

# Microbes, Immunity, and Behavior: Psychoneuroimmunology Meets the Microbiome

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There is now a large volume of evidence to support the view that the immune system is a key communication pathway between the gut and brain, which plays an important role in stress-related psychopathologies and thus provides a potentially fruitful target for psychotropic intervention. The gut microbiota is a complex ecosystem with a diverse range of organisms and a sophisticated genomic structure. Bacteria within the gut are estimated to weigh in excess of 1 kg in the adult human and the microbes within not only produce antimicrobial peptides, short chain fatty acids, and vitamins, but also most of the common neurotransmitters found in the human brain. That the microbial content of the gut plays a key role in immune development is now beyond doubt. Early disruption of the host-microbe interplay can have lifelong consequences, not just in terms of intestinal function but in distal organs including the brain. It is clear that the immune system and nervous system are in continuous communication in order to maintain a state of homeostasis. Significant gaps in knowledge remain about the effect of the gut microbiota in coordinating the immune-nervous systems dialogue. However, studies using germ-free animals, infective models, prebiotics, probiotics, and antibiotics have increased our understanding of the interplay. Early life stress can have a lifelong impact on the microbial content of the intestine and permanently alter immune functioning. That early life stress can also impact adult psychopathology has long been appreciated in psychiatry. The challenge now is to fully decipher the molecular mechanisms that link the gut microbiota, immune, and central nervous systems in a network of communication that impacts behavior patterns and psychopathology, to eventually translate these findings to the human situation both in health and disease. Even at this juncture, there is evidence to pinpoint key sites of communication where gut microbial interventions either with drugs or diet or perhaps fecal microbiota transplantation may positively impact mental health.

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## INTRODUCTION

Although the field of psychoneuroimmunology emerged in the 1970s, it took a number of decades for the concept of the bidirectional communication between the immune system and central nervous system to gain mainstream attention in medicine (Ader *et al*, 1995; Glaser and Kiecolt-Glaser, 2005). There is now an overwhelming body of evidence to support the view that immune alterations occur in response to both psychological and physical stress and dysregulation of the immune system is viewed by many as a key component of psychiatric disorders such as major depression (Dinan, 2009). Over the past decade, the brain-gut axis has become an increasing focus of neuroscience research, and there is a large volume of data to indicate that the immune system is a major communication pathway between gut microbes and brain, which may play an important role in stress-related

psychopathologies and thus provides a potentially fruitful target for novel psychotropic development (El Aidy *et al*, 2015). The focus of this review is to explore the mechanisms through which the brain-gut-microbiota axis regulates immune responses and in so doing impacts behavior, both normal and pathological. We will describe the microbiota changes from infancy to old age and examine ways in which gut microbes may be manipulated to improve mental health.

## GUT MICROBIOTA ESTABLISHMENT

The gut microbiota is a complex ecosystem with a diverse range of organisms and a sophisticated genomic structure. Bacteria within the gut are estimated to weigh in excess of 1 kg in the adult human and the microbes within, produce not just antimicrobial peptides but short chain fatty acids (SCFAs), vitamins and intriguingly most of the common neurotransmitters found in the brain. That the microbial content of the gut plays a key role in immune development is generally acknowledged.

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The composition and function of the intestinal microbiota has been the subject of intense scrutiny and debate, initially using culture-dependent microbiological techniques that have significant limitations (Vaughan *et al*, 2000) and more recently, using culture-independent 16S rRNA gene sequence-based techniques, which allow greater insight into the microbial composition and diversity of this complex ecosystem and as a result has greatly expanded knowledge in the field (Qin *et al*, 2010). Such advances in metagenomic technologies have revealed the composition of the human gut microbiota from early infancy (Palmer *et al*, 2007) through to old age (Claesson *et al*, 2011), but far less is known about the physiological impact this microbiota has on host health. Advances in our knowledge of microbial structure have far outpaced our understanding in relation to function. Indeed, understanding the influence of the gut microbiota on host health is viewed as one of the most important fields across all aspects of biomedicine (Shanahan, 2012). For example, a perturbed microbiota has been implicated in an ever-increasing list of disorders in humans, from necrotizing enterocolitis (NEC) in infants to obesity, autism spectrum disorder, diabetes, metabolic syndrome, irritable bowel syndrome (IBS), and inflammatory bowel disease (Kosloske, 1984; Ley *et al*, 2006; Kassinen *et al*, 2007; Peterson *et al*, 2008; Flint *et al*, 2010). As a result, the gut microbiota is increasingly becoming a target for drug and dietary therapy to treat such disorders, as well as a source of novel drugs and bioactive compounds (Shanahan, 2012).

While the GI tract of the fetus was thought to be sterile, recent evidence suggests that bacteria are present in the amniotic fluid (Jimenez *et al*, 2008) and also the placenta (Wassenaar and Panigrahi, 2014). During the delivery process microbes are acquired from the mother's fecal and vaginal flora and subsequently from the environment, in most developed countries the hospital environment. These begin to colonize the infant gut with a dense and intricate microbiota which subsequently develops in the full-term infant (Adlerberth and Wold, 2009). This early colonization is critical for the development of the immune system. This rapid colonization process and the microbial composition of the neonatal gut are influenced by a number of factors including antibiotic use, diet, mode of delivery, environmental factors, and the maternal microbiota (Adlerberth and Wold, 2009; Marques *et al*, 2010). The microbiota of formula-fed infants has been reported to be more diverse than breast-fed infants, containing higher proportions of *Bacteroides*, *Clostridium*, and *Enterobacteriaceae*. While vaginally delivered infants are colonized by the fecal and vaginal bacteria of the mother, infants delivered by caesarean section are colonized by other bacteria from environmental sources including health-care workers, air, equipment, and other newborns (Penders *et al*, 2006; Adlerberth and Wold, 2009). Efforts to replicate the intestinal microbiota of breast-fed infants via formula-feeding has been a major challenge for the food industry and has led to the incorporation of prebiotics in infant formula; nondigestible food ingredients that selectively stimulate the growth and/or activity of one or

more bacterial species in the colon beneficially affecting the host (Gibson and Roberfroid, 1995). It has been reported that a combination of galacto-oligosaccharides (GOS) and fructo-oligosaccharides (FOS), at ratios similar to the oligosaccharide composition of human breast milk, can stimulate the growth of bifidobacteria and influence species distribution in the gut microbiota, as well as altering fecal pH and SCFA production levels to mimic those of breast-fed infants (Fanaro *et al*, 2005; Haarman and Knol, 2005). Prebiotics are also known to play a role in the neonatal immune system and protect against pathogens (Boehm and Moro, 2008; Saulnier *et al*, 2009). Preclinical data from our laboratory indicate that such prebiotics can dramatically impact stress responses both endocrine and behavioral (Burokas *et al*, in press).

The intestinal microbiota of newborn infants is characterized by low diversity and a relative dominance of the phyla *Proteobacteria* and *Actinobacteria* in the early postnatal period. With the passage of time, the microbiota becomes more diverse with the emergence and dominance of *Firmicutes* and *Bacteroidetes* (Eckburg *et al*, 2005; Qin *et al*, 2010; Backhed, 2011). Full-term, vaginally delivered babies born to healthy mothers who are breast fed and nonantibiotic treated have an optimal development of the neonatal microbiota (Penders *et al*, 2006). In these infants, facultative anaerobes such as enterobacteria, staphylococci, and streptococci are the earliest to colonize, taking advantage of the redox potential and available oxygen of the newborn GI tract (Stark and Lee, 1982; Mackie *et al*, 1999). Earliest colonizers consume the oxygen in the GI tract and generate an anaerobic environment allowing the proliferation of strict anaerobes, *Clostridium*, *Bacteroides*, and *Bifidobacteria*, with the latter becoming dominant and outnumbering all other bacterial groups in the first weeks of life (Harmsen *et al*, 2000; Penders *et al*, 2006). The neonatal microbiota is highly dynamic and is characterized by rapid change and low diversity (Penders *et al*, 2006; Adlerberth and Wold, 2009). By the end of the first year of life, infants possess a microbial profile distinct for each infant, converging towards the characteristic microbiota of an adult and within 3 years of age the microbiota fully resembles that of an adult in terms of composition (Palmer *et al*, 2007; Koenig *et al*, 2011; Barrett *et al*, 2015).

The characteristic intestinal microbiota observed in healthy full-term infants is disturbed in preterm infants (Dennison, 1976), who are frequently delivered by C. section, receive antibiotics, and may have problems feeding (Hoy *et al*, 2000). Furthermore, preterm infants possess a functionally immature gut with low levels of acidity in the stomach, due to insufficient gastric acid secretion and their requirement for more frequent feeding (Sondheimer and Clark, 1985; Sondheimer *et al*, 1985; Hoy *et al*, 2000). These events lead to an increase in the prevalence of potentially pathogenic bacteria in the GI tract and less microbial diversity than full-term infants (Chang *et al*, 2011; Jacquot *et al*, 2011; Arbolea *et al*, 2012). It has been opined that the dysregulated intestinal microbiota of preterm infants is a risk factor for the increased susceptibility to systemic infection and the development of NEC, which remains a common

cause of morbidity and mortality in preterm infants (Claud and Walker, 2001; Westerbeek *et al*, 2006; Deshpande *et al*, 2007; Neu and Walker, 2011). NEC is a life-threatening GI tract illness, causing necrosis of the intestine and can affect other organs of the body, including the brain with subsequent consequences which are far worse than the damage to the GI tract. NEC affects 5–10% of infants born with a birth-weight of less than 1500 g and despite advances in neonatal care is fatal in up to 30% of cases (Fitzgibbons *et al*, 2009) and associated with long-term intellectual disability. Recently, the use of probiotics in the treatment of NEC has been advocated (Embleton and Yates, 2008; Mshvildadze and Neu, 2009; Gareau *et al*, 2010).

## ADULT MICROBIOTA

The adult microbiota, while more complex than that of the neonate, is relatively stable over time (Vaughan *et al*, 2000). There are large interpersonal differences in the microbiota of healthy adults, even between monozygotic twins, but nonetheless a shared core gut microbiome exists, exerting a common functionality within the host (Turnbaugh *et al*, 2010; Burke *et al*, 2011). The adult microbiota is dominated by members of the *Bacteroidetes* and *Firmicutes* phyla (Turnbaugh *et al*, 2006) and recently three different ‘enterotypes’ have been described (Arumugam *et al*, 2011). The three distinct ‘enterotypes’ are dominated by *Prevotella*, *Ruminococcus*, and *Bacteroides*, and are described as independent of sex, age, nationality, and body mass index. Although there are those who advocate this enterotype classification, it can be criticized on the grounds of oversimplification. What seems clear is that the composition of the human microbiota is determined by an interaction of host genetics and diet with the environment, which includes chemicals such as antibiotics (Rawls *et al*, 2006; Benson *et al*, 2010). It has been suggested that diet may be the easiest way to alter the host microbiota and that such changes may come about rapidly (Wu *et al*, 2011). However, a recent study in rodents suggests this may not be as easy as it seems (Sonnenburg *et al*, 2016). They raised mice in a germ-free (GF) environment and then transplanted a human microbiota. When fed a low-fiber diet, the animals showed a substantial drop in gut microbial diversity. Furthermore, mice kept on a low-fiber food and allowed to breed produced offspring with an even lower diversity and subsequent generations of low-fiber-fed mice continued to lose whole groups of microbes. By the fourth generation, the mouse microbiota seemed to have reached a stable low-diversity, with only a quarter of the diversity of the first generation. Perhaps the most important finding in the study is that none of the low-fiber generations could be ‘rescued’ by reintroducing high-fiber food. If these findings are replicated in humans it raises major questions regarding poor diet, especially in families of low socio-economic status and the potential for trans-generational negative health consequences.

**TABLE 1** Gut Microbiota Composition Throughout the Life Span

	Composition	References
Neonatal	Low diversity and a relative dominance of the phyla <i>Proteobacteria</i> and <i>Actinobacteria</i>	Eckburg <i>et al</i> (2005), Qin <i>et al</i> (2010)
Adult	Dominated by members of the <i>Bacteroidetes</i> and <i>Firmicutes</i> phyla	Tumbaugh <i>et al</i> (2006)
Old age	<i>Bacteroides</i> and <i>Firmicutes</i> are the dominant phyla Significant loss of diversity especially in frail elderly	Claesson <i>et al</i> (2011)

## ELDERLY MICROBIOTA

Given the fact that the ageing population is increasing rapidly as people live longer in most developed countries, the state of the gut microbiota and the relationship with common medical conditions seen in the elderly is of major importance (Mariat *et al*, 2009). In reality our knowledge of the mutating microbiota from adulthood into old age is poorly understood. It seems clear that the adult microbiota remains stable into old age except when confronted with antibiotics or other insults which include disease pathology, dramatic changes in diet or medications used to treat a variety of illnesses (Mariat *et al*, 2009; O’Toole and Claesson, 2010; Clemente *et al*, 2012). Differences in the microbiota of elderly subjects across Europe have been reported upon (Mueller *et al*, 2006). In Ireland the Eldermet consortium at University College Cork was established to characterizing the gut microbiota of elderly Irish subjects and to develop functional foods for this population. The consortium used pyrosequencing to examine over 40 000 reads per sample from 161 subjects. *Bacteroides* and *Firmicutes* were identified as the dominant phyla in the distal gut of the elderly population, accounting for 97% of the assigned sequences. However, there were major variations at phylum level from one subject to the next: *Firmicutes* varied from 8 to 80% and *Bacteroidetes* varied from 14 to 92% (Claesson *et al*, 2011). When subjects in nursing homes were compared with those in the community, large-scale differences were detected. Those in nursing homes have a far less diverse microbiota and this was attributed to a less varied diet. However, it is possible that pathological factors that lead to admission into nursing homes such as deteriorating cognitive function might have played an important role in the decreased microbial diversity and not the less diverse diet Table 1.

## MICROBES AND IMMUNITY

Until the late nineteenth century microbes were viewed as harmful pathogens that cause infections and the host immune system was seen as developed to recognize and eradicate these invaders (Surana and Kasper, 2014). We now know that the development of the immune system is pivotally dependent on colonization with commensal microbes (Kostic *et al*, 2013). While multiple pathways exist for bacteria to communicate with their host, pattern

recognition receptors (ie Toll-like receptors, TLRs) on host cells play a pivotal role. At least 10 TLRs are present on cells of the innate immune system in man and recognize characteristic molecular patterns (Frieri and Stampfl, 2016). These receptors are the gateway to the innate immune system and are a first step in the cascade leading to cytokine production. They are also universally distributed on neurons, thus allowing them to respond to bacterial and viral components (Reis *et al*, 2015). While the intestinal epithelium and the mucus layer acts largely as a barrier to translocation of microorganisms into the internal milieu, the nervous system is prepared and capable of responding to such interactions (Johansson *et al*, 2013).

The initial microbial colonization results in significant changes in mucosal and systemic immunity. The maturation of the immune system, initiated within the fetus, is highly dynamic in character and mutates over time through the first neonatal months and into childhood (Newburg and Walker, 2007). Neonates have low expression of co-stimulatory molecules, diminished dendritic cell (DC) differentiation, impaired phagocytosis as well as defective interaction between DCs, T lymphocytes and regulatory T cells and impaired cytotoxic activity of T cells (Kotiranta-Ainamo *et al*, 2004; Velilla *et al*, 2006). Furthermore, the activity of the transplacentally derived maternal immunoglobulin G antibodies results in a deficiency of specific humoral responses, which include minimal levels of IgA in neonates (Keles *et al*, 2010). Similar findings have been reported in animal studies. For example, in GF mice and those in the first days of colonization smaller Peyer's patches and low level cellularity of the lamina propria and low Cd4<sup>+</sup> and Cd8<sup>+</sup> T cells are reported (Wei *et al*, 2010; El Aidy *et al*, 2012). Furthermore, intraepithelial lymphocytes and plasma cells are rare in the small intestinal mucosa while secretory immunoglobulin A (sIgA) levels are significantly lower with suppressed expression of genes and markers of activation for intestinal macrophages (Mikkelsen *et al*, 2004; El Aidy *et al*, 2012). Interestingly, recent evidence using GF animals also points to a role for the maternal microbiota in influencing early postnatal innate immune development in the infant. In this study monocolonization of GF animals with *Escherichia coli* HA107 during pregnancy altered the numbers of early postnatal intestinal innate leukocytes and increased small intestinal innate lymphoid cell (ILC) proportions and total numbers compared with GF controls, particularly the NKp46<sup>+</sup>RORγ<sup>t</sup> ILC3 subset (Gomez de Aguero *et al*, 2016).

Secretory immunoglobulin A provides a good example of how the microbiota mediates host physiology through immune modulation. Secretory immunoglobulin A binds to luminal bacteria and prevents microbial translocation across the epithelial barrier (Macpherson *et al*, 2005). It also plays a role in influencing the balance of immune and metabolic pathways in the intestinal epithelium through a microbiota-dependent mechanism (Shulzhenko *et al*, 2011). In the absence of IgA, a shift towards the expression of genes involved in host defense emerges, with excessive production of antimicrobial proteins and pro-inflammatory responses to

compensate for the deficiency in microbial compartmentalization via the sIgA. In marked contrast, when IgA is present, the intestinal microbiota alters the expression of genes involved in lipid metabolism and storage; as a result there is a shift towards a microbiota-immune-metabolic response which is fundamental for homeostasis (Shulzhenko *et al*, 2011; El Aidy *et al*, 2013).

Maturation of the host immune system is dependent upon interaction with the microbiota and requires specific strains of the microbes that flourish in an environment enriched by the inflammatory processes (Chung *et al*, 2012; El Aidy *et al*, 2014). While many questions remain, it seems that these specific bacteria activate mucosal immune priming for the bacterial sampling process in order to minimize their exposure to the systemic immune system by transient breaching of the epithelial barrier, thus stimulating the production of antimicrobial molecules and significant immune pro-inflammatory and regulatory immune proteins (Mazmanian *et al*, 2005; Galindo-Villegas *et al*, 2012; El Aidy *et al*, 2014). The initial close highly regulated contact benefits the host by augmenting gut barrier function and enhancing protection against subsequent invading pathogens. Segmented filamentous bacteria (SFB) are a well-studied intriguing example of initial microbial-host interaction. In mice, it has been shown that after weaning SFB adhere transiently to the surface epithelium of the ileum and Peyer's patches (Gaboriau-Routhiau *et al*, 2009) inducing Th17 cells in the intestinal lamina propria by a mechanism that involving serum amyloid A (Gaboriau-Routhiau *et al*, 2011). Segmented filamentous bacteria not only induce Th17 in the GI tract, but also the effect seems to expand to the CNS (Lee *et al*, 2011). Understanding this complex process has implications not just for understanding gut pathology but brain immune disorders also. Studies in GF and ex-GF mice have helped increase understanding of how the gut microbiota programs the intestinal mucosa to maintain a balanced immune response within a few days of colonization (El Aidy *et al*, 2012; El Aidy and Kleerebezem, 2013). There is now no doubt that early life results in the most extensive alterations in host biology in response to the colonization of the microbiota. As we have previously pointed out the host encounters challenges, which elicit responses that involve activation of gene networks associated with several illnesses (El Aidy *et al*, 2016). Abnormal shifts during the infant's developmental path through this early rapidly mutating phase may thus have long-term health consequences (Costello *et al*, 2012). We have suggested that there is a critical window of development during the early life that allows for a full-scale establishment of an adequate host-microbial homeostasis; aberrant responses during this window can result in significant pathology later in life (Borre *et al*, 2014).

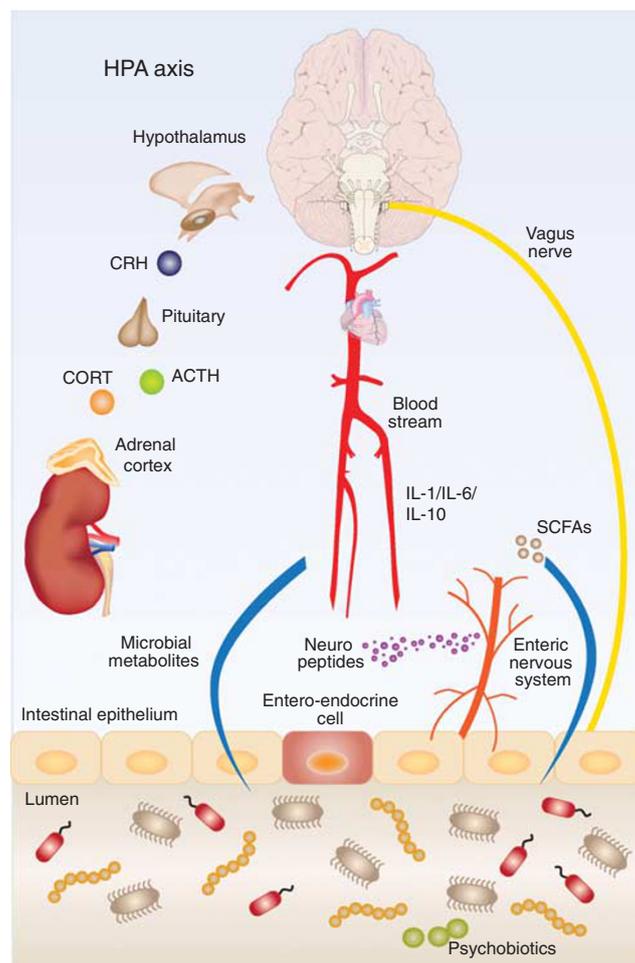
After initial priming by the gut microbiota, the immune system is continuously stimulated by several structural components of the microbial cells via TLRs and responds by creating a diverse range of lymphocytes and cytokines. In the absence of stressors, the gut microbiota produces a

chronic state of ultra-low-level activation of the immune system, where bacterial components stimulate macrophages and T cells to create pro-inflammatory cytokines such as IL-1 $\beta$ , tumor necrosis factor alpha (TNF- $\alpha$ ), and IL-18 (Heumann *et al*, 1994; Ulevitch and Tobias, 1995). These cytokines generate a basal state of immune activation commencing at the intestinal mucosal surface but eventually affects the entire body. It is estimated that the adult human contain 1 g of lipopolysaccharides and the exposure of immune cells to these cell wall molecules is essential for the maintenance of mucosal homeostasis (Bested *et al*, 2013). The precise molecular mechanisms that underlie aspects of host immune-microbe interaction lack clarity. However, one important factor is the regulation of the chemokine CXCL16, which is important for natural killer (iNK) T-cell migration and homeostasis. This chemokine regulation has been shown to occur through epigenetic changes involving the reduction of methylation pattern of the CXCL16 gene and in so doing reducing the number of invariant NK T cells in the colonic lamina propria (Olszak *et al*, 2012). *Bacteroides fragilis* (*B. fragilis*) provides another example of a factor critical for the normal function of the immune system by producing glycosphingolipid which in turn inhibits natural killer T cells proliferation in the colonic lamina propria (Wieland Brown *et al*, 2013). *B. fragilis* polysaccharide A (PSA) is recognized by TLR2 on DCs and this interaction induces colonic regulatory T cells (Tregs) (Round and Mazmanian, 2009). Such Tregs are also induced by Clostridium species, possibly by production of SCFAs (Atarashi *et al*, 2011). There is a marked difference between Tregs induced by *B. fragilis* and Clostridium species in that the latter stimulate Tregs in both normal and inflamed tissue while *B. fragilis* induces Tregs only in the presence of inflammation (Surana and Kasper, 2014). These findings indicate that when commensals leave their normal environment they can become pathogenic.

## MICROBIOTA AND HUMORAL RESPONSES

Communication between the gut microbiota and the brain can occur via multiple routes that include the vagus nerve, gut-secreted neuropeptides, sensory nerves, cytokines, tryptophan, and SCFAs (Holzer and Farzi, 2014). See Figure 1. The brain in turn can influence gut commensals directly via the receptor-mediated signaling and signaling molecules released in the gut lumen from immune cells or epithelial cells, in particular enteroendocrine cells, or indirectly via changes in the intestinal motility and secretion (Rhee *et al*, 2009). Recent data suggest that the activity of microbes can also be influenced by the release into the gut lumen and uptake by microbes of miRNAs (Liu *et al*, 2016). While bacterial genes may be regulated by miRNAs, bacteria do not themselves produce such miRNAs.

Until relatively recently the brain was considered outside the remit of the peripheral immune system, a view now known to be incorrect. GF mice have an altered expression of genes involved in neuropeptide production (El Aidy *et al*,



**Figure 1.** Routes of communication between the gut microbes and the brain include the vagus nerve, cytokines, short chain fatty acids, and tryptophan. Acting through some or all of these routes, psychobiotics induce an anti-inflammatory response and alter behavioral responses to stress.

2013) together with genes involved in brain development and behavior (Diaz Heijtz *et al*, 2011; Stilling *et al*, 2014). GF mice have significant neurochemical alterations, which are reflected in markedly altered behavior patterns including stress responses. For example they exhibit increased spontaneous motor activity compared to conventional mice (Diaz Heijtz *et al*, 2011) and show altered exploratory behavior, following fecal transfer from mice with anxiety-like behavior (Bercik *et al*, 2011). *Lactobacillus rhamnosus* (JB-1) reduces stress-induced corticosterone hormone levels when fed to mice. Moreover, the same strain makes the mice less anxious, an effect which is mediated by the vagus nerve and not seen following vagotomy (Bravo *et al*, 2011). It is also worth noting that there is an efferent vagal pathway that has been shown to have marked anti-inflammatory responses (Olofsson *et al*, 2012). The central mechanisms by which this bacterial strain reduces corticosterone levels has not been explored but may involve reduced release of corticotropin-releasing factor (CRF), though alterations in negative feedback in the axis cannot be ruled out.

The gut microbiota has been shown to produce a range of beneficial metabolites such as bacteriocins, which can help defend against bacterial infections (eg thuricin has a powerful impact against *Clostridium difficile*), vitamins, such as folate, cobalamin, vitamin K, riboflavin, and thiamine, all of which are essential for human survival, and exopolysaccharides, which have prebiotic, immuno-stimulatory, anti-tumoral, and blood cholesterol-lowering effects (Conly and Stein, 1992; Cerning, 1995; Corr *et al*, 2007). More specifically a number of further microbial metabolites or 'pharmabiotics' can interact with the host's immune, neural and endocrine systems and have a profound effect on health (O'Hara and Shanahan, 2007; Shanahan, 2011). Tryptophan which is the precursor of 5-HT is increased in the plasma of animals treated with a *B. infantis* (Desbonnet *et al*, 2008). This may be significant given the limited storage capacity for tryptophan in the mammalian brain and the requirement for a constant dietary supply.

$\gamma$ -Amino butyric acid (GABA) is the main inhibitory neurotransmitter in the human brain and the target of a wide range of drugs including the benzodiazepines. GABA also plays a role in the regulation of movement, blood pressure, heart rate, and pain perception. It has been implicated in anxiety and depression (Mody *et al*, 1994; Cryan and Kaupmann, 2005; Schousboe and Waagepetersen, 2007). Demonstrated as involved in the control of growth hormone secretion (Volpi *et al*, 1997), it has protective effects against glycerol-induced acute renal failure in rats (Kim *et al*, 2004) and microbes secreting GABA may have antidiabetic properties (Soltani *et al*, 2011). Interestingly, foods enriched with GABA have been defined as 'foods for specified health use (FOSHU) in Japan' (Sanders, 1997), leading to the development of fermented products containing GABA derived from dairy, soybean, kimchi (a fermented vegetable), and juice products. However, it must be noted that GABA does not cross the blood brain barrier. It has recently been suggested that microbially produced GABA in the gut may impact the brain-gut axis (Bienenstock *et al*, 2010). GABA is produced by the enzyme glutamate decarboxylase and it has been hypothesized that *in vivo* GABA production by intestinal bacteria could have potential health benefits (Mills *et al*, 2009; Lyte, 2011). Lactobacilli have the necessary capacity to produce GABA and do to varying extents depending upon the individual strain and the environment. Indeed, the GABA-producing *Lactobacillus brevis* DPC 6108 strain has recently been shown to alter the development of diabetes in a streptozotocin rat (Marques *et al*, 2016).

Conjugated fatty acids, natural components of milk and tissue fat from ruminants, plant seed oils, and marine algae are positional and geometric isomers of polyunsaturated fatty acids with one or more conjugated double bonds (Chisholm and Hopkins, 1967; Burgess *et al*, 1991; Kohno *et al*, 2004; Yasui *et al*, 2005). Of these conjugated linoleic acid (CLA) is the most extensively studied with multiple putative health benefits including an antiatherosclerotic effect, an antiobesogenic impact, and probable anticarcinogenic properties (Bassaganya-Riera *et al*, 2002; Belury, 2002;

Ip *et al*, 2003). A number of *Lactobacillus*, *Propionibacterium*, and *Bifidobacterium* strains have the ability to produce these bioactive fatty acids, and in addition, *Roseburia* spp. has been identified among the most active producers in the gut (Devillard *et al*, 2007). However, there are indications that a high intake of CLA may have a pro-inflammatory action. Unlike polyunsaturated fatty acids, so far no clear mental health benefits from CLA have been identified.

Short chain fatty acids are water soluble, readily absorbed organic fatty acids of one to six carbon atoms long and are produced in the human gut as the end products of anaerobic bacterial fermentation of carbohydrates (Cummings *et al*, 1987). Acetate, butyrate, and propionate are the major SCFAs found in the human large intestine (Oltmer and Engelhardt, 1994). SCFAs are a major source of energy for colonic epithelial cells, are involved in the prevention of DNA and cell damage, can stimulate sodium absorption, and impact immune and inflammatory responses, while sodium butyrate has been shown to elicit an antidepressant effect in mice (Schroeder *et al*, 2007). It has been postulated that SCFAs can help prevent colorectal cancer and they have been reported to alter gene expression of cell cycle regulators, *in vitro* (Scheppach *et al*, 1995; Siavoshian *et al*, 1997; Stilling *et al*, 2014). As well as playing a role as epigenetic regulators by HDAC inhibition, SCFAs bind to G-protein coupled receptors (GPCRs) (Maslowski *et al*, 2009). Bacteria belonging to the phylum *Firmicutes* have been identified as the main butyrate producers in the colon, with the two most abundant groups related to *Eubacterium rectale*/*Roseburia* spp. and *Faecalibacterium prausnitzii*, with *Faecalibacterium prausnitzii* reportedly reduced in prevalence in Crohn's disease (Louis and Flint, 2009). Animal studies suggest that propionate acting centrally may play a role in the emergence of autistic-type behavior patterns and butyrate shows antidepressant effects in animal models. However, despite the fact that SCFAs can act on GPCRs the view that they have significant direct CNS effects is highly controversial. The GPCRs on which the SCFAs act are only sparsely distributed in the mammalian brain.

Serotonin (5-HT) is the most widely studied neurotransmitter in relation to psychiatric illness. Produced from tryptophan it plays a key role in multiple processes and only a small percentage of 5-HT is found in the brain with over 90% found in the GI tract (Keszthelyi *et al*, 2009). In the gut it is mainly stored in the enterochromaffin cells of the intestinal mucosa and the remainder in the serotonergic neurons of the enteric nervous system (Keszthelyi *et al*, 2009). Most antidepressants currently marketed impact 5-HT (Cryan and O'Leary, 2010). It has been shown that the gut microbiota plays a role in the production of serotonin (Wikoff *et al*, 2009) and the plasma serotonin levels of conventional mice are almost three-fold higher than GF mice (Wikoff *et al*, 2009). Furthermore, *B. infantis* has been reported to increase levels of the serotonin precursor, tryptophan, in the plasma of rats and shows antidepressant activity in rodent tests such as the forced swim test (FST) (Desbonnet *et al*, 2008). Recently, Hsiao and colleagues

have shown that certain strains of spore-forming bacteria produced metabolites which affect the production of serotonin that in turn impacts host physiological functions (Yano *et al*, 2015).

Endocannabinoids are lipid molecules whose receptor sites are found widely distributed in the brain (Piomelli, 2003). Such receptors also bind  $\delta^9$ -tetrahydro-cannabinol, the active ingredient in cannabis, a plant long known for its psychotropic activities including a tendency for the emergence of psychotic symptoms (Piomelli, 2003). Endocannabinoids, along with their receptors, play a role in the bioactive lipid signaling system in the central nervous system and in various peripheral organs and have been implicated in the pathogenesis of cardiovascular diseases (Batkai and Pacher, 2009). There is increasing evidence to indicate that the endocannabinoid system and the gut microbiota can affect the development of obesity and related disorders. They also have the potential to modulate pain perception. *Lactobacillus acidophilus* has been reported to modulate expression of cannabinoid receptors and reduce abdominal pain in rodents (Rousseaux *et al*, 2007).

One investigated mechanism through which immune activation may alter behavior is via actions on serotonergic systems (Lowry *et al*, 2007). The nonpathogenic *Mycobacterium vaccae* leads to activation of the peripheral immune system. The Th1 and Treg, but not Th2, mediated immune activation stimulates a subset of 5-HT neurons in the dorsal raphe nucleus of mice and increases serotonin turnover in the ventromedial prefrontal cortex (Lowry *et al*, 2007). This finding illustrates the fact that the type of peripheral immune response determines the effects on central 5-HT neurons. Furthermore, the findings suggest that the immune-responsive subpopulation of serotonergic neurons in the dorsal raphe play a role in the regulation of mood. It has been suggested that afferent fibers within the vagus nerve are involved in transferring signals of peripheral immune activation to the CNS. Immune activation under normal conditions stimulates a subset of 5-HT neurons, different from those activated under pathological states. Immune activation can induce depression and anxiety and is also associated with disorders of the brain-gut axis such as irritable bowels syndrome (Clarke *et al*, 2009). Immune activation induced by interferon therapy frequently results in the emergence of depressive symptoms. Treatment with selective serotonin reuptake inhibitors prevents the onset of depressive symptoms in such situations (Capuron and Miller, 2004). Moreover, recent data have shown that the anti-TNF antibody infliximab can reduce features of depression.

Local and systemic depletion of the 5-HT precursor, tryptophan is associated with elevation of the immunomodulatory enzyme indoleamine-pyrrole 2,3-dioxygenase (IDO), which occurs during immune activation (Moffett and Namboodiri, 2003) and transiently during primary gut colonization. The ability of the gut microbiota to control host tryptophan metabolism along the kynurenine pathway is gaining attention (O'Mahony *et al*, 2015). This reduces the

fraction available for serotonin synthesis and increases the production of neuroactive and immunoresponsive metabolites (O'Mahony *et al*, 2015).

Local and systemic increases in pro-inflammatory cytokines such as IL-1 and IL-6 bring about activation of the hypothalamic-pituitary-adrenal (HPA) axis and production of CRF, corticotropin-releasing factor, which is also elevated by psychological stress, causes disturbance to other neuropeptides, leading to changes in mood and behavior (Dinan and Cryan, 2012). It is interesting from an evolutionary perspective that both physical stressors such as microbial pathogens and psychological stress activate the HPA, albeit through somewhat different mechanisms. CRF stimulates the anterior pituitary gland to release the stress hormone; ACTH, which in turn stimulates release of cortisol from the adrenal gland. Cortisol, through a feedback loop, regulates the levels of CRF and ACTH. Importantly, immune cells, through the COX-2 pathway and production of PGE-2 during (pro-) inflammation, stimulate the adrenal gland to produce cortisol, which supports the  $\beta$  adrenergic pathway in its anti-inflammatory effect (Straub *et al*, 2006). This indicates that under normal conditions the neuroendocrine and immune systems coordinate to maintain homeostasis.

Bacterial by-products that come in contact with the gut epithelium stimulate the production of several neuropeptides including peptide YY, neuropeptide Y, cholecystokinin, glucagon-like peptide-1,2, and substance P and oxytocin (Furness *et al*, 2013). Following secretion, neuropeptides diffuse throughout the lamina propria, which is occupied by a variety of immune cells, until they reach the blood stream, stimulate sensory neurons or act on the vagus nerve (Cummings and Overduin, 2007; Okano-Matsumoto *et al*, 2011). The exact mechanisms by which neuropeptides communicate with the CNS are poorly understood. Bohorquez *et al* described a neuroepithelial circuit that acts as both sensory channels for food and gut microbes and transmits messages from the lumen to the ENS and CNS and also in reverse (Bohorquez *et al*, 2015). Neuropeptides overall represent an important route of dialogue between but microbes and the host.

## MICROBES, GUT BARRIER, AND INFLAMMATION

The main function of the intestinal barrier is to regulate the absorption of nutrients, electrolytes, and water from the lumen into the circulation and to prevent the entry of pathogens and toxins (Kelly *et al*, 2015). Furthermore, regulation of the exchange of molecules between the environment and the host through the intestinal barrier influences the equilibrium between tolerance and immunity to self and non-self-antigens (Fasano, 2011). From a structural perspective these functions are preserved by a number of features including a mucus layer and a monolayer of epithelial cells interconnected by tight junctions. The mucus layer containing sIgA and antimicrobial peptides

cover the epithelial cell lining which functions to facilitate GI transport, and as a protective layer against bacterial invasion. The colonic mucus layer is composed of two layers, an outer and inner layer composed of gel forming highly glycosylated proteins termed mucins. These are produced and maintained by goblet cells, which renew the inner mucus layer on a regular basis (Johansson *et al*, 2011). The dynamic processes are subject to extensive and continuous interplay with the gut microbiota, disruption of which may have implications for the maintenance of key barrier functions (Bischoff *et al*, 2014).

Tight junctions are complex protein structures that consist of transmembrane proteins such as claudin, occludin, and tricullulin (Cording *et al*, 2013). These transmembrane proteins connect with the opposing plasma membrane, thereby forming a mechanical link between epithelial cells and establishing a barrier to paracellular. It has been shown that the structure of the intestinal barrier is formed by the end of the first trimester of pregnancy (Kelly *et al*, 2015). Epithelial cells with microvilli, goblet, and enteroendocrine cells appear by week 8 of gestation and tight junctions are detected from week 10. Continued functional development of the intestinal barrier continues in the after birth and is influenced significantly by diet (Verhasselt, 2010). Disruptions in this process are seen in the underdevelopment of the intestinal barrier of the premature infant, which predisposes to immune disorders. There is an overlapping developmental trajectory of the gut microbiota and intestinal barrier. As described above, the gut microbiota, in the initial days of life, is unstable and lacks diversity. By age three, however, the microbiota composition resembles that of an adult-like profile (Voreades *et al*, 2014). The intestinal barrier acts as a protective defense that can be modified by the gut microbiota or its metabolites. The mechanisms underlying the regulation of the epithelial barrier are complex and only partially understood. The role of the gut microbiota in disrupting the intestinal barrier in stress-related conditions, such as depression, has not been adequately investigated. In animal models of depression gut barrier function is disrupted (O'Mahony *et al*, 2011) and there is a narrowing in the diversity of gut microbes and a pro-inflammatory cytokine profile. It is tempting to speculate that these factors are causally related but this has yet to be convincingly demonstrated.

## MICROBIOTA AS A REGULATOR OF NEUROIMMUNE FUNCTION

Microglia are the resident macrophage-like cells of the brain and thus the key immune cells that sense environmental changes in the brain and regulate neuroinflammatory processes (Yamasaki *et al*, 2014). Microglia once activated are critically involved in neuronal events at various stages in development and adulthood, including synaptic remodeling to improve neuronal network signaling (Schafer and Stevens, 2015). An increasing body of evidence suggests a link

between microglia and the microbiota-gut-brain axis which has implications for many stress-related and neurodegenerative disorders (Cryan and Dinan, 2015). There is a close link between HPA axis activation and the regulation of the peripheral and central immune responses, leading to monocyte priming and trafficking, subsequent alterations in microglia phenotype and, ultimately resulting in neuroinflammation. Indeed such neuroinflammatory responses are implicated in a number of psychiatric disorders, including anxiety and depression (Hodes *et al*, 2015; Miller and Raison, 2015). Microglia activation and subsequent release of cytokines plays a causal role in the manifestation of behavioral phenotypes in a variety of rodent stress models (Kreisel *et al*, 2014).

Intriguingly, a critical role for gastrointestinal microbiota in all aspects of microglia maturation, morphology, and immunological function has been uncovered (Erny *et al*, 2015). In contrast, the absence of a complex host microbiota (as evidenced by GF models or using antibiotic suppression of microbiota) led to increased microglial populations; defects in microglia maturation, activation state, and differentiation; alterations to microglia morphology (with longer processes and increased branching, terminal points and clubbing at synaptic boutons); and a compromised immune response to bacterial or viral infection (Erny *et al*, 2015). The observed alterations in microglial phenotype were reversed with recolonization of gut microbiota, following 6 weeks co-habitation of GF mice with control mice. The findings from this study have redefined the construct of targeting brain processes through the gut. It should also be noted that minocycline, a semi-synthetic, second-generation tetracycline analogue can effectively cross the blood-brain barrier and has been reported to exert neuroprotective effects in cerebral ischemia, traumatic brain injury, amyotrophic lateral sclerosis, Parkinson's disease, kainic acid treatment, Huntington's disease, multiple sclerosis, and Alzheimer's in various preclinical animal models; reportedly through suppression of microglia activation (Tikka *et al*, 2001). However, minocycline has a dual action as an antibiotic and thus may be influencing centrally mediated events indirectly through microbiota depletion, where it acts on both gram-positive and gram-negative bacteria.

## MICROBIOTA AND STROKE

Given the accumulating role for microbiome in regulating key processes involved in neuroimmune function such as microglia activation and blood brain barrier integrity (Braniste *et al*, 2014) in addition to those brain processes critically regulated by neuroinflammation such as adult hippocampal neurogenesis and myelination (Hoban *et al*, 2016), it is thus not too surprising that a link with the microbiome and stroke/brain injury has been put forward. In an intriguing recent study antibiotic-induced alterations in the microbiota resulted in a markedly reduced ischemic brain injury in mice, an effect transmissible by fecal

transplants (Winek *et al*, 2016). Immune mechanisms are driving such effects as alterations in microbiome composition indices, changes in immune homeostasis in the small intestine, leading to an increase in Treg cells and a reduction in interleukin (IL)-17-positive  $\gamma\delta$  T cells through altered DC activity. Moreover, changing microbiota composition unmasks a heretofore unknown suppression of the trafficking of effector T cells from the gut to the leptomeninges after stroke. Additionally, IL-10 and IL-17 are required for the neuroprotection afforded by intestinal dysbiosis (Benakis *et al*, 2016).

Using a different approach recent studies have showed that targeting the microbiome, using a specific bacterial strain that produces SCFAs, *Clostridium butyricum* attenuated the enhanced cognitive impairment, cell damage, and apoptosis induced in a stroke model in both normal (Sun *et al*, 2016) and in a diabetic mouse model (Sun *et al*, 2016).

## MICROBIOTA AND SCHIZOPHRENIA

Dinan *et al* argued strongly that the gut microbiota might play an important role in schizophrenia (Dinan *et al*, 2014). However, to date little systematic analysis has taken place in this field. In a small-scale study of the oro-pharyngeal microbiota alterations were observed (Castro-Nallar *et al*, 2015). A shotgun metagenomic analysis of the oropharyngeal microbiome in 16 individuals with schizophrenia and 16 controls was undertaken. High-level differences were evident at both the phylum and genus levels, with Proteobacteria, Firmicutes, Bacteroidetes, and Actinobacteria dominating both schizophrenia patients and controls, and Ascomycota being more abundant in schizophrenia. The authors conclude that overall the microbiome of schizophrenia patients was characterized by an increased number of metabolic pathways related to metabolite transport systems including siderophores, glutamate, and vitamin B12.

## MICROBIOTA, IMMUNITY, AND DEPRESSION

Depression is a pro-inflammatory state associated with the presence of biomarkers of inflammation such as elevated interleukin IL-6, TNF $\alpha$  and the acute phase protein, C reactive protein (Dinan, 2009). Similar elevated biomarkers of inflammation have been seen in anxiety states and are known to occur as a result of stress. Furthermore, the site at which these pro-inflammatory molecules is produced in depression is not known and it has yet to be established that gut-microbe-immune interactions are central to the elevation. With impairment in gut barrier function and resultant increased gut permeability translocation of gut bacteria across the intestinal wall occurs and into the mesenteric lymphoid tissue. It is speculated that increased exposure of the mucosal immune cells to bacteria can activate an immune response and lead to release of inflammatory cytokines with stimulation of the vagus nerve and spinal afferent neurons. Furthermore, increased permeability of the

gut can also increase the translocation of metabolic products or neuroactive substances produced by the bacteria and cause altered activity centrally. For a detailed review of this process, see Yarandi *et al* (2016). There is evidence from rodent studies to indicate that stress alters the gut barrier function allowing lipopolysaccharide and other molecules to gain access to the bloodstream stimulating TLR4 and other TLRs resulting in the production of inflammatory cytokines (Kelly *et al*, 2015). Formyl peptide receptors (FPRs) may also play a role (Dahlgren *et al*, 2016). These receptors are involved in host defense against microbial intruders and in regulating inflammatory reactions. FPR1 and FPR2 are expressed in neutrophils and bind many structurally diverse activating and inhibiting ligands. If altered barrier function occurs in depression with microbial or microbial metabolite translocation and stimulation of key pattern recognition receptors it would help explain the pro-inflammatory phenotype observed.

While an infectious etiology of mood disorders is not currently a major focus of research, in the past, infectious diseases were thought to be the cause of many psychiatric disorders. Brucellosis, which is associated with dairy farming, provides a good example of an infective agent that can result in depression. It has been shown that approximately 30% of patients with brucellosis are depressed and the presence of depression is independent of whether or not there is CNS involvement (Shehata *et al*, 2010). How is such a syndrome induced and is it possible that commensal bacteria may have a reverse action and alleviate depressive symptoms? As documented above, the evidence points to the fact that immune activation in the periphery can lead to central neurotransmitter changes. Pathogenic bacteria in doses that do not elicit sickness behavior can also bring about central changes. Lyte *et al* (1998) have shown that oral administration of the pathogen *Campylobacter jejuni*, in subclinical doses, which were too low to elicit an immune response, resulted in anxiety-like behavior in mice. They also found that areas of brainstem activation, most notably the nucleus tractus solitarius and lateral parabrachial nucleus, participate in neural information processing that lead to autonomic, neuroendocrine, and behavioral responses.

The gut microbiota has been found altered in the maternal separation model of depression, where a narrowing in diversity has been reported (O'Mahony *et al*, 2009). In a more recent study the gut microbiota was profiled in patients with major depression (Jiang *et al*, 2015). Major depressives had increased levels of Enterobacteriaceae and Alistipes but reduced levels of Faecalibacterium. A negative correlation was observed between Faecalibacterium and the severity of depressive symptoms. In another investigation several correlations between microbial composition and depression were reported in a study of 37 depressed patients and 18 healthy controls (Naseribafrouei *et al*, 2014). The order *Bacteroidales* was over-represented. The altered nutritional status of depressives and the impact of psychotropic medication cannot be entirely ruled out in inducing the changes seen in either of these studies. It is also clear that

given the nature of depression far greater sample sizes need to be recruited.

A small but interesting study has recently further linked mood states with microbiota composition. The research took advantage of the closed human life support system used by astronauts in training during a 105-day period. Gut microbial community structures were analyzed every 2 weeks as was a profile of mood states questionnaire. Bacterial genera *Roseburia*, *Phascolarctobacterium*, *Lachnospira*, and *Prevotella* had potential positive correlation with positive mood, while genera *Faecalibacterium*, *Bifidobacterium*, *Bacteroides*, *Parabacteroides*, and *Anaerostipes* were correlated with negative mood. Among which, *Faecalibacterium spp.* had the highest abundance, and showed a significant negative correlation with mood (Li *et al*, 2016).

## MICROBIOTA-IMMUNE TARGET OF ANTIDEPRESSANTS

It has been hypothesized that the major classes of antidepressants work, in addition to their effects on central monoamines by the generation of the potent immunoregulatory effects and as a consequence suppressing inflammation and the CNS changes associated with depression (Raison and Miller, 2013). In this regard, it is interesting that the immunoregulatory effects of probiotic microorganisms are also thought to occur through the generation of T regs and the synthesis and secretion of IL-10 (Lavasani *et al*, 2010). Feeding of a commensal bacteria to GF mice results in local DC uptake and alteration of phenotype to one which promotes T reg production and IL-10 synthesis. Ingestion of *Lactobacillus GG* has been suggested as a therapeutic in management of several conditions and has been shown to upregulate IL-10 in the plasma. While IL-10 has potent anti-inflammatory properties, it is also thought to act directly as an anti-nociceptive agent, indicating that it has broad neuroimmune effects, but impact on behavior has not to date been reported. The intestinal microbial balance may alter the regulation of inflammatory responses and in so doing may be involved in the modulation of mood and behavior (Cryan and Dinan, 2012; El Aidy *et al*, 2016).

As mentioned above, the hyper-response of the HPA in GF mice is reversed by mono-association with a single organism, *B. infantis*, which is a predominant bacterium in the infant gut and a commonly used probiotic organism. Furthermore, in GF animals the levels of norepinephrine (NE) and 5-HT in the cortex and hippocampus are significantly reduced (Clarke *et al*, 2013). Thus, preclinical data clearly show that commensal bacteria have the capability of altering not only the HPA axis but key neurotransmitters thought to be of relevance in the etiology of depression.

We have assessed the potential benefits of the probiotic *B. infantis* in the rat maternal separation model of depression, a paradigm that has proven to be of value in the study of antidepressant effects (Desbonnet *et al*, 2010).

Maternally separated adult rat offspring were chronically treated with *B. infantis* or a selective serotonin reuptake inhibitor (citalopram) and subjected to the FST to assess motivational state. Cytokine concentrations in stimulated whole blood samples, monoamine levels in the brain, and central and peripheral HPA measures were also analyzed. Maternal separation reduced swim behavior and increased immobility in the FST, decreased noradrenaline content in the brain, and enhanced pro-inflammatory peripheral IL-6 release and amygdala CRF mRNA levels. Probiotic treatment resulted in reversal of behavioral deficits, normalization of immune response, and restoration of basal noradrenaline concentrations in the brainstem. *B. infantis* has been shown in patients with IBS to alter the ratio of pro- to anti-inflammatory cytokines in the plasma (O'Mahony *et al*, 2005). Overall, preclinical data strongly support the view that an aberrant microbiota can alter behavior, immunity, and endocrinology and point to an influential role for *Bifidobacterium* in neural function (McKernan *et al*, 2010). *L. rhamnosus* whose effects have been described previously produces marked anxiolytic effects in animals acting through central GABA (Bravo *et al*, 2011). Vagotomy prevented the emergence of an anxiolytic effect from the probiotic and prevented changes in GABA receptor expression.

Prebiotics that increase the levels of 'good' bacteria in the intestine have been demonstrated to affect stress responses in animal models, but more importantly to reduce the awakening cortisol response in healthy volunteers (Schmidt *et al*, 2015). While the prebiotics FOS and GOS have been found useful in treating the metabolic syndrome, to date they have not been tested in patients with depression or other stress-related disorders.

In a double-blind, placebo-controlled, randomized parallel group study volunteers received either the probiotic combination *L. helveticus* R0052 and *B. longum* or placebo for 30 days and were assessed by the Hopkins Symptom Checklist (HSCL-90), the Hospital Anxiety and Depression Scale (HADS), the Perceived Stress Scale, the Coping Checklist (CCL) (Messaoudi *et al*, 2011). Daily administration of probiotic combination significantly reduced psychological distress in volunteers, as measured by the HSCL-90 scale, the HADS, and by the CCL. Furthermore, urinary-free cortisol levels were significantly reduced by the probiotics, providing a potential mechanism for the improvement in psychological symptoms observed and we have also observed that a strain of *B. longum* significantly reduced the awakening salivary cortisol response in healthy subjects relative to that seen with placebo.

Another study found that the consumption of a probiotic-containing yoghurt improved mood (Benton *et al*, 2007). One hundred and thirty-two physically healthy subjects with a mean age 61.8 years volunteered in response to local media coverage. One hundred and twenty-four completed the trial. For a 3-week period, either a probiotic containing milk drink, or a placebo were consumed daily. Mood and cognition were measured at baseline, and after 10 and 20 days of consumption. When the third with the lowest baseline mood

were considered, they selectively responded by reporting themselves as happy rather than depressed after taking the probiotic.

The focus to date has largely been on the modulation of host-immune interactions using probiotic organisms that might benefit mental health. Such bacteria have been defined as psychobiotic (Dinan *et al*, 2013). However, the possibility that depleting the microbiota of certain potentially melancholic-inducing bacterial strains has also been explored, though to a lesser extent. Minocycline, the tetracycline antibiotic, in small-scale studies has been found effective in treating the symptoms of both depression (Miyaoaka *et al*, 2012) and schizophrenia (Khodaie-Ardakani *et al*, 2014). As mentioned above, these findings are usually explained on the basis that minocycline inhibits the microglia polarization to a pro-inflammatory state and reduces pro-inflammatory cytokine levels (Hinwood *et al*, 2012). Such an analysis, while focused on what is now a definite and frequently reproduced observation, ignores the fact that minocycline is first and foremost an antibiotic that impacts both gram-positive and gram-negative bacteria. It is plausible that the therapeutic effects of minocycline occur primarily by an action on the gut microbiota. Careful studies are required to tease apart the microglial stabilizing effects of the minocycline from its classic antibiotic action.

## MICROBIOTA TRANSPLANTATION

In recent times we have seen a broadening of the indication for fecal microbiota transplantation (FMT) beyond the narrow remit of treating *Clostridium difficile* infection (Xu *et al*, 2015). Recently, a double blind trial of 18 patients with metabolic syndrome was reported. Half received a transplant from a lean donor and half an auto-fecal transplant (Vrieze *et al*, 2012). Transplant from the lean donors resulted in improved insulin sensitivity and alterations in SCFAs. The control group failed to show such changes. Targeting the microbiota has resulted in improvement in a case study of a patient with Parkinson's disease and it has been shown that a full truncal vagotomy decreases the risk of the disorder as individuals enter old age (Gray *et al*, 2015). There are also case reports of improvement in patients with multiple sclerosis and autism following FMT (Xu *et al*, 2015). Proper controlled studies are clearly required. Given the pre-clinical data in animal models of stress-related disorders, it is intriguing to speculate that FMT might be beneficial in conditions such as treatment refractory depression. To date no such studies have been reported and will undoubtedly be subject to serious regulatory scrutiny.

## FUTURE RESEARCH DIRECTIONS: FOCUS ON NOVEL TARGETS

We are still at the very early stages of understanding the complex communication systems between gut bacteria, immunity, and behavior, but it is now established that

certain bacteria within the human gut play a fundamental role in immune development and have the ability to produce molecules with neuroactive properties which can impact brain physiology and behavior. However, while metagenomics has advanced our understanding of the diversity of bacteria within the gut, the reality is that many of these bacteria remain unculturable and only a limited number of bacterial strains, which may be psychoactive, have been tested *in vivo*. Moreover, complex microbial ecosystems such as in the human gut are difficult to mimic and interactions between bacteria cannot be studied by simple culture conditions *in vitro*. We therefore require *in vivo* studies to determine the role of metabolite-producing bacteria and what affect such bacteria, and their components parts and metabolites have on the immune system and subsequently on the brain and behavior. Such studies will facilitate our understanding of the consequences of neuroactive compound production by the microbiota and how probiotic bacteria can influence the CNS, and could furthermore identify probiotic bacteria which have psychobiotic potential for the treatment of psychiatric disorders such as depression. However, while we have seen a recent massive expansion in preclinical studies there is a marked shortage of translational investigations. Nonetheless, immune modulation of behavior via the gut microbiota offers exciting and intriguing potential for innovative treatments in psychiatry.

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