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VIEWPOINT

Melancholic microbes: a link between gut microbiota and depression?

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Key Messages

- The concept that gut microbiota may modulate brain chemistry and behaviour is gaining traction and efforts are now turning to investigate the role of microbiota in animal models of psychopathology.
- Here we summarise the evidence that microbiota may also play a role in depression and susceptibility to chronic stress.
- This may lead to the development of psychobiotic-based therapeutic strategies for psychiatric disorders and gastrointestinal disorders with affective co-morbidities.
- However, further studies investigating the exact alterations in microbiota in depressed patients are now warranted.

Abstract

There is a growing awareness of the potential for microbiota to influence gut-brain communication in health and disease. A variety of strategies have been used to study the impact of the microbiota on brain function and these include antibiotic use, probiotic treatments, fecal microbiota transplantation, gastrointestinal infection studies, and germ-free studies. All of these approaches provide evidence to support the view that the microbiota can influence brain chemistry and consequently behavior. Efforts are now turning to investigate the role of microbiota in animal models of psychopathology. Animal models of depression are thus essential in studying the complex interplay between the microbiota and brain. Recent studies published in this Journal and elsewhere demonstrate that there is a distinct perturbation of the composition of gut microbiota in animal models of depression and chronic stress. This has direct implications for the development of psychobiotic-based therapeutic strategies for psychiatric disorders. Moreover, given that affective co-morbidities, such as major depression and anxiety states, are common in patients presenting with irritable bowel syndrome (IBS), it may have implications for functional bowel disorders also. Further studies require appropriately phenotyped patients with depression and/or IBS using a judicious use of techniques including functional imaging and in depth microbial pyrosequencing.

Keywords *brain-gut axis, IBS, major depression, microbiota, probiotics, psychobiotics.*

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It is increasingly recognized that the brain-gut axis provides a bidirectional homeostatic route of communication which if dysfunctional can have important pathophysiological consequences. This axis is regu-

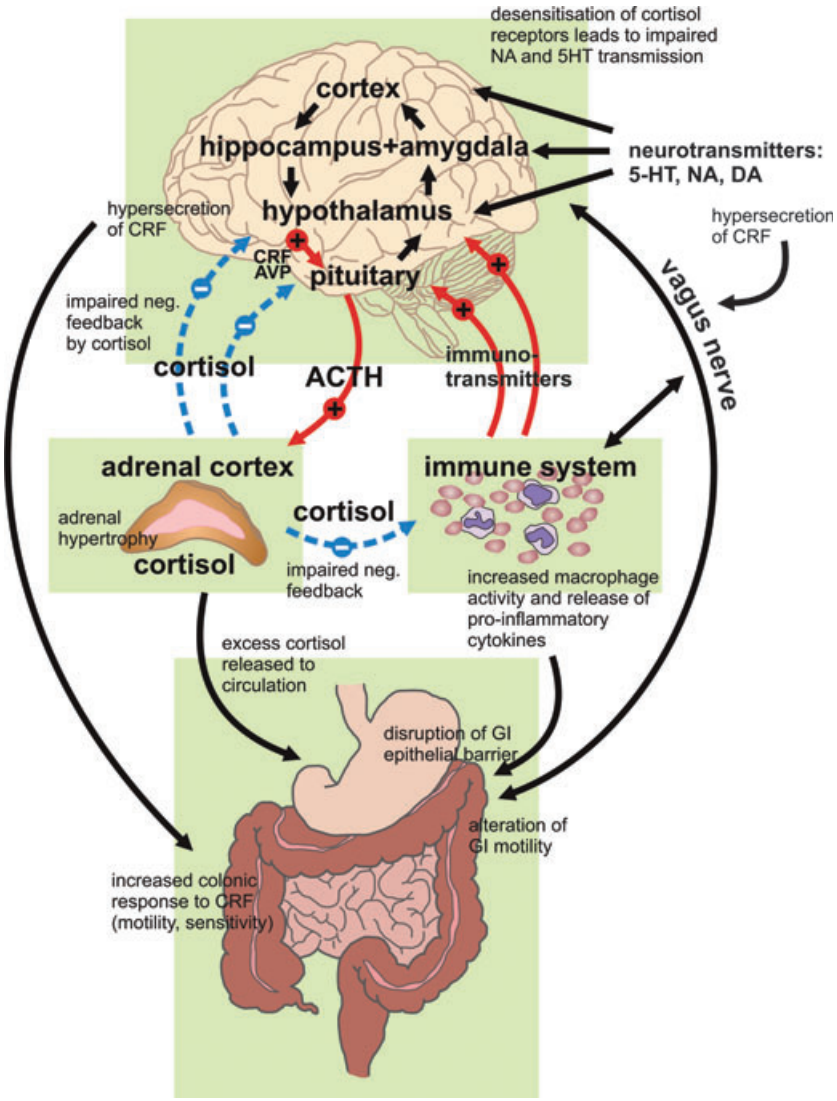

Figure 1 A schematic representation of the effects of chronic stress and depression on brain-gut axis activity. The bi-directional communication allows signals from the brain corticolimbic structures to alter gastrointestinal function. The HPA axis and immune system are key regulators of this axis also.

lated at neural, hormonal, and immunological levels.[1] Whilst much focus on this axis has been on the central regulation of digestive function and satiety; there has been increasing emphasis on its role in other aspects of physiology. It is now clear that alterations in brain–gut interactions are associated with gut inflammation, chronic abdominal pain syndromes, and eating disorders.[1] Indeed, modulation of brain-gut axis function is associated with specific alterations in the stress-response and overall behavior (see Fig. 1). The high co-morbidity between stress-related psychiatric symptoms such as anxiety with gastrointestinal (GI) disorders including irritable bowel syndrome (IBS) and inflammatory bowel disorder (IBD) is further evidence of the importance of this axis.[2] Thus modulation of the brain-gut axis is being seen as an attractive target for the development of novel treatments for a wide variety of disorders ranging from obesity, mood, and anxiety disorders to GI disorders such as IBS.

There is also a growing appreciation that gut microbiota can play a crucial role in maintaining homeostasis in health and contribute to the pathogenesis of a variety of diseases. This is now even extending to disorders of the central nervous system. Indeed, the importance of emotional state and stress processing in the brain has received increasing recognition in the study of GI disorders, and microbiota-gut brain axis dysregulation in stress-related CNS disorders has been the subject of a

number of excellent recent reviews.[3–7] Major depression is a common, debilitating stress-related disorder whereby patients frequently have hypothalamic-pituitary-adrenal (HPA) alterations such as elevated cortisol levels in plasma, elevated corticotropin releasing factor (CRF) levels in the cerebrospinal fluid coupled with a failure to suppress cortisol in response to dexamethasone challenge.[8] Moreover, marked increases in the concentrations of pro-inflammatory cytokines are also common biological hallmark of the disease (see Fig. 1).[8]

Microbes can influence the functioning of the HPA and immune system and thus it is perhaps not so surprising that there could be a link between microbiota and depression. It is now almost a decade since Sudo *et al.* demonstrated that germ-free (GF) mice with a sterile GI tract have an overactive HPA in response to stress. This hyper-response of the HPA is reversed by monoassociation with a single organism, *Bifidobacterium infantis*, which is a predominant bacterium in the infant gut and a commonly used probiotic organism.[9] More recent studies from three independent research groups in Sweden, Canada and Ireland[10–12] have all showed alterations in the levels of key monoamines (or their receptors) involved in depression (noradrenaline [NA] and 5-hydroxytryptamine [5-HT; serotonin]) in corticolimbic regions of the brain. Moreover, there is also evidence for alterations in key neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), involved in depression in the hippocampus of GF mice.[10,13] However, there has been limited direct evidence linking depression and gut microbiota to date.

To this end, Park and colleagues in this issue of the *Journal*[14] attempt to bridge this gap in an interesting series of studies. They show that a lesion-based animal model of depression the olfactory bulbectomised mouse has alterations in the gut microbiota in parallel with a depression-related behavioral and endocrine phenotype. Specifically, the behavior of bulbectomised mice was markedly different to sham-operated animals in a step-down test whereby they had a significant reduction in the latency to step down, and in the tail suspension test whereby they exhibited prolonged immobility or behavioral despair. Olfactory bulbectomised mice also demonstrated hyperlocomotion in the open field test which is one of the most robust behavioral changes in this model. Moreover, a two-fold increase in the basal expression of CRF in the paraventricular nucleus of the hypothalamus was also found suggesting that heightened activation of the HPA axis. With regard to the microbiota cluster analysis of the banding patterns was used to compare microbial profiles in bulbectomised and sham-operated mice. The similarity of the microbial composition was in the order of 60% within the groups of bulbectomised and sham animals which was markedly reduced to 49.1% similarity between the two groups. Interestingly, the difference was mainly due to a change in the proportion of certain bacterial phyla rather than the appearance or disappearance of bacteria at the phylum level following bulbectomy. Overall, these findings indicate a redistribution of the relative abundances of bacterial phyla within the intestinal microbiota in animals with a depression- and anxiety-related phenotype. Although this could be unrelated to the behavioral phenotype, the authors go on to show that central CRF infusion which produces a similar behavioral phenotype altered motility and also reduced the relative diversity of microbiota. Thus together, these findings provide important and novel insights into the relationship between microbiota and major depression. Olfactory bulbectomy is one of the few animal models that responds to chronic, but not acute antidepressant medication and has been widely used as a screening tool for novel therapeutics.[15–18] Moreover, many of the biological hallmarks of depression are also found following bilateral removal of the olfactory bulbs of rodents including neurochemical, neuroendocrine, and neuroimmune changes.[18] Altered reward processing a key symptom of depression is also observed.[19] Now Park and colleagues add altered microbial composition to the list of phenotypic alterations in the bulbectomy model. It is important to note that the bulbectomy model is often criticized as it lacks overt construct validity despite high face and predictive validities.[20] Moreover, alterations in bodyweight and loss of smell are confounding factors in all bulbectomy studies and these were not explicitly controlled for in the current study. Further, the mechanism as to how bulbectomy induces its effects are not clear as it is not due to simple anosmia and is thought to involve limbic degeneration.[18] Indeed, some authors refer it as a model of neurodegeneration as opposed to depression *per se.*[21] Thus, it is important to assess the role of the microbiota in other models of depression and chronic stress.

Indeed the relationship between stress and microbiota composition goes back many decades, to when Tannock and Savage noted that 'stressed mice showed dramatic reductions in these populations of lactobacilli'.[22] We have previously used the maternal separation model of early-life stress and depression in this regard. Adverse early-life events are associated with a maladaptive stress response system and increase the vulnerability to disease in later life. Several disorders

have been associated with early life stress, including both depression and IBS.[23] We assessed the effect of early-life stress on the brain-gut axis. Male rat pups were stressed by separating them from their mothers for 3 h daily between postnatal days 2–12. The control group was left undisturbed with their mothers. Behavior, immune response, stress sensitivity, visceral sensation, and fecal microbiota were analyzed.[24] The early life stress increased the number of fecal boli in response to a novel stress.[24] The key stress hormone, corticosterone, was increased in the maternally separated animals, when they reached adulthood. An increase in the systemic immune response was noted in the stressed animals after an *in vitro* lipopolysaccharide challenge, as evidenced by an exaggerated release of pro-inflammatory cytokines. Increased visceral sensation was also seen in the stressed group.[24] Moreover, there were alterations locally in the GI tract in terms of colonic morphology, increased mucus secretion and an associated elevation in the number of mucosal goblet cells.[25] Moreover and most interestingly, there was a reduction in the diversity of the microbiota as determined by the percentage similarity of the Denaturing Gradient Gel Electrophoresis (DGGE) profiles within maternally separated relative to the non-separated animals.[24] This is in line with the findings of Park *et al.* in the olfactory bulbectomised model which also find a redistribution of the relative abundances of bacterial phyla within the intestinal microbiota. Although the culture techniques used in both studies are being superseded by newer metagenomic approaches they are a useful indicator of overall bacterial population differences. Similarly, both studies reported a pro-inflammatory phenotype associated with the depressed state and enhanced HPA activity, although using different parameters. The former finding increased CRF expression in the paraventricular nucleus and the latter high levels of corticosterone in the plasma.

Moreover, using a primate model of early life stress Bailey and colleagues have shown a significant decrease in fecal lactobacilli on day three postseparation.[26] Other investigators have used chronic stress models in adulthood to investigate brain-gut-microbiota interactions. Chronic social defeat has been shown to induce moderate morphological changes to the colon in mice[27] and a combination of social defeat and overcrowding which induces depression and anxiety-like behaviors[28] increases the severity of an acute DSS-induced colitis.[29] Using deep sequencing methods it has been demonstrated that the community structure of microbiota from mice exposed to chronic restraint stressor was significantly different to that in non-stressed control mice[30] and that the relative abundance of *Bacteroides* was decreased, while that of the genus Clostridium was increased in the caecum. The stressor also increased circulating levels of IL-6 and MCP-1, which were significantly correlated with stressor-induced changes to three bacterial genera (i.e., *Coprococcus, Pseudobutyrivibrio,* and *Dorea*). These data show that exposure to repeated stress affects gut bacterial populations in a cytokine dependent manner.[30] More recently it has been shown that stress can exaggerate the impact of antibiotics on luminal and wall-adhered microbiota and enhance the local expression of visceral sensory-related systems in mice.[31]

It is perhaps premature to extrapolate the current preclinical work to the clinic. Indeed there are no data available on the relative composition of the microbiota in depressed patients to date. However, it is clear that affective co-morbidities in the form of major depression and anxiety states are common in patients presenting with IBS. In many studies over 50% of IBS sufferers have a concomitant psychiatric diagnosis. This level of co-morbidity far exceeds that seen in other GI disorders. The underlying pathophysiology behind the increased rate is poorly understood and attention is now focusing on the role of the microbiota. Results from 16S rRNA-based microbiota fingerprinting suggest both quantitative and qualitative changes of both the mucosal and fecal gut microbiota in IBS patients. However, as Jeffery *et al.* state the contribution of altered Intestinal microbiota composition or function in IBS remains controversial, though the subject of much current research.[32] Subgroup specificity seems to be important with indications from a recent pyrosequencing study in a diarrhea predominant group of IBS patients indicating reduced microbial richness, a decrease in beneficial and an increase in detrimental bacterial species.[33] Interestingly, although a targeted pyrosequencing study looking at the fecal composition did not find a uniform change between a well characterized IBS cohort and healthy controls[32] they did find associations between the microbiota and metadata, particularly in relation to microbial signatures that defined clinical phenotypes such as colonic transit indices and of note co-morbid depression. Not all studies have found disturbances in the microbiota composition of IBS patients and it is currently unclear whether the alterations that have been reported are primary or secondary in nature.

Various strategies to date have been used to study the impact of the microbiota on brain function and include antibiotic use, probiotics treatments, fecal

microbiota transplantation, GI infection studies and GF studies.[6] Interestingly, recently published data indicate that the microbiota are crucial for the programming and presentation of distinct normal social behaviors, including social motivation and preference for social novelty in addition to other cognitive deficits previously described.[34] Given that cognitive deficits are also hallmarks of the bulbectomy syndrome[18,21,35] it will be of interest to assess if the altered microbiota composition plays a role in this aspect of the bulbectomy phenotype. One of the most intriguing and direct way of studying the impact of microbiota on behavior is via fecal transplantation to GF mice. To this end Bercik *et al.* showed that colonization of GF BALB/c mice (anxious strain) with microbiota from NIH Swiss mice increased exploratory behavior and hippocampal levels of BDNF, whereas colonization of GF NIH Swiss mice with BALB/c microbiota reduced exploratory behavior.[13] Levels of arousal in mice therefore seem determined at least in part by the microbiota. Whether fecal transplantation of microbiota from bulbectomised animals or other models of depression could also alter behavior of the recipient is an intriguing possibility.

Probiotic studies are among the most commonly carried out to support a relationship between gut microbiota and brain and behavior. We examined the impact of *Lactobacillus rhamnosus* on behavior and central GABA receptors in mice.[36] Animals fed *L. rhamnosus* demonstrated reduced anxiety on a variety of behavioral measures and altered central expression of both $GABA_A$ and $GABA_B$ receptors. In order to determine the mechanism of action, animals underwent vagotomy or sham surgery and were treated either with *L. rhamnosus* or inactive broth. Vagotomy prevented the emergence of an anxiolytic effect from the probiotic and prevented changes in GABA receptor expression. The study provides compelling evidence to indicate that the vagus mediates the behavioral and neurochemical effects of *L. rhamnosus*.[36] A growing body of data is emerging using different models to support the contention that a variety of other potential probiotics can exert psychotropic potential. Specifically, *B. Infantis* has been shown to reverse maternal-separation-induced increases in immobility in the forced swim test[37] and also increased plasma tryptophan levels.[38] More recently, *L. helveticus* was shown to prevent diet-induced anxiety-like behavior and memory[39] and *B. longum* NCC3001 reversed colitis-induced anxiety in the mouse via the vagus nerve.[40] Moreover, a cocktail of probiotics (*L. acidophilus, B. lactis* and *L. Fermentum*) reversed diabetes-induced cognitive and electrophysiologiocal changes[41] whereas a combination of *L. helveticus* and *B. longum* decreased anxiety[42] and reversed postmyocardial infarction-induced depression in the rat.[43]

Clinical validation of these findings is essential and a recent neuroimaging study has been very important in illuminating the promise of translating the preclinical data into a potential therapeutic reality. Healthy women were recruited with no GI or psychiatric symptoms and randomly assigned them to receive either fermented milk product containing *B. animalis*, *Streptococcus thermophiles, L. bulgaricus*, and *Lactococcus lactis* or a non-fermented milk product or no intervention twice daily for 4 weeks.[44] Participants underwent functional magnetic resonance imaging before and after the intervention to measure brain response to an emotional faces attention task and resting brain activity. Multivariate and region of interest analyses were performed. Probiotic intake was associated with reduced task-related response of a distributed functional network containing affective, viscerosensory, and somatosensory cortices. Alterations in the intrinsic activity of resting brain indicated that ingestion of probiotics was associated with changes in midbrain connectivity, which could explain the observed differences in activity during the task. The data suggest that probiotics can alter brain regions that control central processing of emotion and sensation.[44] Recently, a study assessing the effect of a combination of *L. helveticus* and *B. longum* on both human subjects had beneficial psychological effects with a decrease in serum cortisol.[41] Probiotics with the capacity to positively impact on symptoms of depression or anxiety have recently been termed psychobiotics.[45]

There are to date no published studies of the use of probiotics in major depression whereas they are effective in IBS.[46] Modulating the microbiota using antibiotics has been the subject of some investigation. Some studies have demonstrated the efficacy and sustained improvement of IBS symptoms with rifaximin treatment,[47] while studies in depressed patients indicate the efficacy of minocycline which impacts on both gram negative and positive bacteria.[48] Data indicate that patients with psychotic depression respond to a combination of an antidepressant and minocycline in combination.[48] Interestingly minocycline has also been shown to reveres some of the motor and cognitive effects induced by olfactory bulbectomy.[35] This adds further possibility to the possibility of the behavioral changes in this model maybe due to alterations in microbial composition in the gut. To date there are no reported studies of antibiotic therapy focusing on

psychiatric co-morbidities in IBS. However, it is intriguing that both IBS symptoms and those of depression respond independently to antibiotics, providing important evidence for the role the microbiota plays in regulating brain function.

In summary, using a variety of techniques our understanding of the ways in which the microbiota influences the brain and behavior is gradually unraveling. This will allow for a more complete understanding of the brain-gut axis miscommunication in chronic stress states such as depression and those thought to underlie the common co-morbid affective disturbances associated with IBS. What we require at this point are appropriately phenotyped patients and a judicious use of the various techniques including functional imaging and in depth pyrosequencing of the microbiome.

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DISCLOSURE

TGD has until recently been on the Board of Alimentary Health Ltd. Both authors have spoken at meetings sponsored by food and pharmaceutical companies.

AUTHOR CONTRIBUTION

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