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# The microbiome: stress, health and disease

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**Abstract** Bacterial colonisation of the gut plays a major role in postnatal development and maturation of key systems that have the capacity to influence central nervous system (CNS) programming and signaling, including the immune and endocrine systems. Individually, these systems have been implicated in the neuropathology of many CNS disorders and collectively they form an important bidirectional pathway of communication between the microbiota and the brain in health and disease. Regulation of the microbiome–brain–gut axis is essential for maintaining homeostasis, including that of the CNS. Moreover, there is now expanding evidence for the view that commensal organisms within the gut play a role in early programming and later responsivity of the stress system. Research has focused on how the microbiota communicates with the CNS and thereby influences brain function. The routes of this communication are not fully elucidated but include neural, humoral, immune and metabolic pathways. This view is underpinned by studies in germ-free animals and in animals exposed to pathogenic bacterial infections, probiotic agents or antibiotics which indicate a role for the gut microbiota in the regulation of mood, cognition, pain and obesity. Thus, the concept of a microbiome–brain–gut axis is emerging which suggests that modulation of the gut microflora may be a tractable strategy for developing novel therapeutics for complex stress-related CNS disorders where there is a huge unmet medical need.

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## Introduction

The human body contains a diverse and sizeable community of microbial cells and genetic material, collectively known as the microbiome. There is a growing appreciation that the microbiome plays a vital and active role in the development and function of basic physiological processes including digestion, growth, immune-defense and more recently has been shown to influence brain development (Bengmark 2013; Collins et al. 2012; Cryan and Dinan 2012; Diamond et al. 2011). The growing appreciation that the gut microbiota impacts on multiple aspects of human development is reflected in large-scale projects such as the NIH funded Human Microbiome Project (http://commonfund.nih.gov/hmp; HMPC 2012) and the European Commission's Metagenomics of the Human Intestinal Tract, MetaHIT (http://www.metahit.eu/). The Common Fund's Human Microbiome Project (HMP) aims to characterise the microbial populations that are found at numerous sites on the human body, including skin, oral cavity, nasal passages, urogenital tract and the gastrointestinal (GI) tract and to explore the role of these microbes in human health and disease. The goal of the MetaHit project is to establish associations between the genes of the human intestinal

microbiota and health and disease with particular focus on two disorders of increasing importance in Europe, inflammatory bowel disease (IBD) and obesity.

It is estimated that $10^{14}$ microorganisms reside in the adult GI tract which amounts to ten times the number of human cells in the body, the majority of which are comprised of bacteria from 500 to 1,000 different species that vary in stability, diversity and number throughout development and across different human populations (Arumugam et al. 2011; De Filippo et al. 2010; Eckburg et al. 2005). Interestingly, the catalogue of microbial genes living in the human gut contains 3.3 million microbial genes which amounts to 150-fold more than the human gene complement (Qin et al. 2010).

In this review we summarise how the gut microbiota can influence behaviour. Moreover, the nature and composition of the gut microbiota throughout development and the impact of important influencing factors such as diet, mode of delivery at birth, and stress is discussed in the context of health and disease in the host. Evidence that bacteria residing in the gut can also affect central brain function including neurobiological features and behaviours relevant to various psychiatric disorders is also presented. Finally, we consider the implications this may have for our understanding of this micro-environment and its important contributions to brain development and psychopathology and ultimately the development of novel and improved therapies for these disorders.

## The changing microbiome

Microbial populations reside on numerous sites of the body including the skin, the conjunctiva, the oral cavity, the respiratory tract, the vagina and in the GI tract. The composition and diversity of these populations is characteristic of the location (Grice and Segre 2012). The review will specifically focus on the composition of the gut microbiota and the implications for health and disease.

One of the key points when considering the role of the gut microbiota in health and disease is the composition of the microbiota across the lifespan (Douglas-Escobar et al. 2013). The microbiome is a dynamic entity that is under continuous evolution throughout the host's lifetime in particular the first three years of life during which time a stable microbiome is established (Costello et al. 2012; Gregory 2011; Relman 2012). It is sensitive to a whole array of manipulations such as diet, stress, infection, pharmacological intervention and thus it is clear that the composition of the microbiota is distinct at different milestones of life. Indeed, the microbial composition has been investigated across all stages of the lifespan beginning from the antenatal period. It is known that the developing foetus is effectively sterile up until birth (Adlerberth and Wold 2009; Costello et al. 2012) with microbial penetration of the amniotic space seen as an extremely rare occurrence. However, reports have shown a bacterial presence in the meconium of healthy neonates (Jimenez et al. 2008).

The significance of the mode of delivery, be it vaginal birth or Caesarean-section, has recently been highlighted, where it was noted that the microbial composition of neonates delivered vaginally resembled that of their own mother's vaginal microbiota, with a predominance of *Lactobacillus, Prevotella,* or *Sneathia* spp. Moreover the microbiota of infants delivered via Caesarean-section resembled that of the mother's skin with *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp. dominating (Dominguez-Bello et al. 2010). Although infants delivered by Caesarean-section exhibit a delayed acquisition of the members (*Firmicutes* and *Bacteroidetes*) which dominate the adult microbiome, their microbiome do eventually match that of their vaginally delivered counterparts (Maynard et al. 2012). Despite this normalisation of microbiota composition, infants delivered by Caesarean-section are more likely to suffer from allergies, asthma and diabetes later in life (Cho and Norman 2013; Relman 2012; Romero and Korzeniewski 2013), highlighting that the early life environment is fundamental in the development of a healthy microbiome.

The perinatal period is a key determinant in the establishment of the initial microbiome of the infant in that not only is mode of delivery a significant impact on microbial composition but also significant divergences have been described in preterm and healthy term neonates. Recently, it was reported that preterm infants lacked two of the main bacterial genera in healthy term infants; *Bifidobacterium* and *Lactobacillus,* instead displaying a dominance of the *Proteobacteria* (Barrett et al. 2013). Furthermore, the functional ability of the host microbiome is intrinsically linked to the needs of the infant host. Indeed, infants who are exclusively breastfed display an enrichment of *Bifidobacterium* species equipped to utilise human milk oligosaccharides (Costello et al. 2012). Each dietary juncture as the infant progresses towards adulthood is paralleled by shifts in the microbiota and a predominance of genes specialised towards microbial digestion of that diet (Ursell et al. 2012).

The maternal role has been shown to play a part in the developing infant's microbiome be it via vaginal birth or breast feeding (Parfrey and Knight 2012). It is also likely that both genetic and environmental influences also play a role in defining the adult core microbiome. Studies comparing infant's faecal microbiota with that of the mother at one and 11 months after birth suggests that early colonisers are readily replaced by externally

acquired species (Vaishampayan et al. 2010; Valles et al. 2012). Interestingly, in twin studies whereby the faecal microbiomes of monozygotic twins, dizygotic twins and their mothers were analysed, it was revealed that the microbiota composition was more similar between family members than unrelated individuals. However, compositional profiles indicated both monozygotic and dizygotic twins to have broadly similar core microbiomes which may indicate that environmental factors play a more significant role than genetic background (Lozupone et al. 2012; Turnbaugh et al. 2009) and that microbial ecologies tend to cluster in family members (Maynard et al. 2012). Undoubtedly, the contribution of host genotype and environmental factors to the core microbiome remains to be fully elucidated.

The question of whether or not an infant's particular microbial experience during this early changeable phase bestows health benefits or poses a risk for future disease is currently a matter of intense speculation. Indeed, emerging evidence from animal studies utilising probiotics, antibiotic treatments and germ-free (GF) mouse models indicate that eradication or introduction of specific bacterial strains impacts on various aspects of physiology and neurobiology, and suggest that early postnatal life and adolescence are important periods of microbial influence (Clarke et al. 2013b; Foster and McVey Neufeld 2013; Johnson and Versalovic 2012; Marques et al. 2010).

The gut microbiome is ever-evolving throughout the lifespan and thus it is perhaps not that surprising that profound differences in faecal microbial compositions occurs in an aged population (Claesson et al. 2011). Specifically an enhanced abundance of Bacteroides species and an altered pattern of Clostridium clusters were evident in the elderly population as well as an increased between subject variability (Claesson et al. 2011). More recently, findings by the same group highlighted that compositional groups were evident in an elderly population and these groups correlated with residence location in the community, day-hospital, rehabilitation or in long-term residential care. Furthermore, diet was found to influence microbiota and the health status of the individual (Claesson et al. 2012). Crucially, the loss of community-associated microbiota correlated with increased frailty. The population was geographically and ethnically homogenous but, if confirmed in more diverse cohorts, these results could have important implications for our understanding of the interactions between diet, the microbiota, health and ageing (Kinross and Nicholson 2012). Interestingly, a recent study in aged mice has demonstrated that a diet rich in omega-6 polyunsaturated fatty acids (n-6 PUFAs), a feature of 'Western' diets, can influence microbiota composition and intestinal inflammation (Ghosh et al. 2013).

## Microbiota–brain–gut axis

Interest in the potential involvement of gut microbiota in brain function emerged, in part, due to the emphasis placed on a construct known as the brain–gut axis. This is a relatively well developed concept in the area of food intake and satiety (Sam et al. 2012; Schellekens et al. 2012). However, it has now expanded outside this relatively narrow focus and has become a fast-evolving research topic that has led to a convergence of research efforts in the fields of neuroscience, psychiatry, gastroenterology and microbiology, domains that were previously considered to have distinct and separate research objectives and focus. The initial construct of the brain–gut axis, which describes the complex bidirectional communication system linking the central nervous system (CNS) and the GI tract, preceded any notion that microorganisms resident in the gut played any modulatory role in brain function and development. The brain–gut axis is vital for maintaining homeostasis and dysregulation has been implicated in various disease states (Aziz and Thompson 1998; Bonaz and Bernstein 2013; Cryan and O'Mahony 2011; Davari et al. 2013; Grenham et al. 2011; Mayer 2011). In addition to facilitating the central regulation of digestive function and satiety, impairments in brain–gut axis signalling are associated with gut inflammation, chronic abdominal pain syndromes and eating disorders (Cryan and Dinan 2012; Nicholson et al. 2012). Moreover, modulation of brain–gut axis function is linked to the stress response and behaviour (Clarke et al. 2013b). More and more researchers in this area now acknowledge the microbiome itself as an active and highly-influential contributing factor in this bidirectional communication network (Bercik 2011; Collins et al. 2012; Grenham et al. 2011; Mayer 2011; Rhee et al. 2009).

This complex network of communication between the gut microbiota and the brain encompasses the CNS, and both the sympathetic and parasympathetic branches of the autonomic nervous system (ANS) and the enteric nervous system (ENS) in addition to the neuroendocrine and neuroimmune systems (Grenham et al. 2011). Afferent fibres which project from the gut to cortical centres such as cerebral, anterior and posterior cingulate, insular, and amygdala cortices and as well as effector fibres projecting to the smooth muscle of the gut are the major routes for bidirectional communication along this axis (O'Mahony et al. 2011). Collectively, the microbiota–brain–gut axis communicates not only via neuronal routes but also via humoural signalling molecules and hormonal components. Together, this intricate network exerts effects which alter GI function and indeed brain function (Mayer 2011; Rhee et al. 2009). The vast amount of knowledge regarding signalling along this axis is primarily confined to neuronal communication between the ENS and the CNS, However,

the exact role the microbiota plays has yet to be elucidated. Hindering these efforts to define the role of the microbiota is the fact that not much is known of the microbial composition, in that we do not fully appreciate the vast quantity and diversity of the gut microbiome. Furthermore, the functional capabilities of all these gut microorganisms adds an extra complexity to the equation. However, the metagenomic revolution is destined to address this concern (Clarke et al. 2013a; Fraher et al. 2012). A recent paper by (Matsumoto et al. 2013) highlights the role of gut microbiota in cerebral metabolism, where GF animals were found to have an altered metabolic profile to their conventionally colonised counterparts with 10 of these metabolites thought to be specifically involved in brain function. Concurrent to the metagenomic revolution, efforts to establish the implications for the CNS are continuing swiftly. Our discussion below outlines the principal ideas which are uniting the fields of microbiology and neuroscience.

## Pathways of microbiota–brain–gut communication

### Neural pathways

The gut is innervated by the ENS, a complex network of neurons comprised of sensory, motor, and interneurons, that are capable of independently regulating basic GI functions (motility, mucous secretion, and blood flow). Due to the autonomous nature of the ENS, it is often referred to as the 'brain of the gut'. Nevertheless, coordination of gut functions with the general homeostatic state of the organism, which is important for optimal performance in situations of threat, necessitates continuous communication with the brain. Adrenaline and noradrenaline and acetylcholine are the chief neurotransmitters of the ANS, and efferent and afferent neurons of this system innervate the gut by interacting with the ENS (Mertz 2002). The vagus nerve is the major nerve of the parasympathetic division of the ANS and has been shown to be an important pathway for bidirectional communication between the gut microbes and the brain (Forsythe et al. 2010; Goehler et al. 2005) (Fig. 2). For example, the behavioural effects mediated by two separate probiotic strains in rodents were dependent on intact vagal nerve activation (Perez-Burgos et al. 2013). Specifically, chronic treatment with *Lactobacillis rhamnosus* (JB-1) induced region-dependent alterations in GABA receptor expression in the brain and reduced stress-induced corticosterone and anxiety- and depression-related behaviour, effects that were dependent on vagus nerve integrity (Bravo et al. 2011). Similarly in a colitis model, the anxiolytic effect of *Bifidobacterium longum* was not present in vagotomised mice (Bercik et al. 2011b). In contrast to effects mediated by probiotics, changes in the microbial ecology as a consequence of antibiotic treatment in mice did not show a similar dependence on vagal nerve activity (Bercik et al. 2011a) highlighting the likelihood that other mechanisms are equally involved in microbiota–brain–gut communication (Barrett et al. 2012a).

Electrophysiological studies are lacking in the area of microbiota mediated alteration in ENS functioning. Previous work demonstrated that the probiotic, *Lactobacillus reuteri*, increased excitability and number of action potentials per depolarizing pulse, decreased calcium-dependent potassium channel opening and decreased the slow after-hyperpolarization in sensory after-hyperpolarization (AH) neurons. These results demonstrate that *L. reuteri* targets an ion channel in enteric sensory nerves which may be mediating it's effects on gut motility and pain perception (Kunze et al. 2009). A more recent study has shown electrophysiological properties of myenteric neurons are altered in GF mice specifically; decreased excitability in myenteric sensory neurons was found in the absence of intestinal microbiota. Upon colonization of GF mice with normal gut microbiota, excitability of AH sensory neurons in GF mice was increased (McVey Neufeld et al. 2013). Furthermore, it has been shown that a functional microbiota–neurohumoral relationship occurs during colonization of the GF intestine and the neuronal response elicited occurs only after the microbiota accommodating homeostasis has been accomplished. The neuropeptide NPY is just one emerging target thought to be involved in microbiome brain interactions as it sensitive to microbiota manipulations and can operate both as a neural and endocrine messenger (Holzer et al. 2012). These biologically active peptides are present at numerous locations throughout the brain–gut axis and have a broad array of functions such as regulation of mood, stress resilience and GI motility.

### Tryptophan metabolism

Serotonin [5-hydroxytryptamine (5-HT)] is a biogenic amine that functions as a neurotransmitter in the body. Despite the fact that it is predominantly known for its role in the brain, approximately 95 % of 5-HT in the body is contained within the gut, specifically, in the enterochromaffin cells of the mucosa and in the nerve terminals of the ENS neurons. Peripherally, 5-HT is involved in the regulation of GI secretion, motility (smooth muscle contraction and relaxation), and pain perception (Costedio et al. 2007; McLean et al. 2007), whereas in the brain it has an important function in mood and cognition (Wrase et al. 2006). Not surprisingly, it has been postulated that alterations to the functioning of this neurotransmitter may

underlie the pathological symptoms present in both GI and mood disorders, and, may also explain the high co-morbidity of these disorders (Folks 2004). Indeed, drugs that modulate serotonergic neurotransmission, such as tricyclic antidepressants and SSRI's, have been shown to be effective in the treatment of both affective and GI disorders such as irritable bowel syndrome (IBS) (Creed et al. 2003; Tack et al. 2006; Weilburg 2004).

Serotonin synthesis in the brain is crucially dependent on the availability of tryptophan. Tryptophan within the CNS is dependent on peripheral levels and must be supplied in the diet. Moreover, GF animals have altered peripheral tryptophan availability and altered central serotonin concentrations (Clarke et al. 2013b). Acute tryptophan depletion using tryptophan-restricted diets results in a lowering of plasma tryptophan levels that is paralleled by a decline in central serotonin in healthy human volunteers (Moore et al. 2000) and more recently in animals (Browne et al. 2012). More recently, inhibition of the enzyme that converts tryptophan to serotonin, indoleamine-(2,3)-dioxygenase (IDO), in rats resulted in decreased concentrations of brain serotonin concentrations and associated change in anxiety behaviour in the elevated plus maze (Naslund et al. 2013), demonstrating that peripheral tryptophan can influence brain activity and, more importantly, behaviour. The enzymes IDO and tryptophan 2,3-dioxygenase (TDO) are regulated by inflammatory mediators and corticosteroids respectively (Ruddick et al. 2006). The pro-inflammatory cytokine IFN-$\gamma$ has been shown, both in vitro and in vivo, to be a potent stimulus in the activation of indoleamine-(2,3)-dioxygenase, the enzyme involved in the conversion of tryptophan to kynurenine (Taylor and Feng 1991). Excessive immune-mediated tryptophan degradation may induce depressive symptoms when the availability of tryptophan is insufficient for normal serotonin synthesis. Indeed, depressive illness has been associated with reduced plasma tryptophan concentrations and an enhanced enzyme activity, as reflected by an increase in the kynurenine:tryptophan ratio of (Myint et al. 2007). There is evidence to suggest that the probiotic *B. infantis* affects tryptophan metabolism (Desbonnet et al. 2008) however, administration of *B. longum* had no effect on kynurenine levels (Bercik et al. 2010). Further evidence of a modulatory relationship between the microbiota and tryptophan metabolism has emerged from GF mouse studies whereby the absence of the microbiota in early life results increases plasma tryptophan concentrations, reduces the kynurenine:tryptophan ratio, and induces increases in hippocampal serotonin levels in adulthood (Clarke et al. 2013b). Importantly, the former measures are restored following the introduction of bacteria in GF mice post weaning (Fig. 2).

Therefore, the gut microbiota may play a crucial role in tryptophan availability and metabolism and consequently impact on central serotonin concentrations. These effects are potentially mediated through indirect immune-mediated mechanisms involving the IDO-activating cytokine IFN-$\gamma$ or by more directly modulating tryptophan metabolism at the level of the gut. To date, the specific mechanisms underlying this putative modulatory interaction remain unknown and much work has yet to be done to elucidate the processes involved in this potentially important pathway of communication with the CNS.

## Immune system

It is increasingly recognised that the immune system plays an important intermediary role in the dynamic equilibrium that exists between the brain and the gut (Bengmark 2013). The HPA axis, ANS and ENS all directly interact with the immune system (Bateman et al. 1989; Genton and Kudsk 2003; Hori et al. 1995; Leonard 2005; Nance and Sanders 2007). The gut itself is an important immune organ forming a vital defensive barrier between externally-derived pathogens and the internal biological environment. The gut-associated lymphoid tissues, together, form the largest immune organ of the human body, containing over 70 % of the total immune system (Vighi et al. 2008). Indeed, the link between infection and psychiatric illness has long been known, mainly through the observation that syphilis and Lyme disease are often accompanied by neurological deficits (Biesiada et al. 2012). Also in animals, infectious microorganisms are well documented to affect behavioural measures through activation of the central immune response. For example, the pathogenic bacteria Campylobacter jejuni, when administered to mice at subclinical doses, resulted in anxiety-like behaviour in mice (Lyte et al. 1998). In addition, peripheral administration of pro-inflammatory cytokines in rodents induces depressive-like behaviours, disturbances of sleep, reduced appetite and fatigue, symptoms collectively referred to as sickness behaviours (Bilbo and Schwarz 2012). There has been a growing appreciation of the important role played by gut microbes in maturation and fortification of the immune response in recent years however, the molecular basis of these immunomodulatory mechanisms are still emerging. It is proposed that the immunoregulatory effects of probiotic microorganisms may occur through the generation of T regulatory cell populations and the synthesis and secretion of the anti-inflammatory cytokine, IL-10 (Dinan et al. 2013). In support of this, oral consumption of Bifidobacterium infantis 35624 in humans is associated with enhanced IL-10 expression in human peripheral blood (Konieczna et al. 2012). Furthermore, feeding of a commensal bacteria to GF mice promotes Treg production and IL-10 synthesis in these mice (Macpherson and Uhr 2002).Therefore, it is likely that the intestinal microbial balance closely regulates inflammatory responses in the

host, and disturbances to this microbial balance, particularly in early life (O'Mahony et al. 2009), may result in a chronic inflammatory state that can lead to maladaptive changes in mood and behaviour (Fig. 2).

## The microbiome and behaviour

One of the most exciting areas in examining the role of microbiome in health and disease is the effects of the microbiome on behaviour. Studies in GF animals and also probiotic and antibiotic treated animals have yielded fascinating results again highlighting the importance of the microbiome in both health and disease. Furthermore, diet and stressful events are also implicated in microbiome brain communication.

### Germ free (GF)

GF animals are an exciting research tool to explicitly investigate the role of the host gut microbiota on CNS function. Despite exaggerated neuroendocrine responses to stress, several independent laboratories have demonstrated consistent decreases in anxiety-like behaviour in GF mice when exposed to novel and aversive environments (elevated plus maze, light/dark box, open field) (Clarke et al. 2013b; Diaz Heijtz et al. 2011; Neufeld et al. 2011) (Table 1), an effect that is normalised following post-weaning bacterial colonisation of GF mice and also in the offspring of colonized GF mice (Clarke et al. 2013b; Diamond et al. 2011; Diaz Heijtz et al. 2011). However a recent study by (Nishino et al. 2013) found that GF mice generated on a BALB/c background, and subsequently colonized with normal specific pathogen-free (SPF) microbiota, (EX-GF), were less anxious than GF mice using both the open-field test and marble burying test. Moreover a brief exposure to a non-sterile environment can render these GF mice less anxious. These findings highlight that genotype may play a significant role in the complex microbiome brain–gut axis (Gulati et al. 2012; Olivares et al. 2013). GF mice also show social deficits characterised by reduced sociability with a novel stimulus mouse, in the three compartment arena compared with their conventional counterparts. Furthermore GF mice exhibited repetitive grooming behaviour and social cognition deficits (Desbonnet et al. 2013) (Table 1).

### Probiotics

A growing body of evidence is accumulating for the many diverse effects of probiotics/prebiotics on behaviour. In animal models a range of probiotics have been investigated however, not all bacterial populations show efficacy in modulating behaviour. *Bifidobacteria* and *Lactobacillus* are the main genera thus far investigated for beneficial effects on health. However, even within bacterial genera, not all species have positive effects. Furthermore, the status of the host is critical in the efficacy of probiotics in that some probiotics will only exhibit beneficial effects in states of disease such as IBS and may show no positive effects in healthy states. This area of research is an exciting and rapidly growing field.

In the maternal separation model, chronic treatment with *B. infantis* in adulthood attenuated immune system abnormalities and depressive-like behaviours in the forced swim test to a similar extent as the antidepressant citalopram (Desbonnet et al. 2010) (Table 1). *L. helveticus* ROO52 has also been shown to reduce anxiety-like behaviour and alleviate memory dysfunction in the Barnes maze in both naïve and western diet fed mice (Ohland et al. 2013). *L. rhamnosus* reduced anxiety- and depression-related behaviours in the elevated plus maze and forced swim test respectively (Bravo et al. 2011). Recent work by (Matthews and Jenks 2013) demonstrated reduced anxiety and improved performance on a complex maze task following treatment with live *Mycobacterium vaccae* prior to and during the test. *B. longum* normalizes anxiety-like behaviour in the DSS colitis model (Bercik et al. 2011b). Furthermore *B. longum*, but not L. *rhamnosus* normalizes anxiety-like behaviour in *Trichuris muris* infection (Bercik et al. 2010). Memory dysfunction occurs as a result of *Citrobacter rodentium* infection in C57BL/6 mice exposed to acute stress. This was prevented by daily treatment of infected mice with probiotics (*L. rhamnosus* (R0011) + *L. helveticus* (R0052) (Gareau et al. 2011). Probiotic treatment has also proved efficacious in alleviating visceral pain responses (Johnson et al. 2011; McKernan et al. 2010; Rousseaux et al. 2007; Verdu et al. 2006) (Table 1).

### Antimicrobials

Modern society has seen a massive increase in the prescription and use of antibiotics. However, emerging research has found that chronic antibiotic use can be detrimental to the host. This has been investigated in animal models where it was found that antibiotic treated mice showed more exploratory and less apprehensive behaviour which was reversible after a 2 week wash-out period (Bercik et al. 2011a) (Table 1). However the strain of mouse used in these studies was BALB/c, an innately anxious and stress sensitive strain. When GF mice were treated with antibiotic no alteration in anxiety and exploratory behaviour were observed, further emphasising the role of the host microbiota. More interestingly when GF mice were colonized with the microbiota of BALB/c,

**Table 1** Preclinical studies of microbiota–gut–brain axis

| Model | Species, strain | Treatment | Method, *Microbiota* | Findings | | | References |
|---|---|---|---|---|---|---|---|
| | | | | Microbiota/colon | Behaviour | Neurochemical | |
| Early life stress | | | | | | | |
| Maternal separation | Monkey, Rhesus Macaques (infant) | N/A | Culture, *fecal microbiota* | Decreased Lactobacillus 3 days post stress<br>Infants who displayed numerous stress-indicative behaviours were more susceptible to opportunistic bacterial infection | Reduction in microflora was correlated with the display of stress-indicative behaviours | Large cortisol responses to maternal separation | Bailey and Coe (1999) |
| Maternal separation | Rat, Sprague Dawley | Arachidonic, DHAs,<br>Galacto- fructo-oligosaccharides<br>Lactobacillus paracasei NCC2461 | Culture, *fecal microbiota* | Composition of microbiota was altered in stressed compared with control animals<br>Adapted diet normalized the intestinal permeability, but not intestinal mucin content or microbiota | N/A | Increased intestinal permeability to macromolecules stressed animals,<br>Reduced mucin content in small intestinal tissues of stressed animals | Garcia-Rodenas et al. (2006) |
| Maternal separation | Rat, Sprague Dawley | *Bifidobacterium breve* DPC6330<br>0.5 % (w/w) linoleic acid<br>0.5 % (w/w) α-linolenic acid | N/A | N/A | | *B. breve* DPC6330 significantly altered palmitoleic acid, arachidonic acid and DHA contents in tissues of stressed rats | Barrett et al. (2012b) |
| Maternal separation | Rat, Sprague Dawley | *Bifidobacterium infantis* | N/A | N/A | *Bifidobacterium infantis* reversed stress-induced depression-like behaviours | *Bifidobacterium infantis* normalised the immune response and<br>Basal NA concentrations in the brainstem | Desbonnet et al. (2010) |
| Maternal separation | Rat, Sprague Dawley | N/A | 16S Ribosomal RNA Targeted Polymerase chain reaction–DGGE, *fecal microbiota* | The microbiota differed significantly between stressed animals from their non-stressed counterparts | Stress increased anxiety behaviours<br>And visceral pain response | Stress increased immune response and<br>Plasma corticosterone | O'Mahony et al. (2009) |

**Table 1** continued

| Model | Species, strain | Treatment | Method, *Microbiota* | Findings | | | References |
|---|---|---|---|---|---|---|---|
| | | | | Microbiota/colon | Behaviour | Neurochemical | |
| Maternal separation | Rat, Sprague Dawley | *L rhamnosus* (R0011)<br>*L helveticus* (R0052) | N/A | Significant reduction in *Lactobacillus* species in stressed pups | N/A | Ion transport and macromolecule flux were significantly higher in the colon of stressed pups<br>Increased adhesion/penetration of total bacteria in stressed pups<br>Probiotic administration ameliorated the stress-induced functional abnormalities | Gareau et al. (2007) |
| Adulthood stress | | | | | | | |
| Social disruption | Mouse, CD-1 | N/A | Bacterial Tag Encoded FLX Amplicon Pyrosequencing (bTEFAP), *Cecal microbiota* | Reduction in microbial diversity in the stress+15 hr group<br>Decreased relative abundance of Bacteroides<br>Increased relative abundance of Clostridium | N/A | Stress increased circulating levels of IL-6 and MCP-1 | Bailey et al. (2011) |
| Partial restraint stress | Rat, Wistar | *Lactobacillus farciminis* | N/A | N/A | | *L. farciminis* suppressed stress-induced hyperpermeability, endotoxemia & prevented the HPA axis stress response & neuroinflammation | Ait-Belgnaoui et al. (2012) |
| Antimicrobial treatment (ATM) | | | | | | | |
| N/A | Mouse, BALB/c | Neomycin<br>Bacitracin<br>Pimaricin | culture, 16S Ribosomal RNA Targeted Polymerase Chain Reaction DGGE, *Fecal microbiota* | ATM treatment increased the proportion of *Lactobacilli* and *Actinobacteria* populations<br>Decreased in the γ-proteobacteria and Bacteroidetes populations<br>ATM-induced changes in gut microbiota are reversible (2 week wash-out) | ATM treated mice showed more exploratory and less apprehensive behaviour<br>ATM-induced changes in behaviour which are reversible after a 2 week wash-out<br>no differences in behaviour after oral ATM to GF<br>Reduced exploratory behaviour when GF mice were colonized with microbiota from SPF BALB/c mice | BDNF levels in ATM-treated mice were greatly higher in the hippocampus and lower in the amygdala compared with control mice | Bercik et al. (2011a) |

**Table 1** continued

| Model | Species, strain | Treatment | Method, *Microbiota* | Findings | | | References |
|---|---|---|---|---|---|---|---|
| | | | | Microbiota/colon | Behaviour | Neurochemical | |
| Chronic GI infection/inflammation | | | | | | | |
| DSS induced colitis | Mouse, AKR | *Bifidobacterium longum* NCC3001 | N/A | N/A | *B. longum* normalizes anxiety-like behaviour | *B. longum* had no effect on MPO activity or histological scores.<br>Anxiolytic effect *of B. longum* is dependent on vagus nerve integrity | Bercik et al. (2011b) |
| *Trichuris Muris* infection | Mouse, AKR | *Bifidobacterium longum* NCC3001<br>*L. rhamnosus* | N/A | N/A | Infected mice displayed increased anxiety-like behaviour<br>*B. longum*, but not *L. rhamnosus* normalizes anxiety-like behavior | Dcreased hippocampal BDNF<br>*B. longum,* but not *L. rhamnosus,* normalized BDNF levels<br>*B. longum* did not affect cytokine or kynurenine levels | Bercik et al. (2010) |
| *Citrobacter Rodentium* & acute water avoidance stress | Mouse, C57BL/6, Swiss Webster (GF) | *L rhamnosus* (R0011)<br>*L. helveticus* (R0052) | N/A | N/A | Memory dysfunction occurs in infected C57BL/6 mice exposed to acute stress<br>Memory dysfunction was prevented by daily treatment of infected mice with probiotics<br>GF mice have altered memory at baseline. | N/A | Gareau et al. (2011) |
| Probiotic treatment | | | | | | | |
| Naive | Mouse, BALB/c | *Lactobacillus rhamnosus* (JB-1) (28 days) | N/A | N/A | *L. rhamnosus*<br>(JB-1) reduced anxiety-like behaviours<br>*L. rhamnosus*<br>(JB-1) reduced depression-like behaviours<br>Anxiolytic effect of *L. rhamnosus* (JB-1) is dependent on vagus nerve integrity | GABA(B1b) increases in cortical regions (cingulate and prelimbic) and concomitant<br>Reductions in expression in the hippocampus, amygdala, and locus coeruleus<br>reduced GABA(Aα2) mRNA expression in the prefrontal cortex and amygdala, but increased GABA(Aalpha2) in the hippocampus | Bravo et al. (2011) |

**Table 1** continued

| Model | Species, strain | Treatment | Method, *Microbiota* | Findings | | | References |
|---|---|---|---|---|---|---|---|
| | | | | Microbiota/colon | Behaviour | Neurochemical | |
| Naive | Rat, Sprague Dawley | *Bifidobacteria infantis* | N/A | N/A | No change in immobility in the forced swim test | Significant attenuation of IFN-gamma, TNF-alpha and IL-6 cytokines following mitogen stimulation<br>Increased plasma concentrations of tryptophan and kynurenic acid<br>Reduced 5-HIAA concentration in the frontal cortex and a decrease in DOPAC in the amygdaloid cortex | Desbonnet et al. (2008) |
| Naive | Mouse, 129/SvEv | Lactobacillus helveticus ROO52. | Terminal restriction fragment length polymorphism, *Cecal microbiota* | Increased *Firmicutes*/*Bacteriodetes*, *Proteobacteria* and *Spirochaetes* in Western diet fed mice<br>Alterations are reversed by *L. helveticus* treatment | Increased anxiety-like behaviour and<br>Memory dysfunction in Western diet fed mice<br>These behaviours are reversed by *L. helveticus* treatment | N/A | Ohland et al. (2013) |
| Naive | Mouse, BALB/c | *Mycobacterium vaccae* | N/A | N/A | Probiotic treatment reduced anxiety and improved performance on a maze task | N/A | Matthews and Jenks (2013) |
| GF | | | | | | | |
| Naive | Mouse, Swiss Webster, GF, SPF | N/A | N/A | Colonization of GF intestine does not restore neurochemical measures however does normalise behavioural outputs | Reduced anxiety in male GF mice | Increased hippocampal 5HT and 5HIAA, plasma tryptophan in GF males | Clarke et al. (2013b) |
| Naive | Mouse, NMRI, GF, SPF | N/A | N/A | N/A | GF mice display increased motor activity and reduced anxiety, compared with SPF mice | GF mice exposed to gut microbiota early in life display similar characteristics as SPF mice, including reduced expression of PSD-95 and synaptophysin in the striatum. | Diaz Heijtz et al. (2011) |

**Table 1** continued

| Model | Species, strain | Treatment | Method, *Microbiota* | Findings | | | References |
|---|---|---|---|---|---|---|---|
| | | | | Microbiota/colon | Behaviour | Neurochemical | |
| Naive | Mouse, BALB/c, GF, SPF | N/A | N/A | N/A | Brief exposure to non-sterile environment rendered GF mice less anxious<br>EX-GF mice reduced anxiety-like behaviour | Increased turnover rates of norepinephrine, dopamine, and serotonin in EX-GF mice in most regions of the brain | Nishino et al. (2013) |
| Naive | Mouse, Swiss Webster, GF, SPF | N/A | N/A | N/A | GF mice display reduced anxiety, compared with SPF mice | Decrease in the mRNA expression of the N-methyl-D-aspartate receptor subunit NR2B in the central amygdala<br>Increased brain-derived neurotrophic factor expression<br>Decreased serotonin receptor 1A (5HT1A) expression in the dentate granule layer of the hippocampus | Neufeld et al. (2011) |
| Naive | Mouse, BALB/c, GF, SPF | *Bifidobacteria infantis* | N/A | N/A | | Enhanced HPA axis activity in GF mice following an acute psychological stress<br>Exaggerated HPA response was reversed by *Bifidobacteria infantis*<br>Reduced BDNF expression in cortex and hippocampus | Sudo et al. (2004) |
| Social dysfunction | | | | | | | |
| Naive | Rat, Long–Evans hooded (adolescent) | propionic acid (intracerebroventricularly) | N/A | N/A | Restricted behavioural interest to a specific object among a group of objects, impaired social behaviour, and impaired reversal in a T-maze task | N/A | MacFabe et al. (2011) |

significant increases in anxiety behaviour was observed (Bercik et al. 2011a). Increased visceral hypersensitivity has also been shown in antibiotic treated animals however, this effect was reversed by administration of *L. Paracasei* (Verdu et al. 2006) (Table 1).

## Disorders of the microbiome–brain–gut axis

### Stress, anxiety and depression

Despite the well-established association between stress and psychiatric disorders, the struggle to understand the complex processes by which stress mediates pathological changes that increase vulnerability to disease is on-going (Hornig 2013). Stress was first described by Seyle (Selye 1936) over half a century ago, and is defined as an acute threat to the homeostasis of an organism. An adverse event, or stressor, whether it be the presence of a real threat (physical stressor) or the anticipation of a threat (psychological stressor), will elicit a sequence of physiological, emotional and behavioural reactions that allow one to cope adequately with the situation. However, severe, chronic, and uncontrollable stressors can trigger maladaptive changes in brain structure and function that can have long-term consequences on one's physical and mental wellbeing (Lupien et al. 2009; McEwen 2012; Nutt and Malizia 2004).

The importance of emotional state and stress processing in the brain has received increasing recognition in the study of GI disorders, and the subject of microbiota–brain–gut axis dysregulation in stress-related CNS disorders has been the subject of a number of excellent recent reviews (Bested et al. 2013a, b; Bravo et al. 2012; Collins et al. 2012; Cryan and Dinan 2012; Foster and McVey Neufeld 2013; Scott et al. 2013). The stress response is generated by the complex integration of a series of interconnected brain regions, most notably the amygdala, the hippocampus and the paraventricular nucleus of the hypothalamus, which also receive modulatory inputs from higher cortical regions such as the prefrontal cortex. The major output of the central stress circuitry consists of the neuroendocrine HPA axis, and the ANS.

Depression and general anxiety are disorders with well-established aetiological links to the experience of traumatic life events, particularly when experienced in early life and chronic stress (Caspi et al. 2003; Kendler et al. 2000). Although current clinical literature contains few reports that describe the state of the gut microflora in depressed individuals, data from associated illnesses, such as IBS, which are often accompanied by depressive symptoms, have revealed reduced *Bacteroidetes* and increased *Firmicutes* content in the faecal samples of patients with these disorders (Jeffery et al. 2012; Krogius-Kurikka et al. 2009; Tana et al. 2010). Research from both human and animal studies has shown that emotional stressors can negatively impact on the gut microflora. Animal models of stress-related disorders have also revealed interesting alterations to the gut microflora. Maternal separation, restraint conditions, crowding, heat stress and acoustic stress have all been shown to alter the composition of gut microbiota (Bailey and Coe 1999; Bailey et al. 2011; Collins and Bercik 2013; Garcia-Rodenas et al. 2006; O'Mahony et al. 2009; Suzuki et al. 1983; Tannock and Savage 1974; Timoveyev et al. 2002).

The maternal separation model represents one of the best characterised animal models in relation to the long-term effects of stress in early life on microbiota, and in light of its inherent construct and face validity, it is uniquely suited to model co-morbid depression and IBS (O'Mahony et al. 2009). In light of the mutual relationship that exists between the stress response and microbiota, it is not surprising that the period most critical to HPA axis development and programming of the neuroendocrine stress response, early postnatal life, is also an important time-point for the initial establishment of the core gut microbiota. This model and others have been employed to investigate numerous aspects of brain gut interactions and more recently the effects of probiotic treatment on fatty acid metabolism of the host (Barrett et al. 2012b; Wall et al. 2010; Wall et al. 2012). This data is particularly relevant in the context of depression with an increasing body of evidence highlighting the role of fatty acids such as eicosapentaenoic acid and docosahexaenoic acid (DHA) in depressive disorders (Mocking et al. 2013).

The association between microbiota and stress-responsivity is further supported by findings in GF mice and rodents treated with probiotics and/or antibiotics. The seminal findings of Sudo et al. (2004) demonstrating enhanced HPA axis activity in GF mice following an acute psychological stress, provided the first convincing evidence of the essential role played by microbiota in programming of the stress response. In light of these findings, numerous other independent research groups have gone on to investigate the exact mechanisms which may be at play in this complex network of communication between the gut microbiota and the many other systems including the immune and autonomic, enteric and CNSs. These studies have shown decreased mRNA expression of *N*-methyl-D-aspartate receptor subunits (NR1, NR2A, NR2B) in many brain areas which include the central amygdala, the cortex and hippocampus (Neufeld et al. 2011). Furthermore, decreased serotonin receptor 1A ($5\text{-HT}_{1A}$) expression was also evident in the dentate granule layer of the hippocampus (Neufeld et al. 2011), as well as reduced mRNA and protein expression of BDNF in the cortex and hippocampus.

Increased hippocampal 5-HT and 5-HIAA as well as plasma tryptophan was found GF males (Clarke et al. 2013b). Furthermore, it was also shown that the turnover rates of norepinephrine, dopamine, and serotonin were higher in colonized-GF mice than in the GF mice in most regions of the brain, suggesting that monoaminergic neurotransmission might increase in the colonized GF mice compared with GF mice (Nishino et al. 2013). These neurochemical findings have led to profound differences in the behaviour of GF mice in particular a reduced anxiety phenotype which will be discussed later.

In a similar manner antibiotic administration for 1 week in adult mice, and the consequent alteration to gut microbial diversity characterised by significant increases in *Firmicutes* and Actinobacteria, and a decrease in γ-proteobacteria and *Bacteroidetes,* resulted in comparable reductions in anxious behaviour (Bercik et al. 2011a). This effect of antibiotic treatment was maintained in vagotomised mice suggesting that autonomic neuronal connectivity is not required for antibiotic-induced effect on the behavioural phenotype (Bercik et al. 2011a). Interestingly, when microbiota from Balb/C mice, a mouse strain that reproducibly exhibit an anxious phenotype (Belzung and Griebel 2001), is transplanted to the non-anxious GF Swiss Webster mouse strain, the anxiety-like behaviours are induced. Moreover, when the reverse transplantation, Swiss-Webster into GF Balb/C mice was conducted, the anxiety phenotype was attenuated. These findings emphasise that the microbiota is an attractive target for potential therapeutic strategies whereby altering the host's gut microbiota via probiotics may infer health benefits to the host. The finding that microbiota transfer via faecal transplantation impacts on behaviours also highlights the potential benefits of faecal transplant therapy in disorders of microbiota–brain–gut axis dysregulation and the vast opportunities this method presents for further examination of the role played by microbiota in behaviour and CNS function (Aroniadis and Brandt 2013).

Indeed, animal studies focusing on the effects of chronic probiotic treatment, both in naïve and stress-related animal models of CNS disorders, have also served as invaluable and informative tools in ascertaining the specific contributions made by gut bacteria to anxious and depressive disorders. Probiotic treatment during the postnatal stress period in maternally-separated rat offspring has been shown to normalise basal corticosterone levels (Gareau et al. 2007). When administered to naïve rodents, *Lactobacillus rhamnosus* reduced stress-induced corticosterone which was paralleled by region-dependent alterations in GABA receptor gene expression levels in the brain (Bravo et al. 2011). Moreover, the neurochemical effects were not found in vagotomized mice, identifying the vagus as a major modulatory constitutive communication pathway between the bacteria exposed to the gut and the brain (Bravo et al. 2011). *Bifidobacteria infantis* altered peripheral cytokine levels and concentrations of the serotonin precursor, tryptophan, which may allude to the development of possible protective mechanisms prior to stress exposure (Desbonnet et al. 2008). In clinical studies, probiotic treatment was found to alter activity of brain regions that control central processing of emotion and sensation. Specifically, treatment with a fermented milk product with probiotic reduced the response of healthy volunteers to an emotional faces attention task (Tillisch et al. 2013). However, this has yet to be demonstrated in pathological anxiety. Furthermore *L. helveticus* R0052 and *B. longum* R0175 taken in combination alleviated psychological distress in healthy subjects (Messaoudi et al. 2011). Moreover, (Benton et al. 2007) demonstrated that a probiotic containing yoghurt could improve mood in subjects whose initial mood was poor. The therapeutic potential of probiotics in psychiatric conditions has been the topic of intense discussion however, it is apparent that much more work is required in this area to fully elucidate the role of probiotics in brain function (Craft and Li 2013; Myint et al. 2013).

One of the principal mechanisms proposed to underlie stress-induced alterations is the "leaky gut" phenomenon which has been described by (Maes et al. 2008) in major depression (MDD). The proposed mechanism of action is that the epithelial barrier of the GI tract is compromised as a result of psychological or organic stress, leading to increased intestinal permeability and the consequent translocation of gram-negative bacteria across the mucosal lining to sites where direct interaction with immune cells and the ENS can occur (Gareau et al. 2008). This leads to activation of an immune response characterised by increased production of inflammatory mediators such as IL-6 and IFNγ. In patients with MDD, serum concentrations of IgM and IgA against LPS of enterobacteria was significantly higher than that of healthy controls (Maes et al. 2008). In support of this hypothesis, pre-treatment with the probiotic *L. farciminis* attenuated the effects of acute restraint stress on intestinal permeability and HPA axis responsivity (Ait-Belgnaoui et al. 2012).

Taken together, these data give credence to the view that microbiota, and specific profiles of biodiversity in the gut, significantly influence behavioural, neurochemical and immunological measures that are relevant to stress-related psychiatric disorders and that "psychobiotics" may emerge to treat such ailments (Dinan et al. 2013) (Figs. 1, 2).

## Irritable bowel syndrome (IBS)

In addition to psychiatric disorders, stressful life events are also a predisposing factor for GI disorders. IBS is a common functional gut disorder with an estimated prevalence of 10–20 % (Longstreth et al. 2006). Symptoms include

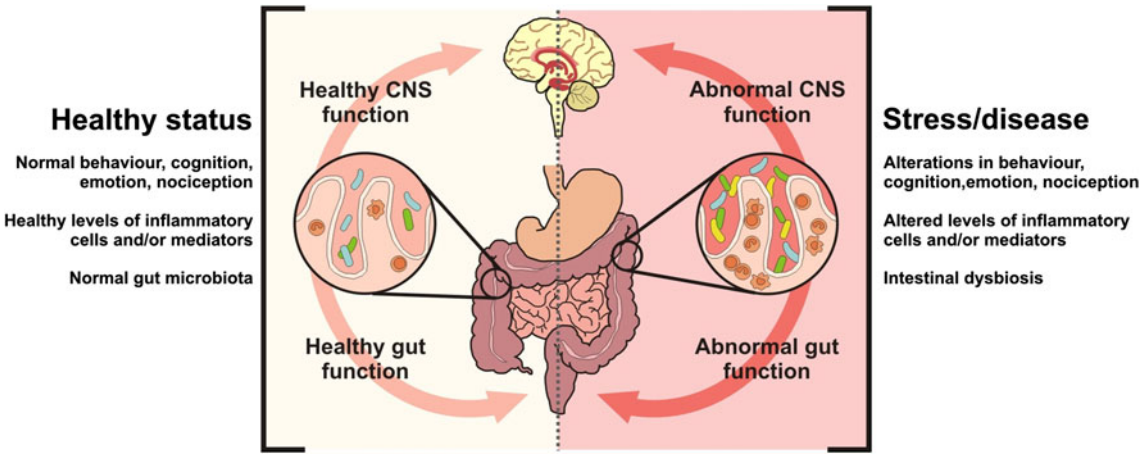


**Fig. 1** Microbiota–brain–gut axis communication in health and disease. A stable gut microbiota is essential for normal gut physiology and contributes to appropriate signaling along the microbiota–brain–gut axis and to the healthy status of the individual. Conversely, intestinal dysbiosis can adversely influence gut physiology leading to inappropriate brain–gut axis signaling and associated consequences for CNS functions and disease states. Stress at the level of the CNS can also impact on gut function and lead to perturbations of the microbiota. Adapted from Grenham et al. (2011)

abdominal pain, altered stool consistency and frequency, bloating and distension. Patients with this disorder experience impaired quality of life, and with consequent increases in work absenteeism and greater demands on health care services, IBS incurs significant costs to society (Hillila et al. 2010). Recent years has seen some progress in our understanding of the underlying pathology of this disorder with wider recognition of the importance of brain–gut communication in the aetiology of IBS (Collins and Bercik 2009). Altered CNS control of visceral pain and inflammatory responses are now considered as integral pathophysiological features. Despite these advances, our current understanding of the pathogenesis of IBS is unsatisfactory. Reliable biomarkers and biological explanations are lacking and hinder the discovery of effective therapies for this disorder (Clarke et al. 2009). The involvement of the CNS and abnormal brain function to disease pathology and to core symptoms in IBS has been confirmed by recent neuroimaging studies that demonstrated thinning in the anterior cingulate and insular cortex in these patients (Davis et al. 2008). Heightened activation of specific brain regions including the thalamus, anterior cingulated and prefrontal cortex was observed in sufferers of IBS relative to healthy controls has also been demonstrated and correlates with symptoms of visceral hypersensitivity (Mertz et al. 2000).

The onset of IBS symptoms after a bout of gastroenteritis or the occurrence of enteric infection represents one of the strongest points of evidence highlighting the importance of gut microbiota in IBS (Spiller and Garsed 2009). Chronic low-grade inflammation is a common feature in many IBS patients and studies have identified

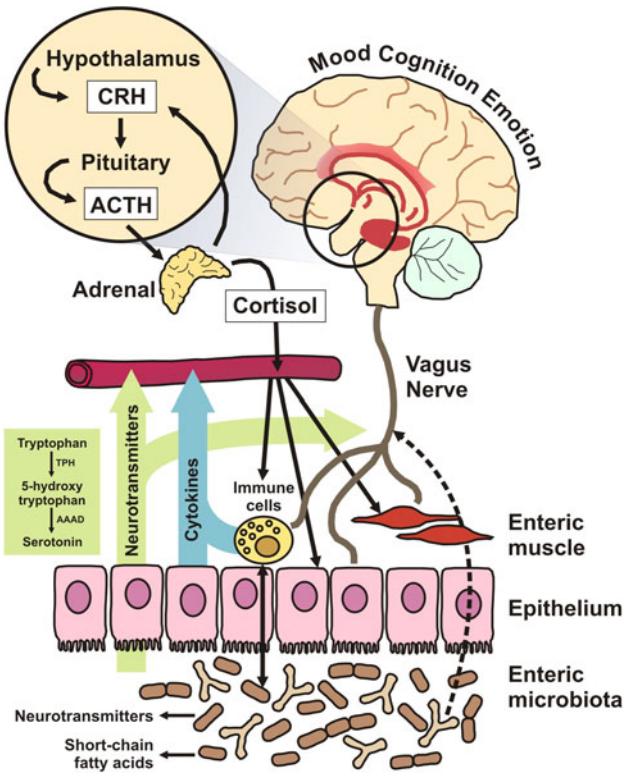


**Fig. 2** Routes of communication along the Microbiota–brain–gut axis. There has been a plethora of proposed mechanisms, including both immune and neural routes, through which the microbiota can modulate signaling along the brain–gut axis, some of which have been summarized in this figure. Adapted from Grenham et al. (2011)

several susceptibility genes for IBS involved in the innate immunity and recognition of bacteria (Zucchelli et al. 2011). It has also been demonstrated that subgroups of IBS

**Table 2** Human studies of microbiota–brain–gut axis

| Group | Sample, method | Diagnostic criteria and subjects | Finding | References |
|---|---|---|---|---|
| Autism | Fecal microbiota, culture | Autistic ($n = 58$), HS ($N = 39$) | Decreased *Bifidobacteria, enterococcus in autistic children*<br>Increased *Lactobacillus* spp. (all strains) in autistic children | Adams et al. (2011a) |
| | Morning blood and urine samples, spectrophotometry, liquid chromatography, Microbiological Assay | Autistic ($n = 55$), HS ($N = 44$) | Low levels of biotin, plasma glutathione, RBC SAM, plasma uridine, plasma ATP, RBC NADH, RBC NADPH, plasma sulfate (free and total), and plasma tryptophan;<br>High levels of oxidative stress markers and plasma glutamate | Adams et al. (2011b) |
| | faecal microbiota, Q-PCR | Autistic ($n = 15$), HS ($n = 8$) | Increased Clostridium bolteae and Clostridium clusters I and XI in autistic children<br>Decreased Clostridium cluster XIVab in autistic children | Desbonnet et al. (2013) |
| | Faecal microbiota, Gastric and duodenal sampling, 16S rRNA gene sequencing and culture | Autistic ($n = 13$), HS ($n = 8$) | Children with autism had nine species of *Clostridium* not found in controls<br>Control children had three species of *Clostridium* not found in autistic children<br>No non-spore-forming anaerobes and microaerophilic bacteria from controls<br>Significant numbers of non-spore-forming anaerobes and microaerophilic bacteria in children with autism | Finegold et al. (2002) |
| | Faecal microbiota, Pyrosequencing | Autistic ($n = 33$)<br>Non-affected siblings ($n = 7$), HS ($n = 8$) | Increased diversity and richness in the autistic GI microbiome<br>Increased *Bacteroidetes* in the severely autistic group<br>Increased *Firmicutes* in the control group<br>Increased *Desulfovibrio* species and *Bacteroides vulgatus* autistic children | Finegold et al. (2010) |
| | Faecal microbiota, bacterial tag-encoded FLX amplicon pyrosequencing | Autistic without GI dysfunction ($n = 23$), Autistic with GI dysfunction ($n = 28$) and their neurotypical siblings ($n = 53$) | Results did not indicate clinically meaningful differences between groups | Gondalia et al. (2012) |
| | Faecal microbiota, 16S rRNA amplicon pyrosequencing | Autistic children ($n = 15$), HS ($n = 7$) | Decreases in *Bacteroidetes*, increases in the ratio of *Firmicutes* to *Bacteroidetes*, and increases in *Betaproteobacteria* | Williams et al. (2011) |
| Preterm Infants | Faecal microbiota, 16S rRNA amplicon pyrosequencing | Preterm infants ($n = 10$) | *Proteobacteria* (46 %), *Firmicutes* (45 %), *Actinobacteria* (2 %) and *Bacteroidetes*(7 %) were detected at much lower levels at week 2<br>Lack of detectable *Bifidobacterium* and *Lactobacillus* genera<br>Large interindividual variation | Barrett et al. (2013) |

**Table 2** continued

| Group | Sample, method | Diagnostic criteria and subjects | Finding | References |
|---|---|---|---|---|
| Elderly | Faecal microbiota, 16S rRNA amplicon pyrosequencing | Adults >65years ($n = 161$), Adults <65years ($n = 9$) | In 68 % of the individuals, the microbiota was dominated by phylum *Bacteroides*, with an average proportion of 57 %<br>Phylum *Firmicutes* had an average proportion of 40 %<br>Proportions of some phyla and genera varied dramatically, including *Proteobacteria*, *Actinobacteria*, and *Faecalibacteria* | Claesson et al. (2011) |
| | Faecal microbiota, 16S rRNA amplicon pyrosequencing | Adults >65years ($n = 178$) | Microbiota of people in long-stay care was significantly less diverse than that of community dwellers<br>Data indicate relationship between diet, microbiota and health status | Claesson et al. (2012) |
| IBD | Faecal microbiota, FISH adapted to flow cytometry | Active CD ($n = 13$), Active UC ($n = 13$), IC ($n = 5$), HS ($n = 13$) | *Clostridium coccoides* was reduced in UC<br>*C. leptum* group was reduced in CD<br>*Bacteroides* group was more abundant in IC | Deng et al. (2012) |
| | Faecal microbiota, 16S rRNA DGGE analysis | Active CD ($n = 5$) Inactive CD ($n = 11$), HS ($n = 18$) | Decreased temporal stability of dominant species for all Crohn's disease patients<br>*Bifidobacterium* spp. were similar in all samples<br>*Clostridiales* and *Bacteroidales* communities are altered in Crohn's disease | Hakem et al. (2011) |
| | Faecal microbiota, DGGE, Q-PCR | CD ($n = 68$)<br>Unaffected relatives ($n = 84$), HS ($n = 55$) | Decreased *Dialister invisus*, an uncharacterized species of *Clostridium* cluster XIVa, *Faecalibacterium prausnitzii* and *Bifidobacterium adolescentis* in CD patients compared with unaffected relatives<br>Increased *Ruminococcus gnavus* in CD patients compared with unaffected relatives<br>Decreased *Collinsella aerofaciens* and member of the *Escherichia coli–Shigella* group in unaffected relatives compared with HS<br>Increased *Ruminococcus torques* in unaffected relatives compared with HS | Weberpals et al. (2011) |
| | Ileal and rectal biopsies, T-RFLP analysis16S rRNA gene, Q-PCR | Monozygotic twin pairs that were discordant ($n = 6$) or concordant ($n = 4$) for CD, HS ($n = 6$) | Predominantly ileal CD vs. co-twins and CD localized in the colon<br>Decreased *Faecalibacterium prausnitzii*<br>Increased *Escherichia coli* | Squires et al. (2011) |
| | Mucosal biopsies, 16S rRNA gene sequencing | Inflamed and non-inflamed intestinal tissue from 6 CD ($n = 12$), 6 UC ($n = 12$), HS ($n = 5$) | Decreased mucosal microbial diversity in IBD<br>Decreased *Firmicutes* in IBD samples and increased *Bacteroidetes*<br>Increased *Enterobacteriaceae* in CD only significant differences in microbial community structure between inflamed and non-inflamed mucosal sites | Toorop et al. (2012) |
| | rRNA sequence analysis and Q-PCR | UC ($n = 61$), CD ($n = 68$) HS ($n = 61$) | Decreased *Bacteroidetes* and *Lachnospiraceae* in IBD<br>Increased *Actinobacteria* and *Proteobacteria* in IBD | Bostrom et al. (2012) |

**Table 2** continued

| Group | Sample, method | Diagnostic criteria and subjects | Finding | References |
|---|---|---|---|---|
| IBS | Faecal microbiota, 16S rRNA amplicon pyrosequencing | IBS Rome II ($n = 37$, IBS-A (12), IBS-D (15), IBS-C (10)), HS ($n = 20$) | Increase of *Firmicutes*-associated taxa and a depletion of *Bacteroidetes*-related taxa | Jeffery et al. (2012) |
| | Faecal microbiota, 16S rRNA gene clone library sequencing | IBS Rome II ($n = 12$, IBS-D (2)), HS ($n = 22$) | IBS-D patients were enriched in *Proteobacteria* and *Firmicutes*, but reduced in the number of *Actinobacteria* and *Bacteroidetes* compared to control<br>In particular, 16S rDNA sequences belonging to the family Lachnospiraceae within the phylum Firmicutes were in greater abundance in the IBS-D clone library | Krogius-Kurikka et al. (2009) |
| | Faecal microbiome, Q-PCR | Rome II and III, $n = 26$ IBS-D ($n = 8$) IBS-C ($n = 11$) IBS-A ($n = 7$) | Increased *Veillonella* and *Lactobacillus* in IBS | Tana et al. (2010) |
| | Fecal microbiome, DGGE | Rome II, $n = 47$ No sub-typing | Significant difference between IBS and healthy controls<br>Significantly more variation in microbiota of healthy volunteers than that of IBS patients | Codling et al. (2010) |
| | Faecal and duodenal microbiome, FISH | Rome II, $n = 41$, IBS-D ($n = 14$) IBS-C ($n = 11$) IBS-A ($n = 16$) | Decreased *Bifidobacteria* in IBS subjects compared to healthy controls | Smith et al. (2012) |
| | Faecal microbiome, Q-PCR | Rome II, $n = 27$, IBS-D ($n = 12$), IBS-C ($n = 9$), IBS-A ($n = 6$) | Decreased *Lactobacillus* spp. in IBS-D subjects<br>Increased *Veillonella* spp. in IBS-C | Kasprowicz et al. (2011) |
| | Faecal microbiome, Culture/DGGE | Rome II, $n = 26$, IBS-D ($n = 12$), IBS-C ($n = 9$), IBS-A ($n = 5$) | Increased number of aerobes in IBS patients<br>Temporal instability in IBS patients revealed by DGGE | Dooley et al. (2011) |
| | Faecal microbiome, Q-PCR, Phylogenic Microarray, | Rome II, $n = 62$ IBS-D ($n = 25$), IBS-C ($n = 18$), IBS-A ($n = 19$) | Increased ratio of the *Firmicutes* to *Bacteroidetes* in IBS<br>Increase in numbers of *Dorea*, *Ruminococcus*, and *Clostridium* spp. in IBS<br>Decreased *Bacteroidetes* in IBS<br>Decreased Bifidobacterium and Faecalibacterium spp.<br>Decreased average number of methanogens in IBS | Woods and Squires (2011) |
| | Fractionation/16S rRNA gene cloning and sequencing, Q-PCR, faecal microbiome | Rome II, $n = 24$, IBS-D ($n = 10$), IBS-C ($n = 8$), IBS-A ($n = 6$) | Significant differences in *Coprococcus*, *Collinsella*, and *Coprobacillus* Phyla in the IBS group compared to controls | Pickett et al. (2012) |
| Healthy subjects | | Healthy subjects ($n = 12$ probiotic, $n = 11$, control) | Probiotic reduced the response of healthy volunteers to an emotional faces attention task | Tillisch et al. (2013) |

**Table 2** continued

| Group | Sample, method | Diagnostic criteria and subjects | Finding | References |
|---|---|---|---|---|
| European/ African | Faecal microbiome, high-throughput 16S rDNA sequencing | Children (European/rural African) | African children showed a significant enrichment in *Bacteroidetes* and depletion in *Firmicutes*<br>African children had a unique abundance of bacteria from the genus *Prevotella* and *Xylanibacter*<br>Significantly more short-chain fatty acids in African than in EU children.<br>Also, *Enterobacteriaceae* (*Shigella* and *Escherichia*) were significantly underrepresented in African than in EU children | De Filippo et al. (2010) |

patients may have an altered microbiota composition relative to healthy individuals, mainly based on the analysis of faecal microbiota (Salonen et al. 2010; Tana et al. 2010). Both an increase (Rajilic-Stojanovic et al. 2007) and a decrease (Codling et al. 2010) in the diversity of the microbiota have been reported in IBS patients (Claesson et al. 2012). Either way, the abnormal variation likely reflects a loss of homeostasis, in which the bacterial community is unable to maintain its normal composition. Although the specific mechanisms by which changes in the gut microbiota lead to IBS symptoms remain unclear, it is hypothesized that higher numbers of microbes such as *Lactobacilli* and *Veillonella* spp. in IBS patients result in a high level of organic acids such as acetic and propionic acid, which in turn may contribute to abdominal pain, bloating, anxiety and poor quality of life (Tana et al. 2010). In light of these promising preliminary findings, it is not surprising that a positive effect of treatment with microbial-based therapeutics (both non-absorbable antibiotics and probiotics) has been demonstrated in IBS (Clarke et al. 2012; Moayyedi et al. 2010; Parkes et al. 2010; Pimentel and Lezcano 2007; Saulnier et al. 2013). For example out of 42 clinical trials 34 have reported beneficial effects of lactic acid bacteria in at least one of the symptoms examined in IBS patients (Clarke et al. 2012) and reaffirms the relevance of gut microbiota and it's compositional changes in IBS. However, inconsistencies in trial design including dosage, probiotic strain selection, and sample size need to be addressed in future clinical studies so that a true evaluation of probiotics for the treatment of IBS can be made (Table 2; Figs. 1, 2).

## Autism

Autism spectrum disorder (ASD) is the collective term used for a diverse group of neurodevelopmental conditions broadly defined by deficits in three behavioural domains: social interaction, communication and the presence of limited, repetitive stereotyped interests and behaviours (Happe et al. 2006). While genetics play a major role in the aetiology of ASD, recent years has seen an emerging interest in the potential role of environmental factors in this disorder (Grabrucker 2012). Amongst the associated environmental risk factors, GI abnormalities and altered microbiota composition have been identified in a number of small-scale studies on children with ASD (Finegold et al. 2002; Finegold et al. 2010; Williams et al. 2011) that directly correlate with symptom severity (Adams et al. 2011a, b). However, there is much controversy in the field and varying results have emerged. Whereas increases in *Bacteroidetes* sp. and decreases in *Firmicutes* sp. has been reported in autistic children presenting with GI symptomology (Finegold et al. 2010), examination of the faecal

flora in a similar cohort revealed no differences in microbiota composition relative to neurotypical sibling measures (Gondalia et al. 2012).

Interpretation of results from these studies is complicated by the knowledge that individuals suffering from ASD have high rates of antibiotic usage and consume diets which often differ from those of healthy populations and may account for reported microbial changes (Cryan and Dinan 2012). For example, it is estimated that upwards of 90 % of children with ASD experience some type of feeding related concern (Ledford and Gast 2006) with food selectivity and preference for starches and processed foods being the most predominant food-related issue in ASD (Sharp et al. 2013).

On the other hand, a parent survey indicating that children who are not breast-fed are at higher risk of developing ASD, suggests that diet-related factors with the capacity to alter gut microbiota composition at a very early age are more likely to play a direct causative role in ASD (Schultz et al. 2006). Other lines of evidence which lend credence to the concept of altered microbiota–brain–gut communication in ASD include studies that show transient improvements of symptoms in regressive onset autism following oral treatment with vancomycin, a minimally absorbed antibiotic that targets gram positive anaerobes in the gut (Sandler et al. 2000). Additionally, altered faecal concentrations of short-chain fatty acids, which are neuroactive microbial fermentation products, has also been reported in ASD (Wang et al. 2012). Of note is that administration of propionic acid, a short-chain fatty acid, to animals via the intracerebroventricular route results in some autistic-like behaviours, albeit it at high doses that might not reflect the clinically observed alterations (MacFabe et al. 2011).

To date, this area of research had not been extensively explored in the preclinical field. Nevertheless, a recent study conducted in GF mice demonstrated robust and reproducible social deficits characterised by social avoidance and deficits in social cognition in addition to increases in repetitive grooming behaviours in these microbiota-depleted mice when compared to mice with conventional bacterial colonisation (Desbonnet et al. 2013). Interestingly, reconstitution of microbiota from weaning onwards normalised social interest in GF mice but had no effect on social cognition in the 3-chambered social test, indicating that the adolescent period is particularly important in the programming of specific aspects of normal social behaviour.

In general, these studies provide promising evidence indicating a more direct and central role for the gut microbiota in the pathogenesis of ASD than previously considered. This is an area of research that has received greater attention in the field of autism in recent years and will without doubt generate more interest and fruitful results in the coming years that may impact on treatment strategies in ASD (de Theije et al. 2011; Louis 2012; Mulle et al. 2013) (Table 2) (Fig. 1).

## Metabolic disorders (obesity/diabetes)

Obesity, a condition characterised by metabolic imbalance, has steadily increased over the past 50 years to reach epidemic proportions in the developed world and is mainly attributable to the increased consumption of carbohydrates and fats and reduced physical activity associated with westernised lifestyles. Changes in the composition of gut microbiota have been reported in obese humans and mice with a relative decrease in the proportion of *Bacteroidetes* in obese versus lean individuals (Ley et al. 2005, 2006; Turnbaugh et al. 2009) suggesting a potential link between microbial diversity and obesity. Therefore, although composition of the gut microbiota varies between individuals, a "core gut microbiome" has been identified that confers greater risk of obesity (Turnbaugh and Gordon 2009). Indeed, it is known in the agricultural domain that subtherapeutic doses of antimicrobial substances administered to farm animals increases body weight by as much as 15 % (Ozawa 1955). More recent work by our own group has identified that anti-psychotic-induced metabolic dysfunction is also associated with an altered microbiota profile (Davey et al. 2012, 2013). In mice, 7 week exposure to low doses of the antibiotics induced taxonomic changes in the microbiome characterised by reductions in the *Bacteroidetes*/*Firmicutes* ratio, which resembles the microbiota profile in obese humans, and was paralleled by increased body fat (Cho et al. 2012). Further support for a causal link between microbiota and obesity is provided by GF mouse studies where GF mice have reduced total body fat relative to their conventionally reared counterparts, and are resistant to diet-induced obesity (Backhed et al. 2007; Rabot et al. 2010). Interestingly, colonisation of GF mice with gut microbes harvested from conventionally-reared mice produces a 60 % increase in body weight within 2 weeks despite decreases in food consumption (Backhed et al. 2004). Probiotic treatment has also been demonstrated to have beneficial anti-obesity effects in both mouse and human studies (Arora et al. 2013). A single dose of *Lactobacillus plantarum* WCFS1 in GF mice maintained on high-fat/high-sugar diet resulted in upregulation of genes involved in carbohydrate transport and metabolism (Marco et al. 2009). Similarly, the presence of the probiotic *Bifidobacterium longum* elicits an expansion in the diversity of polysaccharides targeted for degradation by a prominent component of the adult human gut microbiota, *Bacteroides thetaiotaomicron*, when simultaneously colonised in GF mice (Sonnenburg et al. 2006). A recent study has demonstrated the ability of a specific bacterial strain (Bif

Infantis 35624) to exert an immunomodulatory effect beyond the gut (Groeger et al. 2013). Moreover, it has also demonstrated that treatment with vancomycin can improve metabolic abnormalities associated with obesity (Murphy et al. 2013).

A link between the metabolic disease, diabetes, and bacterial populations in the gut has also been established. Specifically, the proportions of *Firmicutes* and class *Clostridia* were significantly reduced in the diabetic group compared to the control group (Larsen et al. 2010). More recently a correlation between gut microbiome profiles and type 2 diabetes was demonstrated in a Chinese population to the extent that composition of the gut microbiota could predict the disorder in a second cohort (Qin et al. 2012).

There are a number of mechanisms proposed to underlie microbiota modulation of metabolic activity and homeostasis in the host. Certain microbial populations secrete enzymes, not encoded in the human genome, that allow extraction of calories from otherwise indigestible polysaccharides in our diet (Backhed et al. 2005). The consequent increase in bacterial fermentation products, short chain fatty acids (SCFAs) which include acetate, propionate and butyrate influence various aspects of metabolism. The latter SCFA is an energy substrate for cellular metabolism in the colonic epithelium, while acetate and propionate are substrates for gluconeogenesis and lipogenesis in the liver and peripheral organs and through these processes coordinate and promote lipid storage in the host (Backhed et al. 2004). The microbiota has been shown to impact bile acid production, insulin resistance, glucose metabolism and inflammation, processes that contribute to metabolic disease (Tremaroli and Bäckhed 2012).

It is clear from animal studies that disruption of the symbiotic relationship between gut microbes and the host impacts on metabolic homeostasis and contributes in some form to metabolic disorders. Nevertheless, data from clinical studies are more difficult to interpret due to confounding factors including the inclusion of participants from different ethnic origins with associated differences in dietary habits and antibiotic use. Despite these difficulties, the identification of predictive biomarkers for metabolic disease and a core healthy microbiome may pave a path for the development of preventive treatments that target specific microbe populations in obesity and diabetes (Fig. 1).

## Implications and future perspectives

Although this field of research is still in its infancy, evidence emerging from antibiotic, probiotic, infection and GF animal studies strongly suggest that the microbial community residing in the gut plays a key role in the development of various aspects of brain function including anxiety, mood, cognition and more recently in sociability. Whereas some of these behaviours are affected to a greater extent by microbiota disturbances in the early pre-weaning period, adolescence seems to be a more critical time for microbiota modulation of behaviours including anxiety (Foster and McVey Neufeld 2013) and sociability (Desbonnet et al. 2013). In relation to the potential involvement of the microbiome in neurodevelopmental disorders such as autism, and stress-related disorders whereby environmental events in early postnatal life have a crucial bearing on whether brain development is steered along a pathological course, the establishment of periods of vulnerability for the microbiota–brain–gut axis is an important goal. Not only is this crucial to our understanding of the temporal evolution of the composition and diversity of the microbiota and microbial-host interactions, but it will also identify potentially important windows of opportunity in which restoration of the "normal" core microbiota may have therapeutic value in specific psychiatric disorders.

Of course there is much controversy and debate over whether a core healthy microbiota profile exists. Metagenomics, a field of research that applies high-throughput screening techniques to characterise the microbial community at a genome-level in health and disease (Tang and Ho 2007), has undergone revolutionary advancements in recent years and further efforts in this area will undoubtedly shed more light on the composition, diversity and functions of the human microbiome. If indeed a specific combination of bacteria exists that imparts optimal health benefits to the host, this knowledge will open up new doors of opportunity for preventive, diagnostic and therapeutic approaches in disorders of microbiota–brain–gut dysregulation such as those discussed in this chapter. Already there has been some progress in faecal transplant strategies where clinical studies have provided convincing evidence of the benefits of faecal transplants from "healthy" donors in patients with *Clostridium difficile* infection, and also in gut and metabolic disorders (Borody and Khoruts 2012; Damman et al. 2012; van Nood et al. 2013). However, the potential effects of faecal microbiota therapy in CNS disorders with microbiota-related dysfunction have not yet been explored clinically. Well-designed and well-executed randomized trials are now needed to further define these microbiota-related conditions and assess the potential merits of faecal transplants in neurodevelopmental and mood disorders. However much more work is required prior to this procedure being advocated as a treatment and approval by regulatory bodies remains an obstacle, even in other domains where considerable evidence exists for its utility. We must first determine which bacterial populations in the gut microbiota are altered in CNS disorders and with the use of animal models will be able to assess which bacterial populations in the gut microbiota are indeed beneficial to the host. This will aid future probiotic and

prebiotic driven research which many may perceive as a more acceptable form of treatment than faecal transplantation. Indeed, recent findings point to a therapeutic role of prebiotics in both colon cancer and IBD via increased populations of *Bifidobacteria* and *Lactobacillus* in the gut. Although positive reports are emerging for the use of prebiotics more work is needed before any meaningful conclusions can be drawn. (Gibson and Roberfroid 1995; Savignac et al. 2013; Van Loo 2004).

Cognition, which is a general term used to describe thought processes that contribute to learning and memory, is affected in a number of CNS disorders including depression, schizophrenia, autism and Alzheimer's disease. Despite the considerable progress in our understanding of the neurochemical and neuroanatomical correlates of cognitive processes in the brain (Kandel 2012; Squire and Wixted 2011), cognitive deficits remain one of the most difficult symptom categories to address in patients due mainly to the lack of efficacious drug treatments. It is a widely acknowledged fact that chronic or uncontrollable stress experiences in early life strongly influence development of neuronal pathways involved in cognition, and negatively impact on cognitive function in later life (Hedges and Woon 2011) to date, very little is known about the potential contributions of the microbiota to cognitive development. It has been shown that non-spatial memory and social cognition (the ability to distinguish between a novel and previously-encountered mouse) is impaired in GF mice (Desbonnet et al. 2013; Gareau et al. 2011) suggesting that some facets of memory depend on the presence of gut microbiota in early life. In the same vein, supplementation with probiotic bacteria in rodents enhances memory for fear-related contexts (Bravo et al. 2011) and reversed the memory deficits observed in infected *C. rodentium*-infected mice following acute stress (Gareau et al. 2011). These preliminary findings suggest that microbiota may influence cognition and this potential relationship is fast becoming a topic of interest in IBS, a disorder associated with varying degrees of cognitive impairment (Kennedy et al. 2012). Further investigation into the effects of microbiota, antibiotic and probiotic therapy on cognitive performance in both the clinical and preclinical domain are warranted.

Another important feature of psychiatric conditions is the different prevalence rates reported in males and females. For instance, whereas autism is more common in males (4:1 male to female ratio; Fombonne 2005), depression and anxiety are more prevalent in females (Nolen-Hoeksema et al. 1999). To date, only a handful of studies have focused on how sex differences may influence microbiota–brain communication in the context of brain development (Bravo et al. 2011; Clarke et al. 2013b) with the most robust changes in neurochemistry and behaviour observed in males. Interestingly, the social deficits and changes in hippocampal neurobiology (serotonin, brain-derived neurotrophic factor) observed in GF male mice (Clarke et al. 2013b; Desbonnet et al. 2013) is in line with the neuropathology and symptoms observed in autism (Bethea and Sikich 2007). Further studies are required to identify and explore sex-related factors underlying the divergent outcomes in brain neurochemistry and function as a result of microbiota–host interactions in males and females. Establishing the underlying causes of sex differences in microbiota–brain–gut communication may provide key insights into the pathogenesis of these disorders. However, caution is advised when attempting to translate sex-specific preclinical findings to clinical populations.

Finally, the vast majority of studies focusing on the contributions of microbiota and probiotics to brain development and function have been in the preclinical domain, and future work to assess whether this role played by microbiota can be translated to the clinic is a vital next step to progress this field of research. The emerging evidence implicating microbial dysbiosis in stress-related disorders such as depression and in neurodevelopmental disorders is promising but requires validation by way of investigation in the clinical setting. The characterisation of the microbiome using advanced metagenomic and metaproteomic technologies in depressed patients, in addition to assessment of the potential for probiotic treatment and faecal transplant to alleviate the core symptoms of this disorder is essential to justify more comprehensive investigations of specific mechanisms involved in communication, and indeed miscommunication, between microbiota and the host in CNS disorders.

## Conclusions

It would seem that the common saying "healthy in body, healthy in mind", while based on accurate biological principles that harmony of function between the peripheral organs of the body and the brain is important for health and wellbeing, never considered the myriad of other organisms on whose existence we depend for normal brain programming, including the development of brain processes and behaviours at the core of CNS disorders. Recent years has seen a steep rise in research relating to the role of the gut microbiota in brain function, and more importantly, in brain dysfunction, and consequently there is a growing appreciation of the importance of the gut microbiome in the pathogenesis of psychiatric disorders (Bravo et al. 2012; Cryan and Dinan 2012; Forsythe and Kunze 2013). Although several studies have shown that the microbiota influences aspects of behaviour and stress-responsivity that can contribute to psychopathology, particularly in genetically

vulnerable individuals, our understanding of the specific mechanisms involved in transmitting changes in the microbiota to central brain programming, is rudimentary and requires more intensive investigation. With rapid advancements in metagenomic techniques and the development of more cohesive collaborations between researchers in the relevant fields of neuroscience, microbiology, gastroenterology and psychiatry, this fast-evolving and exciting research domain will only expand to shed greater light on the microbiota–host relationship and ultimately create new avenues for microbial-based therapeutics that beneficially influence the CNS.

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