs the vagus nerve in one of several locations, disrupting signalling from various peripheral organs to the brain.

Various animal models of PD are based on either genetic factors or environmental toxin administration to recapitulate human disease aetiology[122], and recent research has investigated the involvement of the gut microbiota in these systems. In a mouse model of human αSyn overexpression, mice lacking a gut microbiota (either through derivation of GF mice or antibiotic treatment) had lower levels of SCFA production and a subsequent reduction in αSyn neuropathology, decreased microglia activation and improved motor performance, suggesting that the gut microbiota was enhancing PD-like symptoms[51]. Transplant of gut bacteria from individuals with PD into GF mice that overexpress human αSyn worsened motor symptoms compared with colonization of congenic mice with microbiotas from healthy individuals, suggesting that the dysfunctional gut microbiota from individuals with PD further exacerbated motor symptoms in mice. Strikingly, there appears to be direct involvement of microbial products in PD pathology stemming from the gut. Pathogenic strains of *Escherichia coli*, the abundance of which are increased in individuals with PD, are capable of producing an amyloid protein called curli that is able to promote αSyn aggregation in the gut and the brain[123]. Curli-dependent αSyn aggregates were associated with motor deficits in mice and, remarkably, the pathology and symptoms required production of *E. coli* curli in the gut[123]. The major subunit protein of curli was sufficient to enhance αSyn aggregation in biochemical assays and in a mouse model of PD. Furthermore, treatment of mice with an oral, gut-restricted chemical inhibitor of amyloid formation ameliorated both motor and constipation-like symptoms, suggesting that the gastrointestinal tract may be involved in the aetiology of PD-like features and offering tantalizing support for Braak's hypothesis. In addition to transgenic models, toxin-based animal models of PD also show alterations in the gut microbiota[124]. The injection of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice led to reduced levels of healthy gut microorganisms, increased levels of Enterobacteriaceae in the gut and altered faecal SCFA concentrations[125], mirroring observations from individuals with PD[114]. Faecal microbiota transplantation from untreated control mice into the MPTP-treated mice improved signs of motor dysfunction and neuroinflammation[125], suggesting that a return to a healthy gut microbiota can prevent PD-like pathology. Moreover, mice treated with rotenone, a pesticide associated with an increase in the prevalence of PD through widespread human exposure, also display altered intestinal microbial composition, with an increased Firmicutes to Bacteroides ratio that is similar to other diseases associated with gastrointestinal inflammation[124]. Specific bacterial species may also be responsible for driving PD-like pathology, such as *Proteus mirabilis*, which promotes motor deficits in mice[60]. Thus, the gut microbiota mediates pathways that can drive neuronal dysfunction and degeneration as well as neuroinflammation in ways that promote PD-like symptomatology in mice.

Some gut bacteria are neuroprotective and can ameliorate signs of PD in mice. The MitoPark PD mouse

model is a mouse line that can reproduce both motor and neurodegeneration signs of PD through genetic inactivation of the mitochondrial transcription factor A in dopaminergic neurons[126]. In this model, PD-like symptoms of motor dysfunction and dopaminergic neurodegeneration can be reduced through administration of a probiotic mix containing *Bifidobacterium*, *Lactobacillus* and *Lactococcus* strains over a period of 16 weeks[127]. Furthermore, a novel probiotic formulation (SLAB51 bacterial lysates) increased cell viability in the human SH-SY5Y cell line, an in vitro model of PD[128]. In the same study, SLAB51 was able to counteract oxidative stress, reduce neuronal death and reverse the motor behavioural phenotype in a toxin-based rodent model of PD induced by 6-hydroxydopamine[128]. These results are promising, and embolden future work to determine whether beneficial gut bacteria can be harnessed as novel therapeutic options for PD.

The gut microbiota appears to have a role in other neurodegenerative diseases, namely AD, the leading cause of dementia worldwide[129]. Shifts in the gut microbiota composition have been identified in individuals with AD, including a decreased abundance of Firmicutes and *Bifidobacterium* spp. and increased Bacteroidetes, *Escherichia* and *Shigella* spp. *Shigella* spp. have been associated with inflammation and increased expression of amyloid proteins in AD[130,131], in a manner that is very similar to PD. The role of the microbiota in ADpathogenesis has been studied in 5XFAD transgenic mice, which are used to model neuronal loss, cognitive deficits and immune alterations similar to human AD[132]. 5XFAD transgenic mice exhibit marked changes in the gut microbiota and metabolism of amino acids. Conversely, microbiota depletion using antibiotics attenuates inflammation and brain pathology in 5XFAD transgenic mice[132] and other animal models of AD, such as the APP/PS1 line[133,134], suggesting that the gut microbiota enhances disease severity. Microbiota effects on learning and memory have recently been demonstrated in 5XFAD transgenic mice through a proposed mechanism involving microglia[135].

Similarly, links between the gut microbiota, immune system and neurodegeneration have been reported in multiple sclerosis, an autoimmune demyelinating disease characterized by degeneration of neuronal signalling throughout an individual's lifetime. Faecal samples from individuals with multiple sclerosis reveal changes in abundance of *Dorea*, *Blautia*, *Pseudomonas*, *Mycoplana* and *Akkermansia* spp. relative to healthy individuals[136]. In preclinical models, GF mice develop attenuated multiple sclerosis-like disease[137] and transplantation of gut microbiota from individuals with multiple sclerosis into mice resulted in more severe experimental autoimmune encephalomyelitis and reduced proportions of anti-inflammatory regulatory T cells compared with mice that received microbiota from healthy individuals[62,138]. Thus, the gut microbiota may be a driver of disease pathogenesis across many neurodegenerative diseases. Integration of these data sets from different diseases, and disease models, will be crucial in identifying conserved pathways that may lead to pathology.

Despite major efforts, the search for therapies for neurodegenerative disorders has been challenging, in part due to the limitations of current animal models and a lack of understanding of disease pathology. For example, mouse models of PD are limited in their ability to recapitulate the totality of human disease (BOX 2). Ideally, an animal model would be progressive in nature and the signs of PD, including the accumulation of αSyn, neuronal degeneration and behavioural symptoms, would develop over relatively long periods of time, as the primary risk factor for PD is age. This timeline is not easily reproduced in animal models of PD. Despite these limitations, further research into a contributing role of the gut microbiota in the pathophysiology of PD and other neurodegenerative disorders, coupled with emerging clinical and epidemiological human data, represents an exciting frontier in biomedicine. These new areas of investigation may inform disease mechanisms linked to neurodegeneration, which may guide the discovery and development of promising treatments to improve the lives of millions worldwide.

Gut microbiota–brain axis in stress, depression and anxiety. Stress, depression and anxiety are highly co-morbid conditions and have overlapping biological mechanisms and manifestations[1]. For this reason, these conditions are often studied together in gut microbiota–brain axis research[1]. Depression and anxiety are prevalent psychiatric conditions worldwide, and are classified as mood disorders related to failures in allostasis, which is the process used by the body to respond to psychological stress and restore homeostasis[139]. Allostasis involves dynamic regulation of the body's stress response systems, including neuroendocrine signalling through the hypothalamic–pituitary–adrenal (HPA) axis, which regulates production of glucocorticoids, and regulation of BDNF, which is important in learning and memory formation[139]. Glucocorticoids, which are released in times of stress from the adrenal glands, control homeostatic conditions throughout the body and can induce anti-inflammatory responses[140]. The gut microbiota helps mediate these stress response systems in mouse models, as GF mice have exaggerated production of glucocorticoids following an experimental stressor, suggesting a sensitization of the allostasis machinery in the absence of microbial signals[18]. The gut microbiota can restore allostasis in rodents, as administration of *Lactobacillus* spp. is able to normalize levels of glucocorticoids following early-life stress[141] and *L. rhamnosus* alleviates anxiety-like behaviours in BALB/c mice[44] (FIG. 2). In addition to *Lacobacillus* spp., *Bifidobacterium* spp. have been shown to ameliorate stress-induced behavioural alterations in rodent models, thus showing that the gut microbiota can affect the stress response pathways in the brain[142,143] (FIG. 2). The connection between the brain and the gut microbiota in stress is bidirectional, as chronic stress is associated with lasting alterations in the composition and function of the gut microbiota, including a correlation between reduced *Lactobacillus* spp. in rhesus macaques experiencing maternal separation early in life and a subsequent increase in stress-related behaviours in offspring[144]. Moreover, maternal separation in

Allostasis
The active process of the body to maintain homeostasis in the face of stress.

Anhedonia
A reduced capacity to experience pleasure.

rats during early life also induces long-term changes in gut microbiota diversity[145,146]. Studies in mice have further supported associations between stress and the gut microbiota by demonstrating that prenatal stress exposure leads to long-term effects on the microbiota composition of offspring and the priming of the HPA axis into adulthood[147].

Depression affects millions of people worldwide[148] and is associated with neurological symptoms of cognitive dysfunction, anhedonia and despair[149]. Major depressive disorder, a form of depression, is associated with physiological changes throughout the body, including changes to gut epithelial permeability and increased systemic inflammation with elevated levels of C-reactive protein, interleukin (IL)-1β, IL-6 and tumour necrosis factor (TNF)[150]. Recent evidence has shown that individuals diagnosed with major depression disorder have altered gut bacterial species relative to healthy adults[57,61,151], including changes in the abundance of common bacterial taxa — such as a reduction in the abundance of Bacteroidetes, Firmicutes and Actinobacteria with a concomitant outgrowth of Proteobacteria[61,151] and a specific increase in *Alistipes* spp.[57] — in individuals with depression. An orthogonal study correlating lifestyle with the gut microbiota across a large Flemish cohort found strong positive associations of *Faecalibacterium* and *Coprococcus* taxa with measurements of quality of life[152], which coincide with the loss of *Faecalibacterium* in individuals with major depression disorder[57]. *Dialister* and *Coprococcus* spp. are decreased in individuals diagnosed with major depressive disorder[152]. These data, when taken together, indicate that there may be conserved changes to the gut microbiota across individuals with differing demographic backgrounds; however, the available literature indicates that the majority of the microbial changes associated with depression are unique to each study, which is a limitation common in many cross-sectional studies and reflects the huge inter-individual microbiome variability in the human population.

Currently available animal models of depression can recapitulate some clinical findings from humans regarding the gut microbiota, as induction of depression-like behaviour in different mouse models (including early life[153] and chronic mild stressors[64,154]) is associated with alterations in the gut microbiota. These changes in the gut microbiota coincide with alterations to the host physiology, including activation of the HPA axis[153] and increases in inflammatory activity in the brain[154]. Transplantation of gut microbiota from individuals with depression into GF mice[151] and rats[61] resulted in an increase in depressive-like behaviours in rodents, thus suggesting that the transplant of gut microorganisms is also able to transfer the phenotypes of depression. Interestingly, the presence of specific bacterial species, namely *Lactobacillus*, have been shown to restore some physiological deficits and diminish behavioural despair in animal models[64]. Furthermore, probiotic administration of *Bifidobacterium longum* NCC3001 significantly improved depression in a cohort of individuals with irritable bowel syndrome in a pilot study[155]. As such, there may be specific bacteria that are capable of exacerbating or alleviating depressive-like behaviours, but the bacterial species responsible for these phenotypes, as well as the mechanisms underlying their presentation, remain elusive.

Approximately 30–40% of the population in the United States will experience an anxiety disorder in their lifetime[156]. The relationship between anxiety and the gut microbiota was initially explored in the context of infection. Enteric infection of mice with the pathogen *Campylobacter jejuni* decreases exploratory behaviour in the elevated-plus maze test, suggesting increased anxiety behaviour[157]. Moreover, exposure to *C. jejuni* activates the amygdala[158], a region of the brain that is crucial to anxiety behaviours. Recently, a large longitudinal epidemiological study examined the relationship between intestinal infection and subsequent onset of anxiety disorder through the Medical Expenditure Panel Survey (MEPS), a publicly available health-related survey set. The results from the study demonstrate an increased likelihood of developing an anxiety disorder in individuals previously exposed to intestinal infection[159], implicating the gut microbiota as a potential 'trigger' for a subsequent anxiety disorder. The influence of the gut

Box 3 | **Examples of biological pathways linking the gut and brain**

Correlations between the gut microbiota composition, brain homeostasis and pathophysiology of various neurological disorders have been established; however, examples of mechanistic explanations underpinning these connections remain limited, although these are steadily increasing. This is due, in part, to the fact that gut microbiota–brain interactions often involve multiple modes of communication (immunological, endocrine, neuronal and so on) and may involve microbial factors that are produced by diverse bacteria (for example, the production of short-chain fatty acids (SCFAs) is common in many lineages of bacteria). As such, care must be taken in experimental design to balance a reductionist versus system-wide approach to fully characterize crucial pathways in model systems while maintaining relevance to human biology. Although this field of modern gut microbiota–brain research is still in its incipience, even relative to other areas of neuroscience, many new tools and novel techniques have been developed that enable molecular characterization of the pathways described in this Review (Supplementary Table 1). These innovations are enabling researchers to move past correlative analyses into detailed experiments to tease apart various biological pathways involved in the gut microbiota–brain axis. Two brief summaries are provided below.

Autism spectrum disorder

The gut microbiota mediates levels of chemical transmitters (for example, γ-aminobutyric acid (GABA), glutamate, oxytocin and serotonin (5-hydroxytryptamine (5-HT))) involved in autism spectrum disorder (ASD). Microbial influence on the immune system may also have a key role in shaping neuroimmune responses in ASD, given that low-grade inflammation is present in individuals with ASD. The extent to which microbial metabolites (for example, 5-aminovaleric acid, taurine, bile acid metabolites and SCFAs) influence ASD symptoms is becoming more clear and novel technologies are being applied to this emerging area of research.

Parkinson disease

The ways in which the gut microbiota may contribute to Parkinson disease (PD) are manifold and involve microbial products that affect protein folding and induction of inflammation, among other effects. For example, the gut microbiota mediates aggregation of phosphorylated α-synuclein (αSyn) in both the intestine and the brain through their metabolic processes and production of structural proteins (for example, *Escherichia coli* production of curli)[118]. Furthermore, gut microorganisms are able to regulate inflammation in several animal models of PD, which is particularly relevant for triggering αSyn pathology and neurodegeneration. Finally, the gut microbiota can modulate the therapeutic efficacy of the primary PD treatment, levodopa (L-dopa), as certain gut bacterial species encode genes for enzymes that are capable of degrading the drug before it reaches the brain[119].

Table 1 | **Gut microbiota–brain axis pathways and neurological disorders**

Neurological disorder	Pathways of the gut microbiota–brain axis	Studies in animal models	Studies in humans
ASD	Metabolic and endocrine pathways	C57Bl/6J mice treated with *p*-Cresol[169a]	[86,88,89,170,171]
		In utero valproic acid mouse model of ASD[83]	
		Maternal immune activation with poly(I:C) mouse model of ASD[52]	
		BTBR T^{+}Itpr3^{tf}/J mouse model of ASD[5,36]	
		Mice humanized with microbiota from individuals with ASD[5]	
	Neuronal signalling	*Shank3b*$^{-/-}$ mouse model of ASD[45,85]	Convincing evidence lacking in human studies
		In utero valproic acid and BTBR T^{+}Itpr3^{tf}/J mouse models of ASD[45]	
	Immune and neuroimmune pathways	Maternal immune activation with poly(I:C) mouse model of ASD[52,59]	Convincing evidence lacking in human studies
Neurodegenerative disorders	Metabolic pathways	Sod1 transgenic mouse model of amyotrophic lateral sclerosis[172]	[113,117,118,173]
		THY1-αSyn mouse model of PD[51]	
	Neuronal signalling	6-Hydroxydopamine PD mouse model[128]	[110,174b]
		Oral administration of *Proteus mirabilis* to C57Bl/6 mice[60]	
		MitoPark PD mouse model[127]	
	Immune and neuroimmune pathways	Mice humanized with microbiota from individuals with multiple sclerosis[62,138]	[112,132,175,176]
		Oral administration of *P. mirabilis* to C57Bl/6 mice[60]	
		Rag$^{-/-}$ and MOG transgenic mouse models of multiple sclerosis[137]	
		HLA-DR3.DQ8 double-transgenic mouse model of multiple sclerosis[136]	
		5XFAD transgenic mouse model of Alzheimer disease[135]	
		$APP_{SWE}/PS1_{\Delta E9}$ transgenic mouse model of Alzheimer disease[133]	
		Rotenone-induced animal model of PD[175]	
	Other microbial factors	THY1-αSyn mice model of PD[51]	Convincing evidence lacking in human studies
		Fischer 344 rats and *Caenorhabditis elegans*[177]	
Mood disorders	Metabolic and endocrine pathways	Neonatal maternal separation in mice[141]	[151,152,179,180]
		Repeated psychosocial stress in mice[178]	
		Unpredictable chronic stress in mice[64]	
	Neuronal signalling	Dextran sodium sulfate colitis in mice[143]	[24,155]
		BALB/c mice[181]	
		Neonatal maternal separation in mice[182]	
	Immune and neuroimmune pathways	Rats humanized with depression patients' microbiota[61]	Convincing evidence lacking in human studies
		Chronic mild stress in mice[154]	

ASD, autism spectrum disorder; MOG, myelin oligodendrocyte glycoprotein; PD, Parkinson disease; poly(I:C), polycytidylic acid; αSyn, α-synuclein. [a]Observations based on preliminary data not yet peer-reviewed at the time of this publication. [b]Microbiota not implicated in these findings.

microbiota in anxiety-like behaviours can be modelled in many different behavioural tests and in multiple animal species, including both rodents as well model organisms such as zebrafish. GF mice[160,161] and GF zebrafish[162] exhibit a 'low anxiety-like' behavioural phenotype, whereas GF rats exhibit 'high anxiety-like' profiles[163]. GF mice display alterations in the fear response and an altered transcriptional profile in brain areas that mediate fear learning, such as the amygdala[164]. Both GF mice and antibiotic-treated mice show an inability to overcome previous negative experiences, a defect known to be associated with anxiety and the inability to cope with stressful stimuli[165]. These studies indicate that the gut microbiota may affect both the 'baseline' anxiety of animals (for example, alterations to the development of the HPA axis) and their resilience to stressful events (for example, resiliency in response to acute stresses). Definition of the gut microbiota as a cause of anxiety-like behaviours is difficult. However, microbiota transplantation from BALB/c mice (a more anxious mouse strain) into GF NIH Swiss mice (a less anxious mouse strain) resulted in an increase in anxiety-like behaviours in the recipient Swiss mice, as well as an increase in BDNF expression in the brain[166], further linking gut microorganisms to anxiety-like behaviours in animals.

Treatments targeting the gut microbiota with different probiotics and prebiotics appear to ameliorate anxiety symptoms in humans and similar behaviours in animal models. For example, a double-blind clinical study of the anxiolytic effects of *Lactobacillus helveticus* R0052 and *B. longum* R0175 in healthy individuals in France found modest improvements in self-reported

symptoms related to stress, anxiety and depression after 30 days of probiotic treatment[167]. However, this study also reported improved stress-related symptoms in the placebo group and also failed to identify any changes in biomarkers of stress in either group (that is, there was no difference in glucocorticoid levels in the participants' urine), which limits the possible application of this candidate intervention[167]. Depression and anxiety disorders are complex and are marked by constellations of varied behavioural, cognitive and physiological symptoms, which may be due to distinct molecular pathways. Given the complex pathophysiology of anxiety and depression, future clinical studies should consider stratifying subpopulations based on constrained behavioural, molecular, demographic or microbiota biomarkers to limit subject heterogeneity. Although preclinical results are promising, animal models of depression and anxiety can only reproduce some features of these complex disorders (BOX 2) and are limited by extrapolation of behaviours between humans and rodents. Nonetheless, the relative tractability and presumed safety of microbiome-based treatments, the large patient population and the potential for improvements in quality of life make research into probiotics for anxiety and depression worthy of further exploration.

Conclusions and perspectives

Mounting information from both clinical and preclinical arenas presents compelling evidence that interaction between the gut microbiota and the mammalian nervous system shapes both adaptive and dysfunctional neurological processes. Three major ways in which the microbiota can influence the development and function of the nervous system include modulation of the immune response; impacts on metabolism, including hormones, neuropeptides and neurotransmitters; and direct effects on neurons and neuronal signalling (BOX 3; TABLE 1). Thus, the co-evolution of animals and their associated microbial communities appears to have resulted in complex biological communications between the gut and the brain, a fascinating perspective that requires more investigation but also provides promising new avenues to modulate behaviour, particularly relevant to the study of psychiatric and neurodegenerative disorders.

Many essential questions regarding the gut–brain axis remain unanswered. Although it appears that microbial metabolites are important for communication along this axis, it is not yet clear what proportion of effects can occur through neuronal and/or hormonal pathways, let alone how many metabolites directly affect the brain after crossing the BBB (FIG. 2). Microbial metabolites may also act directly on peripheral nervous system pathways, such as in the ENS, and thus alter communication between the periphery and the CNS. The obstacles for understanding mechanisms of action in this burgeoning field are intimately connected with the complexities of human neurological disorders and the limitations of animal systems that attempt to model human disease. The vast majority of the studies that causally implicate gut microorganisms in the regulation of behaviour have been described in rodent models and have largely not yet been replicated, thus requiring further validation before translation to humans. For particular microorganisms, data from preclinical studies have been robust and reproducible across model systems and between laboratories (for example, *L. reuteri* for social behaviours[45,85], *Lactobacillus* and *Bifidobacterium* spp. for anxiety-related behaviours[44,154,167,168]), enabling further development and offering hope for successful application in humans.

The fields of microbiology and neuroscience, along with other disciplines, must continue to collaborate to develop comprehensive and relevant approaches to determine mechanisms of action for outcomes that currently remain observational, along with responsible efforts in translating these discoveries to improve human health. An integrative and modern view of classical brain disorders as whole-body conditions, including a major role for the gastrointestinal tract, may lead to strategies that target the gut microbiota to provide new, safe and effective therapeutic options for neuropsychiatric and neurodegenerative diseases. It appears that this exciting concept is poised to be tested in the coming years.

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