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Psychobiotics:

Evolution of novel antidepressants

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Abstract

The gut-brain axis is a bidirectional communication system which allows the central nervous system and gastrointestinal tract to interact with, and respond to each other rapidly and effectively. It is becoming increasingly clear that a major player in this complex system are gut bacteria. The mechanisms of signal transmission from bacteria to brain are complex and not fully elucidated, but include neural, endocrine, immune, and metabolic pathways. It was initially demonstrated in a rodent model of depression that the gut microbiota was altered. This observation has been replicated in patients with major depression who show decreased microbial diversity. Furthermore, when rodents receive a microbiota transplant from a depressed patient their behaviour alters, as does their tryptophan metabolism and immune status. Several studies of psychobiotics (bacteria with a potential mental health benefit) have been conducted in healthy populations and in patients with depression. While some psychobiotics have shown efficacy in treating depression other bacteria have yielded negative findings. Larger scale well designed studies are required. EU funded guidelines recommend that patients with depression or vulnerability to depression should be encouraged to enhance a plant-based diet with a high content of grains /fibres, fermented foods and fish. A significant impact of such a diet is likely mediated through the gut microbiota.

The 1950's represent a watershed in the history of psychopharmacology. In this decade the first antidepressant discovered was the antibiotic isoniazid which was developed in the USA for treating tuberculosis. Unexpected side effects of euphoria, psychostimulation, increased appetite, and improved sleep were observed and prompted an interest in the medication as a potential antidepressant. Subsequent clinical trials confirmed antidepressant efficacy, which was attributed to isoniazid's ability to inhibit monoamine-oxidase (MAO) and, therefore, increase levels of monoamines such as noradrenaline, serotonin and dopamine. The 'monoamine hypothesis of depression' emerged and heralded the development of further antidepressant medications, including the tricyclic antidepressants (TCAs) and serotonin-specific reuptake inhibitors (SSRIs), both of which act to increase central monoamine levels. However, over the past twenty years, few new or novel antidepressants have emerged and the field of psychopharmacology seems to have hit the buffers.

Novel paradigm

The view that the antimicrobial action of isoniazid might be responsible for, or at least contributing to, its antidepressant action has largely been ignored. However, a new paradigm in neuroscience has emerged which challenges the orthodox view of antidepressant action. The new paradigm is referred to as the microbiota-gut-brain axis (MGB) hypothesis which strongly emphasises the important role gut microbes play in regulating brain function and stress responses.

The gut-brain axis is a bidirectional communication system which allows the central nervous system (CNS) and gastrointestinal tract (GIT) to interact with, and respond to, each other rapidly and effectively. Thus, gut homeostasis and function have the ability to reciprocally impact emotional states and behaviour. It is becoming increasingly clear that a major player in this complex system are gut bacteria. Trillions of bacteria reside in our GIT, outnumbering our own human cells and gut viruses add an extra layer of complexity. We now appreciate that the immense collective genetic material of these gut microbes, comprising the 'gut microbiome', has the ability to shape

neurodevelopment and impact psychological functioning to a remarkable extent. While brain-gut communication has been a subject of investigation for decades an exploration of gut microbes as a vector within this context has only recently been addressed. The mechanisms of signal transmission are complex and not fully elucidated, but it is clear they include neural, endocrine, immune, and metabolic pathways [1-3]. Preclinical studies have implicated the vagus nerve as a fundamental route of communication between gut microbes and centrally-mediated behavioural effects, as illustrated by the elimination of central *Lactobacillus rhamnosus* effects following full truncal vagotomy [4]. Interestingly, it has been demonstrated that individuals who underwent a full truncal vagotomy for treatment of peptic ulcer disease have a decreased risk of certain neurological disorders when they enter old age [5]. The gut microbiota also regulates key central neurotransmitters such as serotonin by altering levels of precursors; for example *Bifidobacterium infantis* has been shown to elevate plasma tryptophan levels and thus influence central 5-HT transmission [6]. Tryptophan is the building block of 5HT and the human brain has limited storage capacity therefore requiring a continual supply from the intestine. Of probable evolutionary significance is the fact many bacteria can synthesise and release neurotransmitters. For example, *Lactobacillus* and *Bifidobacterium* species can produce gamma-aminobutyric acid (GABA): *Escheridia*, *Bacillus* and *Saccharomyces spp*. can produce noradrenaline: *Candida, Streptococcus, Escheridia and Enterococcus spp.* can produce serotonin: *Bacillus* can produce dopamine: and *Lactobacillus* can produce acetylcholine [7,8]. These microbe-produced neurotransmitters can cross the mucosal layer of the intestine, though it is improbable that they directly influence brain function. Even if they enter the blood stream, which is by no means certain, they are incapable of crossing the blood brain barrier (BBB), though they might act on brain regions where the BBB is less developed such as the hypothalamus. Their impact on brain function is more likely by acting locally on the enteric nervous system. Short chain fatty

acids (SCFAs), which include butyrate, propionate and acetate are essential metabolic products of gut microbial activity and may exert central effects either through G-protein coupled receptors, though such receptors are sparsely concentrated in the mammalian brain. They may however act as epigenetic modulators through histone deacetylases (HDACs)[9]. Immune signalling from gut to brain mediated by cytokine molecules is another well documented route of communication [10]. Cytokines produced at the level of the gut can travel via the bloodstream to the brain. Under normal physiological circumstances they do not cross the BBB, but increasing evidence indicates a capacity to signal across the BBB and to influence brain areas such as the hypothalamus where the BBB is deficient. It is through the latter mechanism the cytokines interleukin (IL)-1 and IL-6 activate the core stress system, the hypothalamic-pituitary-adrenal axis (HPA), bringing about the release of cortisol. This is regarded as the most potent activating mechanism of the stress system and may be of relevance in conditions such as the depression that emerges with interferon therapy for hepatitis and melanoma [11].

Microbiota in depression

Dinan and his colleagues studied the gut microbiota in a maternal separation model of depression in rats [12]. They reported an overactive HPA response in such animals, together with an increase in pro-inflammatory cytokines and a decrease in the diversity of gut microbes. In a recent study the faecal microbiota was sequenced in a depression study [13]. Forty-six patients with depression and 30 healthy controls were recruited. High-throughput pyrosequencing showed that, according to the Shannon index, increased faecal bacterial alpha-diversity was found in those currently depressed but not in a group who had responded to treatment. Bacteroidetes, Proteobacteria, and Actinobacteria were increased, whereas Firmicutes was significantly reduced. Despite the profound inter-individual variability, levels of several predominant genera were significantly different between the depressives and

controls. Most notably, the depressives had increased levels of Enterobacteriaceae and Alistipes but reduced levels of Faecalibacterium. The authors conclude that further studies are necessary to elucidate the temporal and causal relationships between gut microbiota and depression and to evaluate the suitability of the microbiome as a biomarker. In our study when rats were given a humanised microbiota from depressed patients as opposed to healthy controls they developed a depressive phenotype from a behavioural and immune perspective. The depressed patients had elevated cortisol output together with decreased faecal microbial richness[14]

Development of Psychobiotics

It is notoriously difficult to develop new psychotherapeutics. This is due, in part, to limited knowledge about the neurobiological basis for mental illness and the absence of targetable biomarkers. Many psychotropic medications, such as lithium for mania and chlorpromazine as the first antipsychotic, were discovered serendipitously and coincidentally impact the gut microbiota[15]. The development of 'psychobiotics' has followed a more logical and step-wise course. While the term 'probiotic' refers to a live organism that, when ingested in adequate amounts, exerts a health benefit, a 'psychobiotic' is one which is specifically beneficial for mental health[16]. Most microbiome research over the last decade has focussed on understanding the mechanisms of MGB interaction through in-vitro and animal studies. While ongoing mechanistic studies are needed in the laboratory, a major effort is currently underway to progress the exciting preclinical findings to human studies and clinical trials with psychobiotics. Intriguingly, some psychobiotics, at least in animals, produce effects even when heat killed, suggesting that molecules on their surface which active the immune system are fundamental for their action[17].

The psychobiotic narrative first gained attention in 2004 when a landmark study[18] demonstrated that the gut microbiome could dramatically influence the development and function of the HPA axis, the body's primary stress response system which culminates in the production of cortisol, and has

consistently been shown to be dysfunctional in major depressive disorder (MDD). This Japanese research team demonstrated that germ-free (GF) mice (mice born and housed in sterile conditions, thus lacking a microbiome) exhibited an exaggerated stress response with greater cortisol release in comparison to their control counterparts. Strikingly, the abnormal response was partially reversed by colonisation with faeces from the control mice and completely reversed by feeding with a specific bacterial strain, *Bifidobacterium infantis*. However, the reversal only occurred if recolonization took place at an early stage, indicating a critical time period for normal, microbiome-dependent HPA-axis development. That the gut microbiome could influence the stress response was a seminal discovery which generated much speculation about the antidepressant potential of probiotic bacteria such as *Bifidobacterium*. However, it was early days and much remained to be elucidated about the mechanisms of MGB interaction. A dysfunctional HPA axis is only one component of the multifaceted aetiology of depression and questions remained about whether the microbiome could influence other pathways of interest in depression research such as immune regulation and serotonin metabolism.

The next decade saw great efforts undertaken to increase understanding of the relationship between the gut microbiome and immune system. It was well-recognised that depression is a pro-inflammatory state characterised by elevated pro-inflammatory cytokines such as IL-1, IL-6 and TNF-alpha[19], but the source of these elevated inflammatory markers was unknown. It now appears that the gut microbiome plays a key role in generating this pro-inflammatory state. Under normal homeostatic conditions gut microbes are safely confined to the gut and prevented from extra-intestinal access by the tightly-adherent gut epithelial barrier. However, gut permeability can be increased by various factors, such as chronic stress, a well-established precipitant of depression. The resultant 'leaky gut' can lead to translocation of gut bacteria, and bacterial components such as lipopolysaccharides (LPS), into the bloodstream, thus stimulating the low-grade immune response seen in depression[20]. Such a view is supported by studies that demonstrate the ability of various

probiotics to improve gut barrier function[21] and normalise the immune disturbance seen in animal models of depression[22].

The potential of the gut microbiome as an antidepressant target is further reinforced by evidence that it could influence neurotransmitter pathways. In the first instance, gut bacteria can directly produce many common human neurotransmitters including gamma-aminobutyric acid (GABA), noradrenaline, serotonin, acetylcholine and dopamine[23]. Serotonin, the most well-studied of neurotransmitters in relation to depressive illness, appears to be particularly susceptible to influence by the gut microbiome. A key study revealed that the plasma serotonin levels of GF-mice were almost 3 times less than those of conventional mice[24]. It was subsequently demonstrated that this differential serotonin level was secondary to the remarkable ability of gut microbes to directly promote the synthesis of serotonin from its amino acid precursor, tryptophan, in intestinal enterochromaffin cells. Furthermore, the gut microbiome was also shown to influence serotoninergic levels in the hippocampus, an area of the brain which plays an important role in stress, anxiety and depression[25]. Rats fed a *Bifidobacterium infantis* show significant elevations in blood tryptophan levels and such bacteria have the necessary capability to synthesize tryptophan.

Another possible mechanism of action of psychobiotic bacteria may lie in their ability to produce short-chain-fatty acids (SCFAs) from the fermentation of non-digestible carbohydrates and proteins in the colon. The main SCFAs include propionate, acetate and butyrate and these bacterial metabolites appear to exert far-reaching effects in the body including a role in immune signalling and regulation of plasma lipid levels. Butyrate, in particular, is of major interest given its ability to regulate gene transcription and it has been shown to demonstrate an antidepressant effect in rodents[26]. It is a powerful HDAC inhibitor with the potential for epigenetic modulation[27].

The major challenge in drug development lies in translating what seems promising in the laboratory into the clinical setting. The complexity of human disease means that only a very small fraction of new therapeutics progress through to the drug development process from preclinical evaluation to

successful clinical trials. The challenge of psychobiotic identification and translation is no different. Despite promising preclinical findings, results in human studies have been modest. However, the evidence base is growing and clinical research on the antidepressant properties of psychobiotics is hopeful.

On the positive side, most probiotic bacteria likely to have psychobiotic activity are generally regarded as safe, which means that the costs of toxicology studies are largely eliminated. However, whether or not bacteria can be adequately protected from an intellectual property perspective has yet to be fully determined and until this is done, major pharmaceutical companies may be hesitant to enter the market. After all a company may conduct expensive trials only to find that another company enters the market soon after with a bacteria having only minor genomic differences.

Most human studies investigating the potential of probiotics to improve mood have been conducted in healthy subjects and results have been variable. A combination of *Lactobacillus helveticus* and *Bifidobacterium longum,* administered to 66 healthy adults resulted in slight improvements in mood [28], as did a polybiotic combination of various *Actobacillus, Lactobacillus, Bifidobacterium* and *Streptococcus* strains[29]. *Lactobacillus casei*-Shirota improved mood in healthy adults with low baseline mood scores [30] and, although it did not improve mood in patients with chronic fatigue syndrome, it did reduce anxiety scores[31]. A study of *Bifidobacteria longum* 1714 used a placebo-controlled cross-over design and found an impact on perceived stress, a reduction in awaking cortisol and changes in EEG activity[32]. In a similar study Lactobacillus rhamnosus JB1 was found ineffective[33].

Psychobiotic trials have been undertaken in patients with depression. An Iranian team described improvements in Beck Depression Inventory (BDI) scores in adults with a diagnosis of MDD following 8 weeks of consumption of a polybiotic containing *Lactobacillus acidophilus, Lactobacillus casei* and *Bifidobacterium bifidum* [34]. Interestingly, patients also showed significant decreases in serum insulin and CRP concentrations along with increased plasma glutathione levels, thus demonstrating a

beneficial probiotic effect on metabolic, immune and anti-oxidant parameters alongside the antidepressant action. A major limitation of the study was that no information was provided on the concomitant use of antidepressant medication and so it was unclear whether the probiotic was being used as an adjunctive or sole treatment. Another *Lactobacillus/Bifidobacterium* polybiotic containing alternative species of the genera (*Lactobacillus helveticus* and *Bifidobacterium longum*) found no benefit in terms of improving mood or moderating inflammatory or other biomarkers in patients with depression [35], thereby highlighting the differential antidepressant potential of species within the same bacterial genus. A third clinical trial demonstrated an antidepressant effect of *Bifidobacterium longum* NCC3001 consumed over 10 weeks by patients with comorbid irritable bowel syndrome (IBS) [36]. However, the presence of IBS represented an obvious confounding factor and depressive symptoms were merely self-reported on screening questionnaires with no diagnostic interview performed to establish a clinical diagnosis of MDD. Up to 50% of patients with IBS are clinically depressed but whether this depression has the same biology as regular MDD has yet to be established. A study conducted in New Zealand explored the impact of Lactobacillus rhamnosus HN001 given during pregnancy. A total of 423 women took part and received either probiotic or placebo. The data show a decrease in depressive and anxiety symptoms post-partum in those women taking the probiotic. This finding needs replication but given the high prevalence of post-partum depression the approach offers a safe and potentially effective strategy for reducing the burden of illness.

A study of bipolar patients was conducted at John Hopkins[37]. A total of 66 patients discharged following a manic episode were recruited. Half received a combination of a *Lactobacillus rhamnosus* and a *Bifidobacterium animalis* while the other patients received placebo. Patients remained on their regular medication throughout. The rates of rehospitalisation were 51% in the placebo group and 24% in the psychobiotic group. Those patients who had the greatest inflammatory phenotype showed the greatest response.

When one takes this human data as a whole, it provides tentative but definite optimism for the future of psychobiotics in the treatment of depression, most likely as an adjunctive strategy alongside traditional pharmacological and psychological therapies. Most benefit seems to be derived by those with low mood and depressive symptoms at baseline, a conclusion confirmed by recent meta-analysis [38]. Effects in healthy subjects with normal baseline moods appear to be more limited although the use of psychobiotics as a preventative strategy for depression in those at higher risk must be considered.

The major requirements going forward are:

1. Large placebo-controlled trials with a parallel group design.
2. A determination as to whether single strain psychobiotics or multi-strain probiotics (polybiotics) are more effective.
3. Dose response studies to determine the optimal doses for psychobiotics.

The challenges are analysed in detail elsewhere[39].

Prebiotics

Of course, psychobiotics are only one means of altering the microbiome, and perhaps even, the least effective. While the term 'psychobiotics' originally referred to beneficial live organisms such as bacteria, the definition has been expanded in recent years to include 'prebiotics'. Prebiotics are non-digestible carbohydrates which are selectively fermented by bacteria in the large intestine, and can therefore be used to target beneficial host bacteria by specifically supporting and enhancing their growth (Bindels et al., 2015). Prebiotics include substances such as inulin, fructooligosaccharides (FOS), galactooligosaccharides (GOS), resistant starch and other soluble dietary fibres (though not all dietary fibres are prebiotic, i.e. not all modify selective gut microbiota). Natural sources of prebiotics include fruits and vegetables such as asparagus, leek, banana and chicory, as well as grains such as oats and wheat, foodstuffs which have become increasingly lacking in Western-style diets. Evidence

for the potential of prebiotics to improve psychological health is accumulating. A FOS+GOS combination demonstrated significant antidepressant and anti-anxiety effects in mice exposed to chronic stress[40] and in healthy human volunteers, GOS supplementation for 3 weeks resulted in suppression of the cortisol response and an increase in the processing of positive versus negative attentional vigilance[41]. Clearly prebiotics are important as part of a healthy diet. However, it remains to be demonstrated that an individual with a microbiota of significantly decreased diversity will also benefit from prebiotic administration.

Microbiota transplantation

Another means of modifying the microbiome is through the use of faecal microbiota transplantation (FMT), a process which involves transferring the faecal matter from one individual to another, thereby colonising the recipient with the donor's microbiota. It has been used to explore the potential transference of disease phenotypes to healthy animals by microbiome transplantation from specific human conditions or from animal models of disease. FMT from patients with MDD to microbiota-depleted rats resulted in the recipient rodents developing a depressive phenotype, both behaviourally and biochemically[14]. Similar findings were seen following depression-related FMT to GF-mice[42]. Such studies strongly support an aetiological role for the gut microbiome in depressive illness and have garnered interest in the role of FMT as a therapeutic intervention in psychiatric disorders. However, the studies also stress the necessity for adequate psychiatric phenotyping of microbiota donors when patients with chronic *Clostridium difficile* infection are undergoing treatment.

Most evidence for the therapeutic use of FMT in humans has been in the treatment of refractory *Clostridium difficile* infection , but there is also evidence emerging that it may be beneficial in functional gastrointestinal disorders such as IBS, and in metabolic syndrome. A recent Japanese study observed psychiatric symptoms in 17 patients who underwent FMT for the treatment of IBS, functional diarrhoea or functional constipation. Patients, with elevated depression

scores at baseline, experienced a significant improvement in mood which correlated with an increase in microbiota diversity[43]. Although this was an open-label, uncontrolled, observational study, it does raise the possibility that FMT may be of benefit in depression. There has, to date, been only one FMT interventional study in a neuropsychiatric population. Kang et al[44] administered oral FMT from healthy donors to 18 children diagnosed with autism spectrum disorder (ASD) over a 10-week period. They reported an increase in overall bacterial diversity and significant improvements in both gastrointestinal and autistic behavioural symptoms, which were maintained at assessment 8 weeks after treatment had ended. The use of FMT for psychiatric illness is in its infancy but is no doubt a field which may offer exciting new therapeutic opportunities.

Mediterranean diet

The final, and probably most important, method of introducing microbiota change is by targeting the diet. There is strong evidence that adherence to a Mediterranean diet, as well as lower intakes of food items such as processed meats and trans fats, confer protection against depression. The mechanisms by which specific dietary factors promote resilience to depressive illness are not fully understood, but it is likely that the gut microbiome plays a significant role. It has been shown that a change in diet can dramatically and rapidly alter the microbiome composition and thus, alter levels of health-promoting bacteria and beneficial bacterial metabolites such as SCFAs. However, which components of the Mediterranean diet mediate the effects on mood has yet to be established. High levels of polyphenols or polyunsaturated fatty acids are obvious candidates. EU funded guidelines recommend that patients with depression or vulnerability to depression should be encouraged to enhance a plant-based diet with a high content of grains /fibres, fermented foods and fish[45].

Conclusion:

Antidepressant therapy has come a long way from Isoniazid and the monoamine hypothesis and research in the field has stalled in recent years. However, perhaps the journey will ultimately prove

to have been a circular, rather than linear, one. That the first antidepressant arose from an antibiotic is surely a notable coincidence in the current psychobiotic climate and forces one to reconsider and question widely-accepted concepts with fresh eyes. The traditional view of functional gastrointestinal disorders such as IBS is that these are anxiety- or stress-driven conditions, allowing for the concept of a top-down, brain-gut influence. New insights on the gut microbiome have turned these assumptions on their head and it may well be our gut that is driving the rapidly-escalating rates of depression and anxiety, perhaps due to major changes in the Western diet in recent decades changing microbiota composition.

Nonetheless, the subject of psychobiotics is still relatively new. Much work needs to be done to further delineate MGB interaction mechanisms and establish the extent of influence of this system on physiological and psychological processes. At present the literature is dominated by preclinical studies but more human studies are needed to improve characterisation of normal and 'dysbiotic', or unbalanced, microbiota configurations and to investigate patterns related to specific disease processes. Solid conclusions from probiotic studies are limited by the significant heterogeneity across trials, in particular in relation to the strain of probiotic, dosage levels and duration of treatment. Further studies are required to throw light on these variables and optimise psychobiotic treatment strategies. We may be witnessing another watershed in psychopharmacology.

1. El Aidy S, Dinan TG, Cryan JF. Gut Microbiota: The Conductor in the Orchestra of Immune-Neuroendocrine Communication. Clin Ther 2015;37:954-67.
2. Grenham S, Clarke G, Cryan JF, Dinan TG. Brain-gut-microbe communication in health and disease. Frontiers in physiology 2011;2:94.
3. Dinan TG, Stilling RM, Stanton C, Cryan JF. Collective unconscious: how gut microbes shape human behavior. Journal of psychiatric research 2015;63:1-9.
4. Bravo JA, Forsythe P, Chew MV, et al. Ingestion of Lactobacillus strain regulates emotional behavior and central GABA receptor expression in a mouse via the vagus nerve. Proc Natl Acad Sci U S A 2011;108:16050-5.
5. Svensson E, Horvath-Puho E, Thomsen RW, et al. Vagotomy and subsequent risk of Parkinson's disease. Annals of neurology 2015;78:522-9.
6. Desbonnet L, Garrett L, Clarke G, Kiely B, Cryan JF, Dinan TG. Effects of the probiotic Bifidobacterium infantis in the maternal separation model of depression. Neuroscience 2010;170:1179-88.
7. Lyte M. Microbial endocrinology in the microbiome-gut-brain axis: how bacterial production and utilization of neurochemicals influence behavior. PLoS pathogens 2013;9:e1003726.
8. Lyte M. Microbial endocrinology and the microbiota-gut-brain axis. Advances in experimental medicine and biology 2014;817:3-24.
9. Stilling RM, Dinan TG, Cryan JF. Microbial genes, brain & behaviour - epigenetic regulation of the gut-brain axis. Genes, brain, and behavior 2014;13:69-86.
10. El Aidy S, Dinan TG, Cryan JF. Immune modulation of the brain-gut-microbe axis. Front Microbiol 2014;5:146.
11. Capuron L, Hauser P, Hinze-Selch D, Miller AH, Neveu PJ. Treatment of cytokine-induced depression. Brain, behavior, and immunity 2002;16:575-80.
12. O'Mahony SM, Marchesi JR, Scully P, et al. Early life stress alters behavior, immunity, and microbiota in rats: implications for irritable bowel syndrome and psychiatric illnesses. Biol Psychiatry 2009;65:263-7.
13. Jiang H, Ling Z, Zhang Y, et al. Altered fecal microbiota composition in patients with major depressive disorder. Brain Behav Immun 2015;48:186-94.
14. Kelly JR, Borre Y, C OB, et al. Transferring the blues: Depression-associated gut microbiota induces neurobehavioural changes in the rat. J Psychiatr Res 2016;82:109-18.
15. Cussotto S, Clarke G, Dinan TG, Cryan JF. Psychotropics and the Microbiome: a Chamber of Secrets. Psychopharmacology (Berl) 2019;236:1411-32.
16. Dinan TG, Stanton C, Cryan JF. Psychobiotics: a novel class of psychotropic. Biol Psychiatry 2013;74:720-6.
17. Warda AK, Rea K, Fitzgerald P, et al. Heat-killed lactobacilli alter both microbiota composition and behaviour. Behav Brain Res 2019;362:213-23.
18. Sudo N, Chida Y, Aiba Y, et al. Postnatal microbial colonization programs the hypothalamic-pituitary-adrenal system for stress response in mice. J Physiol 2004;558:263-75.
19. Dinan TG, Cryan JF. Brain-Gut-Microbiota Axis and Mental Health. Psychosom Med 2017;79:920-6.
20. Pereira JDC, Rea K, Nolan YM, O'Leary OF, Dinan TG, Cryan JF. Depression's Unholy Trinity: Dysregulated Stress, Immunity, and the Microbiome. Annu Rev Psychol 2019.
21. Ait-Belgnaoui A, Durand H, Cartier C, et al. Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. Psychoneuroendocrinology 2012;37:1885-95.
22. Desbonnet L, Tighe O, Karayiorgou M, Gogos JA, Waddington JL, O'Tuathaigh CM. Physiological and behavioural responsivity to stress and anxiogenic stimuli in COMT-deficient mice. Behav Brain Res 2012;228:351-8.
23. Dinan TG, Cryan JF. Microbes, Immunity, and Behavior: Psychoneuroimmunology Meets the Microbiome. Neuropsychopharmacology 2017;42:178-92.

24. Wikoff WR, Anfora AT, Liu J, et al. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc Natl Acad Sci U S A 2009;106:3698-703.
25. Clarke G, Grenham S, Scully P, et al. The microbiome-gut-brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. Mol Psychiatry 2013;18:666-73.
26. Tian P, Wang G, Zhao J, Zhang H, Chen W. Bifidobacterium with the role of 5-hydroxytryptophan synthesis regulation alleviates the symptom of depression and related microbiota dysbiosis. J Nutr Biochem 2019;66:43-51.
27. van de Wouw M, Boehme M, Lyte JM, et al. Short-chain fatty acids: microbial metabolites that alleviate stress-induced brain-gut axis alterations. J Physiol 2018;596:4923-44.
28. Messaoudi M, Lalonde R, Violle N, et al. Assessment of psychotropic-like properties of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in rats and human subjects. Br J Nutr 2011;105:755-64.
29. Mohammadi AA, Jazayeri S, Khosravi-Darani K, et al. The effects of probiotics on mental health and hypothalamic-pituitary-adrenal axis: A randomized, double-blind, placebo-controlled trial in petrochemical workers. Nutr Neurosci 2016;19:387-95.
30. Benton D, Williams C, Brown A. Impact of consuming a milk drink containing a probiotic on mood and cognition. Eur J Clin Nutr 2007;61:355-61.
31. Rao AV, Bested AC, Beaulne TM, et al. A randomized, double-blind, placebo-controlled pilot study of a probiotic in emotional symptoms of chronic fatigue syndrome. Gut Pathog 2009;1:6.
32. Allen AP, Hutch W, Borre YE, et al. Bifidobacterium longum 1714 as a translational psychobiotic: modulation of stress, electrophysiology and neurocognition in healthy volunteers. Transl Psychiatry 2016;6:e939.
33. Kelly JR, Allen AP, Temko A, et al. Lost in translation? The potential psychobiotic Lactobacillus rhamnosus (JB-1) fails to modulate stress or cognitive performance in healthy male subjects. Brain Behav Immun 2017;61:50-9.
34. Akkasheh G, Kashani-Poor Z, Tajabadi-Ebrahimi M, et al. Clinical and metabolic response to probiotic administration in patients with major depressive disorder: A randomized, double-blind, placebo-controlled trial. Nutrition 2016;32:315-20.
35. Romijn AR, Rucklidge JJ, Kuijer RG, Frampton C. A double-blind, randomized, placebo-controlled trial of Lactobacillus helveticus and Bifidobacterium longum for the symptoms of depression. Aust N Z J Psychiatry 2017;51:810-21.
36. Pinto-Sanchez MI, Hall GB, Ghajar K, et al. Probiotic Bifidobacterium longum NCC3001 Reduces Depression Scores and Alters Brain Activity: A Pilot Study in Patients With Irritable Bowel Syndrome. Gastroenterology 2017;153:448-59 e8.
37. Dickerson F, Adamos M, Katsafanas E, et al. Adjunctive probiotic microorganisms to prevent rehospitalization in patients with acute mania: A randomized controlled trial. Bipolar Disord 2018;20:614-21.
38. Ng QX, Peters C, Ho CYX, Lim DY, Yeo WS. A meta-analysis of the use of probiotics to alleviate depressive symptoms. J Affect Disord 2018;228:13-9.
39. Bambury A, Sandhu K, Cryan JF, Dinan TG. Finding the needle in the haystack: systematic identification of psychobiotics. Br J Pharmacol 2018;175:4430-8.
40. Burokas A, Arboleya S, Moloney RD, et al. Targeting the Microbiota-Gut-Brain Axis: Prebiotics Have Anxiolytic and Antidepressant-like Effects and Reverse the Impact of Chronic Stress in Mice. Biol Psychiatry 2017;82:472-87.
41. Schmidt K, Cowen PJ, Harmer CJ, Tzortzis G, Errington S, Burnet PW. Prebiotic intake reduces the waking cortisol response and alters emotional bias in healthy volunteers. Psychopharmacology (Berl) 2015;232:1793-801.
42. Zheng P, Zeng B, Zhou C, et al. Gut microbiome remodeling induces depressive-like behaviors thacrough a pathway mediated by the host's metabolism. Mol Psychiatry 2016;21:786-96.

43. Kurokawa S, Kishimoto T, Mizuno S, et al. The effect of fecal microbiota transplantation on psychiatric symptoms among patients with irritable bowel syndrome, functional diarrhea and functional constipation: An open-label observational study. J Affect Disord 2018;235:506-12.
44. Kang DW, Adams JB, Gregory AC, et al. Microbiota Transfer Therapy alters gut ecosystem and improves gastrointestinal and autism symptoms: an open-label study. Microbiome 2017;5:10.
45. Dinan TG, Stanton C, Long-Smith C, et al. Feeding melancholic microbes: MyNewGut recommendations on diet and mood. Clin Nutr 2019;38:1995-2001.

Figure Legend

Figure 1 illustrates some of the ways in which the microbiota may be altered to impact upon mood. Psychobiotics are live biotherapeutics with a mental health benefit. Prebiotics are indigestible fibres metabolised by bacteria. Faecal microbiota transplantation (FMT) is the most radical therapy.