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Review article

Neuropsychiatric disorders: influence of gut microbe to brain signaling

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Abstract: The microbiome gut brain (MGB) axis involves bidirectional routes of communication and has emerged as a potential therapeutic target for multiple medical specialities including psychiatry. Significant numbers of preclinical trials have taken place with some transitioning to clinical studies in more recent years. Some positive results have been reported secondary to probiotic administration in both healthy populations and specific patient groups. This review aims to summarise the current understanding of the MGB axis and the preclinical and clinical findings relevant to psychiatry. The link between the gut microbiome and irritable bowel syndrome (IBS) is well established. Significant differences have been identified between the microbiome of patients with a diagnosis of depressive disorder and healthy controls. Similar findings have occurred in patients diagnosed with bipolar affective disorder. A probiotic containing *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum* produced clinically measurable symptom improvement in patients with depressive disorder. To date some promising results have suggested that probiotics could play a role in the treatment of stress-related psychiatric disease. However, more well-controlled clinical trials are required to determine which clinical conditions are likely to benefit most significantly from this novel approach

Keywords: psychiatry; gut microbiome; probiotics

1. Introduction

The microbiome gut brain (MGB) axis is a relatively new concept in the scientific world. The MGB axis has been revealed as a complex communication system which appears to have multiple influences on affect, motivation and higher cognitive functions (Carabotti et al 2015). Previously the gut microbiome was not appreciated for the role it plays and the far reach of its influence. In recent years it has been recognised that the gut microbiota sends messages to the brain via a variety of routes, and from this understanding the MGB axis has emerged as an area of study and even therapeutic promise.

The bidirectional communication between the gut and the central nervous system plays an important role in maintaining homeostasis (Cryan and O'Mahony 2011). The axis communicates in multiple ways; via the autonomic nervous system, the hypothalamic pituitary adrenal (HPA) axis, the vagus nerve, and the direct production of neurotransmitters and short chain fatty acids (SCFAs). The multiple functions and routes of communication of the MGB axis and how these relate to each other are not yet clear. The MGB axis links the emotional and cognitive centers of the brain with intestinal functions and permeability, immune activation, enteric reflex and endocrine signaling (Carabotti et al 2015). Alterations in the gut microbiota effect the multiple routes of communication which make up the MGB axis.

Several associations have been suggested between the gut microbiota and health status. Loss of diversity in the gut microbiota has been correlated with increased frailty in elderly patients living in long term care (Claesson et al 2012). Certain bacteria are thought to contribute to the pathogenesis of bowel disease and in this context may hold therapeutic promise (O'Hara and Shanahan 2006). Stress

induced alterations in gut microbiota have been linked to sympathetic nervous system activation with cortisol effecting gut wall permeability and microbiota composition (Macedo et al 2017). It is also thought that the makeup of an individual's microbiome may influence their susceptibility to psychiatric illness (Cryan and Dinan 2012). However, it remains unclear if the changes to the microbiome which have been linked to multiple diseases are causal to the disease process or a secondary effect (Cenit et al 2017).

Multiple findings have led to the microbiome being identified as a potential target in the treatment of psychiatric illness. Differences in the microbiota in a depressed population have been noted (Naseribafrouei et al 2014). The influence of bacteria on behavior was explored by Goehler et al (2008), who reported increased anxiety like behavior in mice infected with *Campylobacter jejuni.* Multiple brain regions were noted to be activated during the experiment. A change in gut microbiota can produce behavioural signs of depression (Kelly et al 2016),This was shown via the transplantation of faecal matter from depressed patients into rats with a depleted microbiome. The possibility of antidepressants having antimicrobial effects has been explored by Macedo et al (2017). The production of neurotransmitters by the gut microbiome can be considered in the context of the monoamine theory of depression. Similarly, the role of the HPA axis and inflammation in the pathogenesis of depression may be mediated by the gut microbiome communicating with the brain via the HPA axis. From the possibility of alterations of the gut microbiota producing improvements in symptoms of disease the concept of psychobiotics has emerged. A psychobiotic is a live organism that when ingested in adequate amounts produces mental health benefits (Dinan et al, 2013). Zheng et al (2016) reported significant differences across multiple tests between germ free (GF) mice and specific pathogen free (SPF) mice. They suggest these differences represent decreased anxiety and depression like behaviour in GF mice. Behavioural changes seen in response to changes in the gut microbiota are thought to be medicated by chemicals originating from the microbiota which act directly or indirectly on the central nervous system (Collins et al 2012).

Initial research into the MGB axis examined rodent models. Responses to stress and certain behaviours provide some limited information applicable to human disease models. Psychological sequelae and physiological changes have both been investigated to further understand the role of the gut microbiome and its therapeutic potential. To date GF and SPF mice, trials of infection and probiotics, and faecel transplants have been used to research the role of the gut microbiome (Cryan and Dinan 2012). Faecel transplants have resulted in the transferring of behavioural phenotypes between patients diagnosed with depression and rats with a depleted microbiome. This highlights the possible role modifying gut microbiota could play in treating psychiatric disease according to Cenit et al (2017). The results in human models are currently limited with research ongoing in multiples centres.

2. The human microbiome has been estimated to consist of over 100 trillion microbes, with the majority of these living in the gut (Amon and Sanderson 2016). 70 – 75% of the gut microbiota comprises of bacteriodetes and firmicutes (Mariat et al 2009). The process of gut colonisation starts at birth, and is influenced by many external factors including method of delivery, diet, hygiene and medication (Grolund et al 1999). Although each person's microbiota is different, the abundance of bacterial phylotypes is alike among healthy individuals (Carabotti et al 2015). The populations of bacteria present in the gut appear to remain constant for some people, while others experience change (Holzapfel et al 1998). Both stability and diversity in the gut microbiome are needed to maintain health (Clarke et al 2014). A functioning gut microbiome will balance proinflammatory and antinflammatory responses (Burnet and Cowen 2013). There are multiple examples that environmental factors can affect gut bacteria. Rat pups who experienced early life maternal separation had an altered faecal microbiota when compared to controls (O'Mahony et al 2009). Antibiotics alter the gut microbiota, which results in decreased resistance to colonisation (Clemente et al 2012). This vulnerability to external factors is one of the reasons why the gut microbiota has become a possible target for disease treatment. An

individual's microbiota represents their genetic and environmental history and even contributes to their risk of disease and treatment response (Foster and Neufeld 2013).

How does the microbiome gut brain axis function?

The MGB axis communicates in multiple bidirectional ways. These different modes of communication also interact with each other. The gut microbiota directly and indirectly effects the immune system, in turn the immune system effects the HPA axis and the circulating levels of cytokines (Dinan et al 2015). Cytokines send signals via the vagus nerve to the brain and also act directly on the blood brain barrier. The inflammatory response also sends signals to the brain via the vagus nerve (Sherwin et al 2016).

Hypothalamic pituitary adrenal axis (HPA)

The HPA is stimulated by stress, either physical or psychological.. Initially corticotrophin releasing hormone (CRH) is released from the hypothalamus, which induces the release of adrenocorticotrophic hormone (ACTH) from the pituitary gland and glucocorticoids from the adrenal cortex. This cascade differs in response to acute or chronic stress. Chronic stress levels can lead to sustained high levels of glucocorticoids and prolonged activation of the sympathetic and parasympathetic nervous systems. Chronic stress also decreases the feedback mechanism usually occurring when cortisol is secreted. The secretion of pro-inflammatory cytokines increases levels of glucocorticoids. Stress can impact the gut microbiome through activation of the sympathetic nervous system and slowing of the digestive processes (Gruenwald et al 2002), as well as altering barrier function. The HPA axis has been shown to be hyperactive in the long term in monkeys subjected to early life stress (Coplan et al 1996). The physiological changes in these monkeys are similar to those seen in patients diagnosed with post-traumatic stress disorder. HPA axis alterations have been implicated in the pathophysiology of depression, these can be reversed through the administration of antidepressants (Barden, 2004). Significantly increased stress and immune responses were identified in rat pups exposed to early life maternal separation (O'Mahony et al 2009). The pups exposed also had increased levels of corticosterone and an altered microbiome. These findings suggest stress impacts not only the HPA axis but also the microbiome. The behavioral and humoral changes were noted to indicate that early life stress has a lasting effect which could contribute to the development of a psychiatric illness.

Brain derived neurotrophic factor (BDNF) is a protein which contributes to neuroplasticity. Decreased levels of BDNF and decreased expression of central N Methyl D aspartate (NMDA) receptors have been found in stressed mice (Sudo et al 2004). Gene expression with decreased NMDA receptors in the amygdala and increased BDNF expression in the hippocampus were demonstrated when anxiety like behaviour was studied in GF and SPF mice (Neufeld et al 2010). In patients with depression peripheral BDNF levels have been shown to increase through the administration of antidepressants (Castren et al 2006).

Higher levels of ACTH and corticosterone with lower levels of BDNF have been found in GF mice exposed to stress (Sudo et al 2004). These changes were reversible through monocolonisation by *Bifidobacterium infantis*. Interestingly this reversal was time limited. These notable results highlight the role the microbiome plays in the development and functioning of the HPA axis. They also suggest that the development of the HPA axis is time sensitive and early life events can have long-term physiological consequences. The gut microbiome plays a clear role in the development of the HPA axis from birth and across the lifespan (Allen et al 2017). *Lactobacillus farciminis* has been used to decrease the stress response in female rats by impacting gut permeability (Belgnaoui et al, 2012). Similarly a decrease in the HPA axis stress response was noted in rats after *Lactobacillus farciminis* administration (Ait-Belgnaoui et al 2012). Both peripheral and central effects were noted with decreased ACTH and corticosterone in

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plasma and decreased CRF in the hypothalamus. Liang et al (2015) described improvements in anxiety and depression like behaviours and cognitive functioning secondary to *Lactobacillus helveticus* NS8 in chronically stressed SPF rats. Lower levels of corticosterone, and ACTH were seen along with increased levels of BDNF mRNA. A significant reduction in the diversity of the gut microbiota after exposure to repeated aggression was noted in mice (Bailey et al 2011). A significant increase in the genus *Roseburia* and a significant decrease in the genus *Parabacteroides* was found in mice euthanised 15 hours after the last exposure to aggression, compared to mice euthanised immediately after. Bercik et al (2011) highlighted the influence of the microbiome on the brain through examining alterations in the microbiome and subsequent behaviour. It was suggested that these behavioural changes were independent of the autonomic nervous system, neurotransmitters or inflammation. Increased levels of BDNF were noted after colonisation of GF mice with bacteria from SPF mice treated with antibiotics.

Immune system

Both the innate and the adaptive immune systems are programmed by commensal bacteria (Clemente et al 2012). It has been suggested that disease might be caused by dysbiosis, rather than the presence of a particular bacterium leading to the development of a disease. Some diseases which have been associated with microbial dysbiosis include obesity, inflammatory bowel disease and diabetes mellitus. Changes in the microbiome secondary to alterations in diet, hygiene practices and antibiotic use during the 20th century have a possible role in chronic inflammatory and autoimmune diseases (Belkaid and Hand 2014). The complex relationship between the gut microbiota, the gut wall and the lymphoid tissue and the role this relationship plays in disease has led to the gut microbiota being seen as a potential target for treatment (Rhee et al 2009).

Bailey et al (2011) reported the presence of bacteria in the liver in 47% of mice who had been exposed to stress compared to 14% of mice who had not. The leaky gut theory of depression suggests that changes in gut wall permeability result in translocation of bacteria, which in turn triggers an inflammatory response. The immune response is triggered by the presence of lipopolysaccharide (LPS) which inducee a Toll like receptor (TLR) 4 response. In marked contrast, L*actobacilli* and B*ifidobacterium* are, both probiotics, which have been shown to have health benefits. Neither of these probiotics contain LPS.

Depressed patients had significantly larger IgA and IgM responses to four out of six (gram negative) bacteria when compared to healthy controls (Maes et al 2012). Chronically depressed patients showed significantly larger IgM responses when compared to both patients with acute depression and controls. The authors conclude that these responses affect gut barrier integrity leading to gram negative bacteria entering the systemic circulation. Centrally microglia play a fundamental role in regulating immune responses. Impaired immune responses and defective microglia have been found in GF mice. Gut colonization resulted in improved microglial functioning (Erny et al 2015). Belkaid and Hand (2014) describe the influence microbial ligands have on the immune system, both at times of rest and inflammatory periods. The authors describe bacteria influencing the gene expression of immune cells, although how long these influences last is currently unknown. Significantly increased levels of IL-6 were found in mice repeatedly exposed to aggression. An immune response did not occur when antibiotics were administered prior to the aggression (Bailey et al 2011). A decreased immune cell response with decreased production of cytokines was noted after oral administration of *Bifidobacteria infantis* (Desbonnet et al 2008). Increases in cytokines have previously been linked to depressive symptoms (Felger and Lotrich 2013).

Vagus nerve

The vagus nerve is the main bidirectional pathway between the gut and the brain. It allows signals to move from the gut to the brain and in the opposite direction (Dinan et al 2015). Specific probiotics modulate certain brain functions and behaviours, and in many cases this process is dependent on the vagal nerve (Cryan and Dinan, 2012). The vagal nerve exerts several anti-inflammatory effects via contact with the HPA axis, the cholinergic anti-inflammatory pathway and the splenic sympathetic anti-inflammatory pathway.

The vagal nerve interacts with the gut microbiota both directly and indirectly. Direct contact occurs though short chain fatty acids (SCFAs) which activate afferent vagal fibres. TLRs are expressed on afferent vagal fibres which activate the brain. Vagal afferent fibres do not cross the gut wall epithelium, so signals from the microbiota are indirectly delivered by bacteria or their metabolites crossing the gut wall (Bonaz et al 2018).

Some preliminary data exists that vagal nerve stimulation could be used as an adjunct in the management of treatment refractory depression, post-traumatic stress disorder, and inflammatory bowel disease. Stimulation of the afferent vagal fibres influences monoamines in the brain stem (Breit et al 2018). Psychobiotic bacteria might in the future be used to stimulate the vagus nerve and in so doing bring about an improvement in depressive symptoms.

Bacteria producing and secreting neurotransmitters.

Both neurotransmitters and neuromodulators can be produced by bacteria. GABA is produced by certain species of *Lactobacillus* and *Bifidobacterium*, *Escherichia*, *Bacillus* and *Saccharomyces spp.* all produce noradrenaline, *Candida, Streptococcus, Escherichia* and *Enterococcus spp.* produce serotonin, *Bacillus* produces dopamine and *Lactobacillus* produces acetylcholine (Dinan et al 2015). It has been suggested that multiple neurochemicals necessary for neuronal functioning are regulated by gut microbiota (Erny et al 2015).

GABA is a ubiquitous inhibitory neurotransmitter which is involved in many physiological and psychological processes within the central nervous system. GABA receptor alterations have been implicated in the pathogenesis of both depressive and anxiety disorders. The ingestion of *Lactobacillus rhamnosus* (JB1) has resulted in alterations of central GABA mRNA (Bravo et al 2011). This probiotic also decreased the production of stress induced corticosterone, as well as decreasing anxiety and depressive like behaviours in mice. These results were not found in vagotomised mice supporting the role of the vagal nerve pathway in producing these effects.

Serotonin and tryptophan metabolism

Serotonin is a key neurotransmitter at both ends of the gut brain axis, with the gut microbiota controlling tryptophan metabolism along the kynurenine pathway. The enzymes involved in this pathway are modulated by the immune and sympathetic system, similar to the MGB axis (O'Mahony et al 2015). This is a further example of the interaction of the multiple routes of communication that make up the MGB axis.

Changes in the serotonergic system have long been implicated in the aetiology of depression (Owens and Nemeroff 1994). GF male mice have been found to have increased levels of serotonin and its main metabolite (Clarke et al, 2013). No change in gene expression was found in these mice. Increased levels of tryptophan have been recorded secondary to the oral administration of *Bifidobacteria infantis* in rats (Desbonnet et al, 2008). Circulating concentrations of tryptophan are influenced by the gut microbiota (Clarke et al 2014). Serotonin is usually thought of as a neurotransmitter which exists in the central nervous system, however most of the serotonin in the body is produced in the gut by enterochromaffin cells (EC). Serotonin is released from EC cells secondary to vagal nerve stimulation, ingestion of food, the

presence of acid, amino acids, hypo osmotic or hyperosmotic solutions in the duodenum (Manocha et al 2012). Short chain fatty acids also stimulate the release of serotonin.

Short chain fatty acids

Short chain fatty acids (SCFAs) are produced by gut bacteria when carbohydrates and protein are digested. Examples of SCFAs include butyrate, proprionate and acetate. Butyrate is a histone deacetylase (HDAC) inhibitor. SCFAs also act through G protein coupled receptors (GPCRs) including free fatty acid receptor 2 (FFAR2) and free fatty acid receptor 3 (FFAR3). SCFAs play many roles including cell growth and differentiation, transport and metabolism, and provision of energy for the heart, kidneys and the brain. The production and absorption of SCFAs mainly occurs in the proximal large intestine (Clarke et al 2014). SCFAs also act as signaling molecules which can affect inflammatory responses. De Vadder et al (2014) explored the role SCFAs plays in glucose and energy metabolism. Rat models gained less weight compared to controls when given SCFAs (propionate and butyrate) as part of their diet. The administration of probiotics to increase the amount of butyrate producing bacteria has resulted in decreased anxiety like behaviour in rats and lowered psychological stress in humans (Messaoudi et al 2011). Butyrate influences serotonin release and vagal nerve stimulation (Stilling et al 2016).

Trials involving humans

As one might anticipate, at this point we have far more preclinical than clinical studies targeting the MBG.. However, preliminary human observational and interventional studies have taken place with some promising results reported. Trials involving both healthy populations and specific patient groups have been carried out.

The complex pathology behind many psychiatric disorders is not yet fully understood. Simren et al (2013) considered if IBS and inflammatory bowel disease (IBD) could be part of the same inflammatory spectrum but existing at opposite ends. Faecel transplant from patients with IBS into GF mice has been found to transfer the symptoms of IBS (Crouzet et al 2013). The complexity of the gut microbiome and it's interaction with external factors is highlighted through the use of antibiotics in the treatment of functional bowel disease (FBD). Rifaximin has been trialed in the treatment of IBS (Pimentel et al 2006). Significant improvements in IBS symptoms up to ten weeks after the antibiotic was discontinued were noted. Using antibiotics in the management of a functional bowel disorder and producing positive results suggests that the interaction between external factors, the gut microbiome and psychiatric illness could be used to a therapeutic advantage. At least 50% of patients with IBS have psychiatric co-morbidity. The link between the symptoms of IBS and the microbiome have been previously established with specific organisms contained in probiotics improving the symptoms of IBS (Quigley and Flourie 2006).

Observational studies

Significant differences in the gut microbiota between patients with active major depressive disorder (AMDD) and healthy controls were found at phyla, family and genus levels. Eleven statistically significant differences were discovered between the groups at the family level. Differences were also found at phyla, family and genus levels between patients being treated for MDD (TMDD) and healthy controls. No significant difference in serum inflammatory biomarkers were found between the depressed groups and the healthy controls. BDNF levels in the AMDD and TMDD groups were lower than those in the healthy controls (Jiang et al 2015). Naseribafrouei et al (2014) investigated the links between faecal microbiota and depressive disorder. They found underrepresentation of *Bacteroidales* in the depressed group. An altered microbiome was noted in depressed patients compared to healthy controls with a reduction in

Prevotellaceae (Kelly et al 2016). Zheng et al (2016) carried out faecal transplants from patients with MDD and healthy controls into GF mice. They reported increased Actinobacteria and decreased Bacteroidetes in mice with MDD faecal matter compared to mice transplanted with the healthy control faecel matter. They also noted that Firmicutes was responsible for discriminating MDD from healthy controls, although no significant difference was found between the two groups. Significant differences have been found between the global microbiome and specific operational taxonomic units in patients diagnosed with bipolar disorder affective disorder compared to controls (Evans et al 2017).

Interventional studies

Singh et al (2018) did not find any change in gut microbiota in healthy women during or after the administration of a probiotic. However, one type of T cell (Th17) was significantly reduced when measured after probiotic ingestion. A significant decrease was seen in IL6 and IL10 in vitro. They suggested these results show a possible role for probiotics in immunoinflammatory diseases such depression. Reductions in stress and exhaustion have been described after the oral ingestion of a probiotic for six months. Significant increases were seen in concentration, elation and introversion and significant decreases in fatigue, agitation, sensitivity, anxiety and depression (Gruenwald et al, 2002). These changes were observed in patients diagnosed with stress and exhaustion rather than a psychiatric illness. Decreased anxiety levels were also noted in a healthy population after a 12 week trial of probiotics containing *Lactobacillus gasseri* and *Bifidobacterium longum* (Nishihira et al 2014). A probiotic containing Lactobacillus rhamnosus HN001 was used in a randomised control trial. Significantly lowered anxiety and depressive scores were noted in the interventional group in the postpartum period when compared to the placebo group (Slykerman et al 2017).

Certain subscales of the Hopkins Symptom Checklist 90 (somatization, depression and anger-hostility) were significantly decreased after ingestion of a probiotic formulation (PF) consisting of *Lactobacillus helveticus* R0052 and *Bifidobacterium longum* R0175 (Messaudi et al 2011). The cortisol output of the probiotic treated group decreased significantly overtime and the control group's remained static. This result supports the use of probiotics in the treatment of psychiatric disorders where the HPA axis has been implicated as part of the pathophysiology. Significant improvements in the Beck Depression Inventory scores were found in patients diagnosed with depressive disorder after eight weeks ingestion of a probiotic capsule containing *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium bifidum* (Akkasheh et al 2016). A significant improvement in insulin metabolism and decreased oxidative stress was also found. These results suggest that probiotics could improve the physical sequelae that many patients with MDD experience, as well as improving the actual symptoms of MDD. Aizawa et al (2016) found significantly lower levels of *Bifidobacterium* in patients with major depressive disorder compared to healthy controls. They also reported that patients who consumed fermented milk less than once a week had significantly lower levels of *Bifidobacterium* compared to those who consumed it more than once a week. Dickerson et al (2014) studied the effect of probiotic administration in patients with a diagnosis of schizophrenia, the probiotic contained *Lactobacillus rhamnosus* and *Bifidobacterium animalis.* No change in the symptoms of schizophrenia was noted. Administration of probiotics earlier in the course of the patient's illness was suggested as a strategy for further study.

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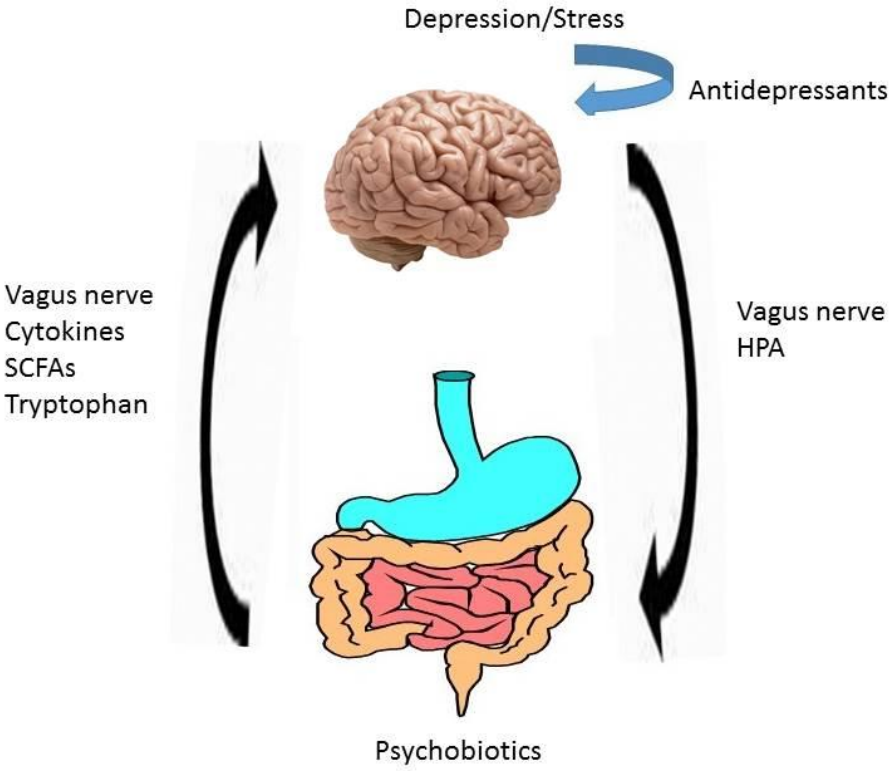

Figure Legend

Figure 1 shows the bidirectional routes of communication between brain and gut microbes and the reverse, gut microbes and brain. SCFAs=short chain fatty acids, HPA=hypothalamic-pituitary-adrenal axis. Also illustrated is the central site of antidepressant action in contrast to the action of psychobiotics.

Discussion

The possibility that the gut microbiota plays a role in the genesis of psychiatric illness and may be an appropriate therapeutic target is a new paradigm in mental health. Some promising results have been reported particularly in preclinical trials. However, we clearly require far more clinical studies to determine the real validity of this novel paradigm. Will psychobiotics or other means of modulating the microbiota be the psychiatric treatments of the future? Certainly this approach shows enormous promise and may provide alternatives to the currently available psychotropic medications and psychological therapies.

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