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EXPERT REVIEW

From gut dysbiosis to altered brain function and mental illness: mechanisms and pathways

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The human body hosts an enormous abundance and diversity of microbes, which perform a range of essential and beneficial functions. Our appreciation of the importance of these microbial communities to many aspects of human physiology has grown dramatically in recent years. We know, for example, that animals raised in a germ-free environment exhibit substantially altered immune and metabolic function, while the disruption of commensal microbiota in humans is associated with the development of a growing number of diseases. Evidence is now emerging that, through interactions with the gut–brain axis, the bidirectional communication system between the central nervous system and the gastrointestinal tract, the gut microbiome can also influence neural development, cognition and behaviour, with recent evidence that changes in behaviour alter gut microbiota composition, while modifications of the microbiome can induce depressive-like behaviours. Although an association between enteropathy and certain psychiatric conditions has long been recognized, it now appears that gut microbes represent direct mediators of psychopathology. Here, we examine roles of gut microbiome in shaping brain development and neurological function, and the mechanisms by which it can contribute to mental illness. Further, we discuss how the insight provided by this new and exciting field of research can inform care and provide a basis for the design of novel, microbiota-targeted, therapies.

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INTRODUCTION

The disruption of the microbes that are resident in our gastrointestinal tract has long been implicated in the development or exacerbation of mental disorders. There is, for example, a long history of anecdotal reports of psychiatric side-effects of antibiotics, even in those without a premorbid psychiatric history.[1] There have also been attempts to influence the composition of the gut microbiota to achieve clinical benefit. For example, in the first decades of the twentieth century, probiotic preparations containing *Lactobacillus* strains were marketed widely as a means to improve mental health or treat psychiatric disorders.[2] These approaches fell from favour in the 1920s because of a lack of mechanistic understanding and their link to the increasingly unfashionable 'autointoxication' model. However, the interest in the role of gut microbes in mental health, and our ability to improve psychiatric wellbeing through their manipulation, is resurgent.[2,3]

In this review, we consider the potential of dysbiosis to contribute to psychopathology and the evidence linking disruption of gut microbiota with specific psychiatric disorders. We examine the role of the microbiome in neurological development and regulation, and consider its contribution to aging-related morbidity. Finally, we discuss the potential for modification of the gut microbiome to provide clinical benefit in the context of altered brain function.

REGULATION OF NEUROLOGICAL FUNCTION BY THE GUT MICROBIOME

The potential contribution of bidirectional communication between the gut and central nervous system (CNS) is suggested by high rates of comorbidity between gastrointestinal and psychiatric illnesses.[4,5] For example, mood disorders affect more than half of all patients with irritable bowel syndrome,[6] with antidepressants being one of the most common pharmaceutical interventions for irritable bowel syndrome.[4] The gut–brain axis consists of a bidirectional communication network that monitors and integrates gut functions and link them to cognitive and emotional centres of the brain. It encompasses the central, autonomic and enteric nervous systems, as well as the neuroendocrine, enteroendocrine and neuroimmune systems.[7,8] It mediates the effects of both genetic and environmental factors on brain development and function, and has been implicated in the aetiology of a number of psychiatric disorders.[9–12]

In recent years, we have increasingly understood the contribution made by the gut microbiome not only in the regulation of host physiology, particularly metabolism and immunity,[13–17] but also the CNS and brain function.[11,18,19] Given mounting evidence that the microbiome has a key role in influencing the development and function of the nervous system through its interaction with the gut–brain axis, it has been suggested that a 'microbiome–gut–brain axis' may be a more appropriate model.[19–22]

The delicate balance between the human microbiome and the development of psychopathologies is particularly interesting given the ease with which the microbiome can be altered by external factors, such as diet,[23] exposure to antimicrobials[24,25] or disrupted sleep patterns.[26] For example, a link between antibiotic exposure and altered brain function is well evidenced by the psychiatric side-effects of antibiotics, which range from anxiety

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and panic to major depression, psychosis and delirium.[1] A recent large population study reported that treatment with a single antibiotic course was associated with an increased risk for depression and anxiety, rising with multiple exposures.[27] Bercik *et al.*[28] showed that oral administration of non-absorbable antimicrobials transiently altered the composition of the gut microbiota in adult mice and increased exploratory behaviour and hippocampal expression of brain-derived neurotrophic factor (BDNF), while intraperitoneal administration had no effect on behaviour. Alteration of brain function may therefore add to the many reasons that inappropriate antibiotic use should be avoided. It should be noted though that unchecked bacterial infection also represents an acute stressor, and has been shown to be associated with memory dysfunction in mice.[29]

Diet is another important determinant of gut microbiota composition and function that is strongly linked with psychopathological outcomes. For example, consumption of high fat diet (HFD) is associated with altered microbial diversity and reduced synaptic plasticity,[30–31] with increased vulnerability to anxiety-like behaviour in mice,[32] while altered microbial diversity upon consumption of a diet high in sucrose results in significantly impaired development of a spatial bias for long-term memory, short-term memory and reversal training.[33] In contrast, adolescent rats fed a low-calorie diet show augmented neurogenesis and BDNF levels, and improved cognition in adulthood,[34] and a diet that increases microbiota diversity is associated with improved cognitive ability.[35] Although human data have shown reduced microbial diversity in individuals is linked with increased adiposity, insulin resistance, dyslipidaemia and more pronounced inflammatory phenotype,[36,37] strong evidence of a direct microbiome effect comes from studies using conventionally housed mice subjected to a microbiome depletion and/or transplantation paradigm using microbiota isolated from donors on either an HFD or control diet. Following re-colonization, mice given the HFD exposed microbiota showed significant and selective disruptions in exploratory, cognitive, and stereotypical behaviour.[38] Although it is not possible to exclude the direct effect of host metabolism on brain function, such findings do suggest that diet-induced changes in the intestinal microbiome substantially influence brain function.

Diet and antibiotic exposure are only two factors that potentially influence brain function through shaping the gut microbiome (Figure 1). An array of common variables may be equally important. For example, alcohol consumption,[39,40] smoking habits[41] and disruption of diurnal rhythm,[26] have all been shown to substantially affect microbiota composition. As such, how wider influences on the microbiome contribute to dysregulation of brain function is an area of growing interest.

THE MICROBIOME IN SPECIFIC PSYCHIATRIC CONDITIONS

While the links between the microbiome and specific psychiatric conditions have been reviewed elsewhere,[18,42–45] a brief examination of the contribution of inter-kingdom interactions to two particularly distressing neuropsychiatric disorders provides a useful illustration.

Major depressive disorder (MDD) is typified by markers known to be influenced by the microbiome. For example, depression-associated changes seen in the hypothalamic-pituitary-adrenal (HPA) stress response,[46] and altered levels of depression-associated monoamines (or their receptors) in corticolimbic regions of the brain, have both been demonstrated in germ-free (GF) mice.[28,47–50] The increased concentrations of pro-inflammatory cytokines seen in MDD[46] may also result from interactions with gut microbes. Levels of serum antibodies against lipopolysaccharide from gram-negative enterobacteria are higher in patients with MDD than in controls,[51] and cause stress-associated with increased gut permeability and bacterial

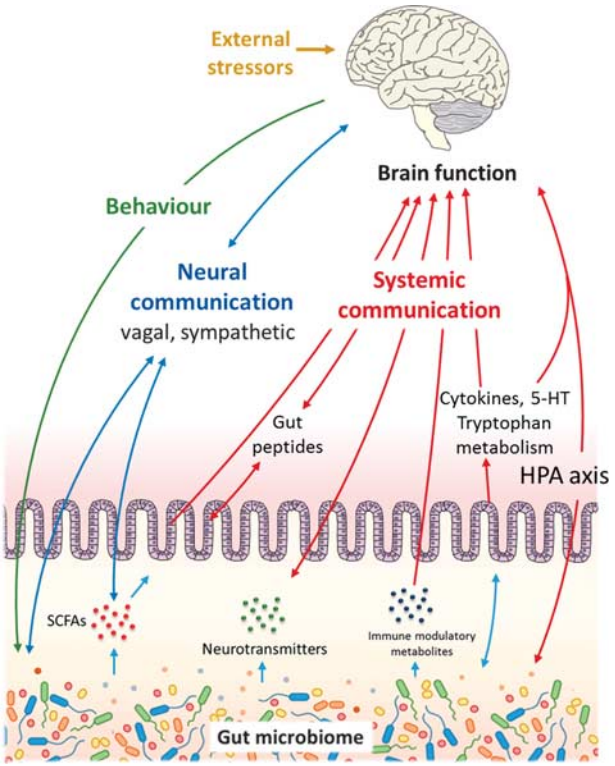

Figure 1. Communication pathways linking the gut microbiome with brain function.

translocation in animal models.[22,52] Evidence also exists that depression alters the gut microbiota, as demonstrated in mice in which chronic depression- and anxiety-like behaviours has been induced by olfactory bulbectomy,[53] suggesting a feedback loop between depressive states and dysbiosis. A reflection of the importance of this circular relationship may be the existence of host mechanisms that regulate microbiota composition.[54,55]

Similar parallels between dysbiosis and psychopathogenesis exist in schizophrenia (reviewed by Dinan *et al.*[56] and Nemani *et al.*[44]). Many of the strongest associations identified between genetic risk and schizophrenia relate to genes involved in immunity,[57,58] paralleling clinical studies that report an upregulated immune and inflammatory status in schizophrenia patients.[59–68] Serological markers of bacterial translocation are also substantially elevated in schizophrenia subjects and significantly correlated with systemic inflammatory markers.[69] In turn, cytokine levels are correlated with the severity of clinical symptoms,[59,70,71] and it has been suggested that the resulting neuroinflammation is involved directly in schizophrenia pathogenesis.[72–74]

As described later, the microbiota also modulate a range of neurotrophins and proteins involved in brain development and plasticity.[48,49,75] There is evidence that such alterations are central to the pathophysiology of schizophrenia. For example, BDNF expression is believed to have a role in the molecular mechanism underlying altered cognition,[76] and through its influence on brain plasticity, may contribute to the *N*-methyl-D-aspartate receptor dysfunction seen in schizophrenia.[77]

TREATMENT INTERACTIONS WITH THE MICROBIOME IN MENTAL ILLNESS

In addition to influencing psychopathogenesis directly, the gut microbiome makes an important contribution to drug metabolism, and potentially explains much of the inter-individual

variability in treatment efficacy and side-effects.[78,79] For example, the gut microbiota has been implicated in the reductive metabolism of psychotropic medications, including benzodiazepine clonazepam,[80] risperidone[81] and levodopa.[82] In addition, the gut microbiome is also able to influence the gene expression of hepatic enzymes that aid in the metabolism and detoxification of drugs outside of the gut.[83,84]

A reciprocal interaction also exists, with drugs used to target psychiatric or neurological disorders having the potential to affect the composition and function of the gut microbiome. For example, the atypical antipsychotic olazapine has been shown to affect microbiota composition in rats, as well as triggering inflammatory effects and weight gain,[85,86] with the co-administration of antibiotics shown to attenuate these physiological effects.[87] The impact of atypical antipsychotics on the gut microbiota may therefore explain to some extent the increased levels of cardiac and metabolic disease in patients receiving these medications.[88–90]

The clinical implications of these pathways remain poorly understood, but suggest the utility of a precision approach to therapy, as has been advocated in psychiatry[91,92] and other disease contexts.[93]

THE ROLE OF THE MICROBIOME IN BRAIN DEVELOPMENT

Prenatal neurodevelopment

Brain development spans the prenatal period to post adolescence and involves the interplay of genetic and environmental factors.[94] Disruption of these interactions can alter normal developmental trajectories and contribute substantially to neuropsychiatric outcomes in later in life.[95,96]

Neural development begins early in embryonic life with a number of important stages occurring before birth.[94] Areas of the brain undergoing these events exhibit greater fragility[97] and the significant impact of insults that occur during gestation is increasingly recognized.[98] During this period, maternal immunity and metabolism represents a link between neurodevelopment in the womb and the external environment. Challenges to maternal homoeostasis, such as infection, poor nutrition or prenatal stress (PNS), are associated with neurodevelopmental disorders, including anxiety, autism, attention deficit hyperactivity disorder, depression and schizophrenia.[99–109] Disruption of the maternal microbiome, or 'dysbiosis', appears to act as a link between external stressors and fetal development, either by altering normal developmental cues, or through the presentation of inappropriate developmental stimuli.

The precise nature of relationships between maternal microbiome interactions, altered neurodevelopment and subsequent psychopathologies, remain poorly defined. To a large extent, this is due to the challenge of determining the relative contribution of parallel and overlapping pathways that link multiple interacting systems. Even in animal models, it is extremely difficult to identify the relative contribution of pathways by which a single factor can lead to an array of behavioural disorders. As an illustration, the consumption of a HFD during pregnancy is associated with subsequent behavioural disorders.[110,111] However, HFD has been shown to influence multiple regulatory pathways in the immune,[112,113] metabolic[114] and neuroendocrine[110] systems, through both microbiome dependent and independent mechanisms, as well as resulting in the vertical transmission of the associated dysbiosis.[25] Further, the impact of an insult such as HFD consumption depends on the developmental stage at which it occurs, with similar adverse events during early or late periods associated with different outcomes.[105,115,116]

One important contributor to aberrant neurodevelopment appears to be the disruption of the immuno-regulatory role of the gut microbiome, resulting in a pro-inflammatory maternal state. Increased levels of circulating cytokines during pregnancy have been shown to negatively affect neural development [110] and could act by altering the fetal immune milieu (reviewed in detail elsewhere[94,117–119]).

Immune-dysregulation could result from factors that ablate the normal microbiota, such as antibiotics, thereby suppressing microbial interactions with toll-like receptors and Treg cells in the gut[120–122] or the production of immuno-regulatory metabolites, such as short-chain fatty acids (SCFAs).[122–124] Alternatively, factors that trigger dysbiosis, such as high fat consumption, could act by promoting the production of pro-inflammatory bacterial metabolites.[125] In addition, the dysbiotic changes in the gut microbiota could influence inflammation and CNS function through changes in activation of vagal and/or spinal nerve pathways.[22,108,126,127] The contribution of such a microbiota-immune interaction to stress-associated pathologies is supported by the observation that exposure to repeated stress affects the gut microbiota in a manner that correlates with changes in levels of pro-inflammatory cytokines.[128]

The maternal HPA axis is likely to represent another important link between prenatal insults and developmental abnormalities. The HPA axis is affected by factors such as PNS[129,130] and infection,[131] which are risk factors for a wide range of neurodevelopmental disorders.[132–136] In animal models of early-life postnatal stress, hyper-responsiveness of the HPA axis is coupled with altered visceral pain sensitivity and impaired intestinal barrier function,[137,138] while aberrant dietary protein:carbohydrate ratios during gestation have moderate long-term effects on the function of the HPA and sympatho-adrenomedullary axes in offspring.[139] It is useful to note direct responses to *in utero* stressors such as hypoxia also involve the adrenal system[140,141] and are essential to fetal survival and neurodevelopment.[142] Whether the maternal microbiome can influence these pathways remains unknown.

The manner in which a hyperactive maternal HPA stress response influences fetal development is unclear; however, an emerging hypothesis involves maternal cortisol crossing the placenta in a quantity sufficient to affect gene expression in fetal brain cells.[143] This model is supported by *in vitro* analysis of human fetal brain aggregates[144] and the observation that the effects of PNS on offspring can be partially mimicked by giving pregnant animals a synthetic glucocorticoid or adrenocorticotropic hormone.[130,145] However, the interaction of the HPA axis with the maternal microbiome is likely to be complex. In addition to affecting fetal neurodevelopment directly, stress-induced alterations to the HPA axis trigger maternal gut dysbiosis.[146] These changes in the gut microbiota could further influence HPA axis dysfunction through altered tryptophan metabolism, as well as contributing to other dysbiosis-associated dysregulatory pathways.[94] In addition, there is evidence that the gut microbiome influences the function of the placenta via the HPA axis, thereby altering fetal exposure to specific compounds in maternal circulation.[147–150]

The maternal gut microbiota could also affect fetal neurodevelopment by influencing levels of circulating 5-hydroxytryptamine (5-HT). The gut microbiome regulates 5-HT biosynthesis by enterochromaffin (EC) cells in the gut.[13] In turn, 5-HT regulates fetal neuronal cell division, differentiation and synaptogenesis[151] and its depletion results in altered brain development.[152] Furthermore, maternal plasma serotonin is required for proper neuronal morphogenesis during developmental stages that precede the appearance of serotonergic neurons, with embryos depending more on maternal plasma serotonin than their own during *in utero* development.[153] Maternal gut dysbiosis is also likely to influence blood–brain barrier (BBB) formation, a critical component in CNS development, ensuring an optimal microenvironment for neuronal growth and specification.[154] This is suggested by analysis of the embryos of GF mice, where the BBB has been shown to be substantially compromised.[155]

Postnatal neurodevelopment

Neurodevelopment continues outside the womb with the neonatal period characterized by substantial neurological development, including morphological changes, cell differentiation and acquisition of function.[156,157] Synaptogenesis begins shortly after birth and reaches maximum levels by around 2 years of age, before a process of synaptic refinement and elimination reduces the number of synapses in the postnatal brain to adult levels by mid-adolescence.[158] Remodelling continues well into the third decade of life,[159] providing a lengthy window of vulnerability to external perturbations. This critical period of neurodevelopment parallels the establishment and maturation of the microbiome, a process now known to be essential for the establishment of normal immune function,[160–164] the neuroendocrine system[165] and metabolic regulation.[166,167] Disruption of the microbiome in early life therefore has the potential to influence neurodevelopment and long-term mental health outcomes, particularly through its interaction with the immune system and the gut–brain axis.

Gnotobiotic animal models have been important in demonstrating the contribution of the developing microbiome to early-life neurodevelopment and the establishment of appropriate stress responses. For example, GF mice have an exaggerated hypothalamic-pituitary response to mild restraint stress, with elevated plasma adrenocorticotropic hormone and corticosterone and reduced BDNF expression levels in the cortex and hippocampus.[49] Furthermore, mice that develop in the absence of microbes exhibit increased motor activity and reduced anxiety, associated with differential expression of synaptophysin and PSD-95, proteins that are specifically involved in synaptogenesis pathways.[48] Microbial colonization is also required for programming and presentation of normal social behaviours, and is important for the regulation of repetitive behaviours,[168] the development of non-spatial memory,[29] and the development of pain signalling from the body.[169] It is important to note that the absence of appropriate microbial developmental cues in early-life can result in aberrant mental development that is not corrected by later microbial exposure (Neufeld *et al*.).[170]

It is clear from these and other GF animal studies that the absence of a commensal microbiota during early-life substantially affects both neurophysiology and the risk of abnormal behaviour development. However, while a useful tool for highlighting mechanistic pathways, the GF animal poorly reflects the types of microbiome disruption that may occur in humans. As such, other investigations have attempted to recreate real-world early-life insults in the controlled context of animal models. For example, while associations between caesarean-section delivery, altered early life microbial colonization[171,172] and the incidence of behavioural disorders and abnormal cognitive development in humans[173–175] have been known for some time, the extent to which a direct causal relationship exists is difficult to discern, given the number of other potentially contributing variables. However, when vaginally delivered mouse pups are compared with those delivered via caesarean section they show an altered gut microbiome and increased anxiety, social deficits and repetitive behaviours reminiscent of autism spectrum disorder-like behaviours in humans.[176]

Even in animal models though, the line between pre- and post-delivery periods is blurred by factors such as the vertical transmission of microbiota, the influence of the maternal microbiome of milk composition,[177] and the continuation of stressors in the external environment. An example of this complexity is the impact of PNS on neurodevelopment. PNS has been shown to alter the composition of the gut[178] and maternal vaginal microbiota in mice,[98,179] thereby altering the pool of microbes that can be passed to the neonate (an analogous situation has been described in humans, where PNS has been shown to affect the composition of the human infant gut microbiota over the first 110 days after birth[180]). As above although PNS also alters prenatal development, and therefore the nature of interactions between the neonate and microbes in early life. Determining the relative contribution and timing of contributory pathways to long-term psychopathological outcomes is therefore challenging.

The lasting impact of antibiotic exposure on the microbiome, whether during pregnancy,[181,182] intrapartum[183] or in the neonatal period[24,184] is an example of a further complex factor. There is clear potential for antibiotic dysbiosis to contribute to maternally mediated antenatal neurodevelopment, while antibiotic dysbiosis is also heritable.[25] Early-life exposure to antibiotics has been shown to result in long-term immune dysregulation[185] and visceral hypersensitivity.[186] Further, the developmental impact of antibiotic dysbiosis is not limited to the neonatal period, with adolescent rats exhibiting an altered tryptophan metabolic pathway, reduced anxiety and cognitive defects.[187]

Diet-induced maternal dysbiosis may also affect early-life neurodevelopment through milk composition. For example, the offspring of mice fed an HFD during lactation show developmental and neurobehavioral changes that suggest possible disruption of physical and sensory-motor maturation, and increased susceptibility to depressive and aggressive-like behaviour.[188] These observations suggests further work in in relation to dietary inputs will be important in understanding brain function determinants in humans.

MECHANISMS OF INTERACTION

Activation of inflammatory pathways appears to be a particularly important link between the microbiome and neonatal neurodevelopment. The gut microbiota can affect the immune system directly via activation of the vagus nerve,[22,126,189–191] in turn triggering bidirectional communication with the CNS.[192] In addition, indirect effects of the gut microbiota on the innate immune system can result in alterations in the circulating levels of pro- and anti-inflammatory cytokines that directly affect brain function.

Bacterial metabolites from the gut have a substantial influence on the regulation of the gut–brain axis and local and systemic immunity. SCFAs, produced by the bacterial fermentation of dietary carbohydrates, have immunomodulatory properties[121,123,124,193] and can interact with nerve cells by stimulating the sympathetic and autonomic nervous system via G-protein-coupled (GPR) receptor 41 (GPR41)[194] and GPR43.[195] In addition, they can cross the BBB, modulate brain development and behaviour[196–198] and have been implicated in the development of autism.[199] Further, gut microbiota derived SCFAs have been shown to regulate microglia homoeostasis,[200] necessary for proper brain development and brain tissue homoeostasis.[201–203] GF mice display global defects in microglia with altered cell proportions and an immature phenotype, leading to impaired innate immune responses in the CNS.[200] SCFAs also regulate the release of gut peptides from enteroendocrine cells,[204] which in turn affect gut–brain hormonal communication.[205,206] SCFAs have recently been shown to regulate the synthesis of gut-derived 5-HT from EC cells.[13] The gut provides ~95% of total body 5-HT,[207] most of which exists in plasma. Although this source of 5-HT has intrinsic roles within the gut[208,209] and peripherally in metabolic control,[210] EC cell 5-HT can activate afferent nerve endings to signal to the CNS.[211] Furthermore, this source of 5-HT has significant links to psychiatric disorders with the most commonly used antidepressant, fluoxetine, blocking the transport of gut 5-HT into plasma, while elevated plasma serotonin is observed in 25–50% of children with autism[212–215] and an inverse correlation between high plasma serotonin and low serotonergic neurotransmission has been demonstrated in young male adults with autism spectrum disorder.[216] In addition to SCFAs, gut bacteria are also capable of producing an array of other neuroactive and

immunomodulatory compounds, including dopamine,[217] γ-aminobutyric acid,[218] histamine[219] and acetylcholine,[220] while the gut microbiome is an important regulator of bile acid pool size and composition,[221] and, in turn, BBB integrity and HPA function.[222]

The gut microbiota could also contribute to the regulation of brain function by influencing tryptophan metabolism (reviewed by O'Mahony and colleagues[95]). Tryptophan is an essential, diet-derived, amino acid,[223] required for serotonin synthesis in the CNS.[224] Once absorbed from the gut, tryptophan can cross the BBB and participate in serotonin synthesis.[224] However, there are many other pathways through which tryptophan can be metabolized,[224] including the largely hepatic kynurenine pathway[225] and the major serotonin synthesis pathway in gut EC cells.[226–228]

The availability of tryptophan is heavily influenced by the gut microbiota. GF mice have been shown to have increased plasma tryptophan concentrations,[47,48] which can be normalized following post-weaning colonization.[47] Resident gut bacteria can utilize tryptophan for growth[229] and in some cases, production of indole,[230,231] or serotonin (reviewed by O'Mahony and colleagues[95]), while the microbiota might also affect tryptophan availability by influencing host enzymes responsible for its degradation.[47] By limiting the availability of tryptophan for serotonin production in the CNS (EC-derived serotonin does not cross the BBB), the gut microbiota could influence serotonergic neurotransmission.[95] In vulnerable populations, reducing the circulating concentrations of tryptophan has been shown to affect mood, and to reinstate depressive symptoms in patients who have successfully responded to selective serotonin reuptake inhibitors.[232,233] The gut microbiota could also influence the production of both neuroprotective and neurotoxic components of the kynurenine pathway.[224]

Other pathways by which the gut microbiota could influence the development and activity of brain tissue include regulation of the release of gut peptides from enteroendocrine cells,[204] which in turn affect gut–brain hormonal communication,[205,206] and, as described above, the regulation of microglia homoeostasis.

Two recent, related papers by Wong *et al*. and Zheng *et al*. indicate that the microbiota–gut–brain axis functions in a bidirectional manner in the regulation of depressive-like behaviours. Data in the paper by Wong *et al*.[234] demonstrate that changes in behaviour caused by increased stress levels, knockout of caspase 1 leading to decreased inflammasome function, or pharmacological treatments result in changes in the gut microbiome. The paper by Zheng *et al*. shows three key findings: (i) the absence of gut microbiota in GF mice resulted in decreased immobility time in the forced swimming test relative to conventionally-raised healthy control mice. (ii) From clinical sampling, the gut microbiotic compositions of MDD patients and healthy controls were significantly different from that of MDD patients. (iii) Faecal microbiota transplantation of GF mice with 'depression microbiota' derived from MDD patients resulted in depression-like behaviours compared with colonization with 'healthy microbiota' derived from healthy control individuals. Moreover, the concerned authors showed that mice harbouring 'depression microbiota' primarily exhibited disturbances of microbial genes and host metabolites involved in carbohydrate and amino acid metabolism, indicating that the development of depressive-like behaviours is mediated through the host's metabolism.[235] The combined findings of these two papers suggest that the microbiota–gut–brain axis is fully bidirectional, functioning in a manner through which changes in microbiota affect behaviour, while conversely, changes in behaviour brought about by chronic stress, genetic manipulation, or pharmacological intervention, result in alterations in microbiota composition. Novel approaches to target this bidirectional interface of gut microbiota and depressive-like behaviour may offer novel approaches for the treatment of major depression.

THE ROLE OF THE MICROBIOME IN AGE-RELATED COGNITIVE DECLINE

Despite fluctuating in response to external influences, the gut microbiota is thought to remain relatively stable during adulthood.[236] However, just as the microbiome has a critical role in the development of the nervous system in the neonate, it also appears to have a substantial influence on CNS degeneration in old age. Aging affects the brain on both cellular and functional levels, and is associated with decline in sensory, motor and higher cognitive functions.[237–239] This period of life is also associated with marked changes in the microbiome.[240,241] In keeping with dysbiosis arising from a range of insults, age-related changes in gut microbiota composition appear to involve a reduction in microbial diversity, with an increased relative abundance of Proteobacteria and a reduction in bifidobacteria species, and reduced SCFA production.[239]

It has been suggested that the processes of age-related dysbiosis and neurological decline are linked through the former mediating chronic low-grade inflammation as a common basis for a broad spectrum of age-related pathologies, or so-called 'inflamm-aging'.[242] Inflammation has a substantial role in cognitive decline, not only in the context of normal aging but also in neurological disorders and sporadic Alzheimer's disease.[243] There are a number of ways in which gut dysbiosis could contribute to this process, including direct inflammatory stimulation, the production of pro-inflammatory metabolites, and the loss of immune-regulatory function. In addition, the gut microbiome is essential to the bioavailability of polyphenols, unsaturated fats and antioxidants, all of which may help protect against neuronal and cell aging role under normal circumstances (reviewed by Caraccciolo *et al*.[239]). Notably, dysbiosis-associated inflammation is also strongly implicated in obesity and diabetes, both of which have been shown to exacerbate normal cognitive decline.[244–247]

Age-related changes in the brain are most pronounced in the amygdala, hippocampus and frontal cortex,[248] whose function is heavily dependent on serotonergic neurotransmission,[249] potentially implicating microbiome-influenced changes in tryptophan metabolism. Further, altered serotonin systems could represent a common link with changes in sleep, sexual behaviour and mood in the elderly, as well as disorders such as diabetes, faecal incontinence and cardiovascular diseases.[94,250]

An association between loss of microbiome function, specifically genes that encode SCFAs, and increased levels of circulating pro-inflammatory cytokines, has been shown in healthy elderly people.[251] Further, markers of microbiome change are significantly correlated with diet, and with indices of frailty and poor health among long-term institutionalized people,[251] while feeding cognitively healthy elderly individuals a diet low in meat and meat products is associated with subsequent increases in brain volume and cognitive function.[252] Interestingly, in mice, the same HFD predisposes to physiological and anxiety-like effects in adults, while aged mice display deficits in spatial cognition,[253] suggesting the effect of stressors changes during the aging process.

With a growing appreciation of the healthcare implications of an aging global population[254–256] obtaining a better understanding of how the bidirectional interaction between the microbiome and gut–brain axis that influences age-related changes in brain function, must be a priority.

MODIFICATION OF THE GUT MICROBIOTA TO AFFECT THERAPEUTIC CHANGE

As described above, studies in mice have shown that alteration of the microbial composition of the gut can induce changes in behaviour, raising the possibility of therapeutic manipulation of the microbiome. What approach might be appropriate depends on the specific role of the microbiome in pathogenesis.

In instances where the absence of particular bacterial species is linked to altered brain function, the addition of discrete microbes may be clinically effective. For example, in rats deprived of maternal contact at an early age, treatment with *Bifidobacterium infantis* results in normalization of the immune response, reversal of behavioural deficits, and restoration of basal noradrenaline concentrations in the brainstem,[257] while in a mouse model of gastrointestinal inflammation and infection, exposure to *B. longum* normalizes anxiety-like behaviour.[258,259] The effects of psychosocial stress are also reversed in mice following probiotic treatments.[260,261] Such effects are not limited to rodent models; in healthy women, a probiotic cocktail alters activity of brain regions that control central processing of emotion and sensation.[262] Broadly, such probiotic effects appear to mediate behavioural changes through stimulation of the vagus nerve[22,191,258] or through modulation of cytokine production.[263]

Probiotic therapies have limitations, including a poor ability to establish a stable population within the recipient. Further, in many instances, pathogenesis may be contributed to by broad functions conserved across many different species, such as the ability to produce metabolites that are immunomodulatory, or that directly influence brain activity.[264,265] Here, it may be the absence of suitable drivers of beneficial behaviour that is limiting, rather than the absence of microbes capable of exhibiting them. In such instances, the broad-scale alteration of the microbiome using selective dietary microbial growth substrates, or prebiotics, may be more appropriate and result in longer lasting change. For example, consumption of fructooligosaccharides or a non-digestible galactooligosaccharide formulation (BGOS) elevates BDNF levels and NMDAR subunit expression in rats.[266] BGOS consumption also reduces anxiety in mice injected with lipopolysaccharide to induce sickness behaviour, an effect that appears to be related to the modulation of cortical interleukin-1β and 5-HT_{2A} receptor expression.[267] In humans, daily consumption of BGOS for 3 weeks results in a significantly lower salivary cortisol awakening response compared with placebo and a decreased attentional vigilance to negative versus positive information.[268] Pusceddu *et al*.[269] showed that long-term supplementation with n-3 polyunsaturated fatty acids corrected dysbiosis seen in maternally separated female rats, and was associated with an attenuation of the corticosterone response to acute stress. Interestingly, while the supporting evidence for the efficacy or such approaches is only now emerging, the consumption of wholegrain and high fibre foods, essentially prebiotics, is already recommended to patients.[270]

Demonstrations of the transmissibility of behavioural traits between animals by faecal microbiota transfer are also intriguing. Faecal microbiota transfer is employed increasingly widely in the treatment of conditions such as recurrent *Clostridium difficile* infection.[271] Its ability to influence behaviour suggests that it might also have a role in the treatment of psychopathology (reviewed by Collins *et al*.[272]). It is important to note, however, that these observations also raise important questions about current approaches to donor screening for therapeutic faecal microbiota transfer.

FUTURE DIRECTIONS

The advances in our understanding of the role of the microbiome in neurodevelopment and mental health, particularly in the past 5 years, have been remarkable. The implications of this new insight are only beginning to become apparent; however, the potential value of microbiome analyses in revealing mechanisms that underpin altered brain development and mental illness is hugely exciting. There is now a need to close the gap between practice, including the increasing use of pro- and prebiotics, and the supporting science. The importance of achieving this is reflected in the substantial investments made to 'microbiome–gut–brain axis' research by both the US government and the European Union.[273]

Achieving a better understanding of the contribution of the microbiome to mental health will require further development of analytical approaches. Studies based on reductive animal models, particularly those involving GF animals, have been important in identifying underlying mechanisms; however, they exclude the complexity of real-world interactions. The rapidly falling costs of 'omics' approaches to microbiome analysis now allow them to be applied to large human cohorts within life-course studies, with data generated assessed in the context of detailed genetic, epigenetic, demographic and clinical assessments. Exploiting these opportunities will result in substantial improvement in our understanding of altered brain function and mental illness in the relative near-term.

In addition to changing analytical strategies, the conceptual framework within which these data are assessed must also continue to develop. A 'three-hit' model of vulnerability and resilience to mental health issues, based on genetic predisposition, the prenatal environment, and later life experiences, has been proposed.[274] However, just as the gut–brain axis might be extended to include the microbiome, such developmental pathways must also take into consideration points of interaction with our resident microbiota. Refining these models based on empirical data now represents a key challenge in understanding the processes behind altered brain function and mental illness.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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