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Gut instincts: microbiota as a key regulator of brain development, ageing and neurodegeneration

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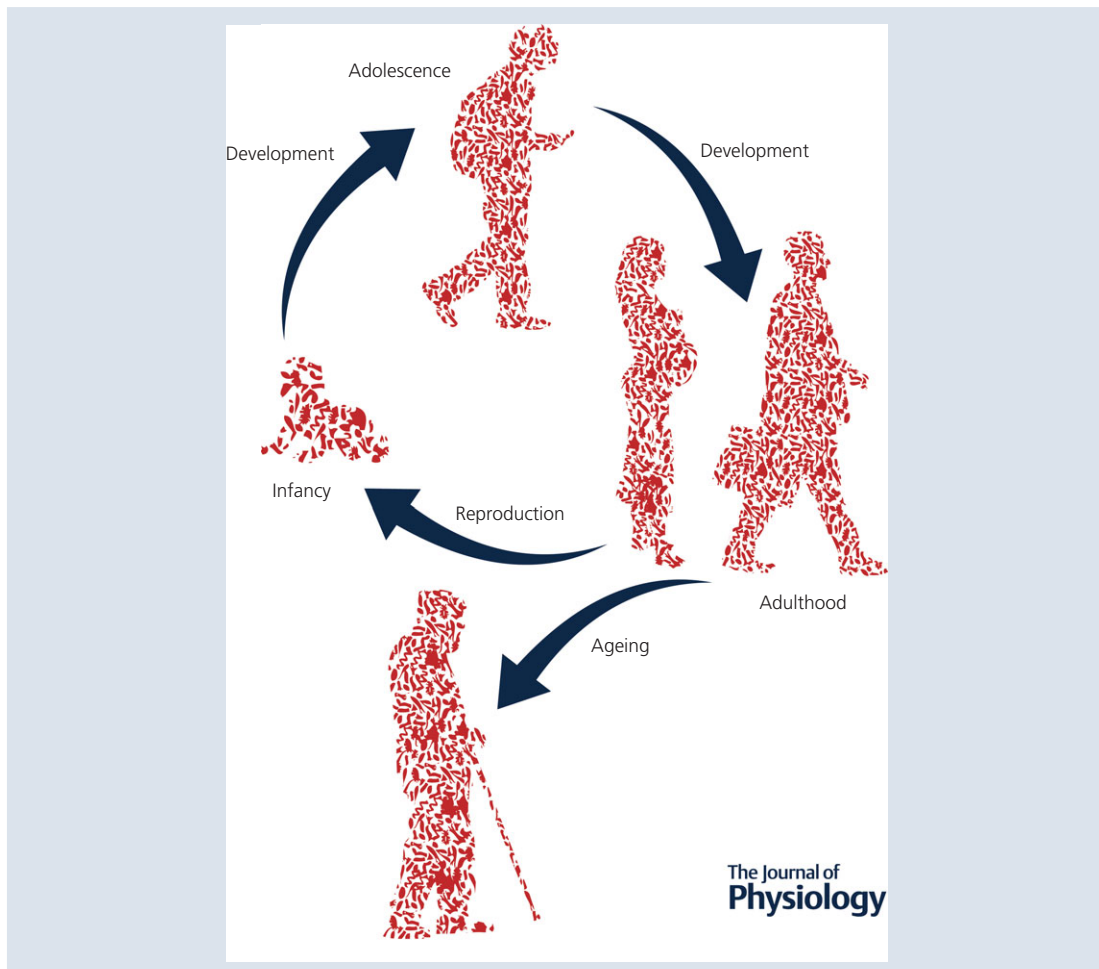
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Abstract   There is a growing realisation that the gut–brain axis and its regulation by the microbiota may play a key role in the biological and physiological basis of neurodevelopmental, age-related and neurodegenerative disorders. The routes of communication between the microbiota and brain are being unravelled and include the vagus nerve, gut hormone signalling, the immune system, tryptophan metabolism or by way of microbial metabolites such as short chain fatty acids. The importance of early life gut microbiota in shaping future health outcomes is also emerging. Disturbances of this composition by way of antibiotic exposure, lack of breastfeeding, infection, stress and the environmental influences coupled with the influence of host genetics can result in long-term effects on physiology and behaviour, at least in animal models. It is also worth noting that mode of delivery at birth influences microbiota composition with those born by Caesarean section having a distinctly different microbiota in early life to those born *per vaginum*. At the other extreme of life, ageing is associated with a narrowing in microbial diversity and healthy ageing correlates with a diverse microbiome. Recently, the gut microbiota has been implicated in a variety of conditions including depression, autism, schizophrenia and Parkinson's disease. There is still considerable debate as to whether or not the gut microbiota changes are core to the pathophysiology of such conditions or are merely epiphenomenal. It is plausible that such neuropsychiatric disorders might be treated in the future by targeting the microbiota either by microbiota transplantation, antibiotics or psychobiotics.

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Abstract figure legend   The developmental trajectory.

Introduction

Claude Bernard, William James, Ivan Pavlov and Walter Cannon, the fathers of modern physiology, have all described the bidirectional communication between the gut and the brain and its importance in maintaining homeostasis (Aziz & Thompson, 1998; Mayer, 2011). However, over the past decade increasing emphasis has been placed on the role of intestinal micro-biota in regulating the gut–brain axis. Concurrently, a growing body of evidence points to the microbiota playing a significant role in modulating brain function and behaviour and that the microbiota–gut–brain axis is poised as a bidirectional communication pathway enabling gut microbes to communicate with the brain, and the brain with the gut (Rhee *et al.* 2009; Canfora *et al.* 2015; Foster *et al.* 2016). The mechanisms of communication are complex and are slowly being unravelled; they include immune, neural, endocrine and metabolic pathways (Grenham *et al.* 2011; Mayer *et al.* 2014*a*; El Aidy *et al.* 2015; Koh *et al.* 2016; Sommer & Backhed, 2016). Pre-clinical studies have implicated the vagus nerve as a key route of neural communication between gut microbes and centrally mediated behavioural effects, as demonstrated by the prevention of the *in vivo* effects of specific bacterial strains by selective vagotomy (Bravo *et al.* 2011; Bercik *et al.* 2011*b*). The gut microbiota also regulates key central neurotransmitters by altering levels of precursors;

for example *Bifidobacterium infantis* has been shown to elevate plasma tryptophan levels and thus influence central 5-HT transmission (Desbonnet *et al.* 2008; O'Mahony *et al.* 2015*a*). Furthermore, synthesis and release of neurotransmitters from bacteria has been reported; the inhibitory neurotransmitter γ-aminobutyric acid (GABA) can be produced by *Lactobacillus* and *Bifidobacterium* species, whereas *Escherichia*, *Bacillus* and *Saccharomyces* spp. can produce noradrenaline (norepinephrine). On the other hand *Candida, Streptococcus, Escherichia and Enterococcus* spp. have been reported to produce serotonin, and *Bacillus* can produce dopamine, whereas certain *Lactobacillus* spp. can produce acetylcholine (Lyte, 2013, 2014; Wall *et al.* 2014). These microbially synthesised neurotransmitters can cross the mucosal layer of the intestines, and possibly mediate physiological events in the brain.

Short chain fatty acids (SCFAs), which include propionate, butyrate and acetate, are important metabolic products of gut microbial activity and may exert central effects indirectly or directly either through G-protein coupled receptors or in the case of butyrate as an epigenetic modulator acting through histone deacetylases (HDACs) (Galland, 2014; Stilling *et al.* 2014, 2016; Paul *et al.* 2015). Moreover, SCFAs are involved in a plethora of physiological functions ranging from energy balance and metabolism to the modulation of adipose tissue, liver tissue and skeletal muscle function (Canfora *et al.*

2015). Immune signalling from gut to brain mediated by cytokine molecules is another documented route of communication (El Aidy *et al.* 2014). The epithelial layer of the gut and its mucous layer have many functions, including the regulation of nutrient and fluid absorption from the gut lumen in addition to serving as a physical barrier from invading pathogens or harmful substances (Farhadi *et al.* 2003; Scaldaferri *et al.* 2012; Johansson *et al.* 2013; Kelly *et al.* 2015). Interactions between microbes and the immune system of the gut help the latter to identify self and non-self antigens and potentially harmful pathogens (Fasano, 2012; Sonnenberg & Artis, 2012; Kamada *et al.* 2013; Sommer & Backhed, 2013).

The hypothalamic–pituitary–adrenal (HPA) axis, which provides the core regulation of the stress response, can significantly impact the microbiota–gut–brain axis (O'Mahony *et al.* 2009, 2011, 2015*b*; Scott *et al.* 2013; Moloney *et al.* 2014; Tillisch, 2014; Wang & Kasper, 2014). It is increasingly clear and probably of relevance in a number of pathological conditions that psychological or physical stress can significantly dysregulate the microbiota–gut–brain, for example in irritable bowel syndrome (Dinan *et al.* 2006).

Multiple lines of approach have been used to interrogate the microbiota–gut–brain axis, especially in animal model systems; these include the use of microbiota deficient animals known as germ-free mice, specific bacterial species often incorrectly referred to as probiotics, antibiotics, animals exposed to pathogens and the use of stress to determine the effects of dysregulating the HPA axis. There is an emerging consensus, at least from animal studies, that the gut microbiota plays a pivotal role in regulating early brain development (Bercik *et al.* 2012; Collins *et al.* 2012; Mayer *et al.* 2014*a*; Borre *et al.* 2014; Sampson & Mazmanian, 2015). Determining the mechanisms and pathways underlying microbiota–brain interactions is an exciting and evolving area of research that may yield novel insights into individual variations and perhaps enable the development of new treatments for a range of neurodevelopmental and neurodegenerative disorders, ranging from autism to Parkinson's disease. Moreover, there is increased emphasis on understanding the factors contributing to healthy brain ageing and the microbiome is poised to play a crucial role here. Here we will review important recent findings in the field.

Maturation and decline of bacterial gut microbiota

It is generally estimated that the gut is inhabited by 10^{13}–10^{14} microorganisms, although the ratio of microbial to human cells has been recently revised downward (Sender *et al.* 2016). In terms of genes we have over 100 times as many genes in our microbiome as we have in our genome. The total weight of these gut microbes is 1–2 kg, which is similar to the weight of the human brain

(Stilling *et al.* 2014). Mammals have never existed without microbes, except in laboratory situations. The reality is that we have co-evolved, and we are fundamentally dependent upon our colonisers for survival, as of course are they on us (Bordenstein & Theis, 2015).

With over 1000 species and greater than 7000 strains the microbiota is an ecosystem dominated by bacteria, mainly strict anaerobes, but also includes other micro-organisms such as viruses and bacteriophages, protozoa, archaea and fungi (Lankelma *et al.* 2015). At a phylum level the microbiome is largely defined by two dominant bacterial phylotypes, Bacteroidetes and Firmicutes with Proteobacteria, Actinobacteria, Fusobacteria and Verrucomicrobia phyla present in relatively low abundance (Qin *et al.* 2010; Lankelma *et al.* 2015).

Colonisation of the infant gut for the most part begins at birth, when delivery through the birth canal exposes the infant to its mother's microbiota and thus initiates an initial maternal signature to the micro-biota (Donnet-Hughes *et al.* 2010; Collado *et al.* 2012; Matamoros *et al.* 2013; Backhed *et al.* 2015). The impact of mode of delivery, be it vaginal birth or Caesarean section (C. section) on neonatal microbial composition is now being appreciated. Indeed, C. section results in an altered microbial composition in the neonate resembling that of the mother's skin with *Staphylococcus*, *Corynebacterium* and *Propionibacterium* spp. dominating in comparison with vaginally delivered infants whose microbiome is more akin to that from the mother's vagina, which have a predominance of *Lactobacillus, Prevotella* or *Sneathia* spp. (Dominguez-Bello *et al.* 2010). Moreover, this signature has been shown to persist into adulthood with a distinctly different faecal microbiota composition being detected in those individuals who have been born by C. section (Goedert *et al.* 2014), compared with their natural born counterparts (but see Yassour *et al.* 2016). Significant differences in micro-biota composition have been described between preterm and normal-term neonates (Barrett *et al.* 2013, 2015). Barrett and colleagues (2013) showed that preterm infants, albeit just two infants, lacked two of the main bacterial genera seen in normal-term infants: *Bifidobacterium* and *Lactobacillus,* with a compensatory dominance of the Proteobacteria observed. It is worth noting that there is a growing appreciation of the connection between micro-bial composition and the nutritional needs of the infant host (Voreades *et al.* 2014). Indeed exclusively breastfed infants display an increase in the relative composition of *Bifidobacterium* species that have evolved specifically to utilise human milk oligosaccharides (Costello *et al.* 2012; Hinde & Lewis, 2015). Each diet induces a specialised microbiota with the capability of microbial digestion of that specific diet. Indeed, an increase in the relative composition of strict anaerobes as a function of diet and environment occurs early in life, and a complex

adult-like microbiome emerges by the age of 1 year old. Although inter-individual variations in the composition of gut microbiota is apparent, an internal balance exists that confers a propensity towards health benefits (Gilbert *et al.* 2016), whereas perturbations in this ecosystem has the potential to negatively impact health, increasing vulnerability to a range of diseases (Sankar *et al.* 2015; Gilbert *et al.* 2016). Infection, disease, diet and antibiotics are among the many factors that may change microbial composition (Borre *et al.* 2014). However, the composition tends to revert to its previous level of diversity once the distorting factor has subsided (Vandenplas, 2015). This is especially true in relation to antibiotic exposure (Blaser, 2014), but far less so in relation to dietary patterns which tend to be relatively permanent. Taken together it is clear that infancy is a critical period for both microbiota colonisation and neurodevelopment. Thus, the time is now ripe for longitudinal studies to assess the impact of altered microbiota composition in early life on neurocognitive function in humans (Yang *et al.* 2016).

Major shifts in diet in adults likewise show dramatic changes in microbiota composition (David *et al.* 2014). It is clear from studies of remote hunter-gatherer tribes that Western guts have undergone a significant reduction in bacterial diversity and globalisation is driving this trend forward (Leach, 2013; Schnorr *et al.* 2014; Clemente *et al.* 2015; Martinez *et al.* 2015; Rampelli *et al.* 2015; Sonnenburg *et al.* 2016).

As we age, it has been shown that the core microbiota undergoes a dynamic shift (Claesson *et al.* 2011). Such age-related changes in the composition, and in particular the diversity, of the microbiota correlate with health outcomes in the elderly, especially in the context of frailty measures (O'Toole & Jeffery, 2015; Zapata & Quagliarello, 2015). Of interest is the fact that the number of probiotic *Bifidobacteria* strains decreases with age (Rondanelli *et al.* 2015). Recent studies in centenarians have reinforced the importance of microbial diversity in maintaining health as we age (Biagi *et al.* 2016). Thus it is becoming clear that maintaining a healthy microbiome is crucial to having a healthy brain across the lifespan from cradle to grave (see Fig. 1).

Microbiota and brain development

Germ-free mice have been instrumental in highlighting a key role of the microbiota in early brain development (Gareau, 2014; Sampson & Mazmanian, 2015; Luczynski *et al.* 2016*a*). Key changes in multiple neurotransmitter systems and their receptors have been described in a variety of different brain regions in germ-free mice. Using a genome-wide transcriptomic approach Diaz Heijtz and colleagues (2011) demonstrated that germ-free mice have an upregulation of genes associated with a variety of plasticity and metabolic pathways including synaptic

long-term potentiation, steroid hormone metabolism, and cyclic adenosine 5-phosphate-mediated signalling. Of note, the cerebellum and hippocampus were the two most sensitised brain areas to such changes in gene expression, with the hypothalamus being relatively resistant. The serotonergic system is particularly vulnerable to early-life manipulations of the microbiome. We have shown that germ-free mice have a marked increase in 5-HT concentrations in the hippocampus (Clarke *et al.* 2013), whereas Neufeld and colleagues have shown a decreased 5-HT_{1A} receptor gene expression in the hippocampal dentate gyrus of female germ-free animals using *in situ* hybridisation (Neufeld *et al.* 2011). Brain-derived neurotrophic factor (BDNF) is an important plasticity-related protein that promotes neuronal growth, development and survival and plays a key role in learning, memory and mood regulation. In germ-free mice, *Bdnf* expression is lower in the cortex and amygdala compared with controls (Diaz Heijtz *et al.* 2011). In the hippocampus, the changes in *Bdnf* levels documented are inconsistent with some studies reporting an increase (Neufeld *et al.* 2011), whereas most others show a decrease in expression (Sudo *et al.* 2004; Diaz Heijtz *et al.* 2011; Gareau *et al.* 2011; Clarke *et al.* 2013).

Somewhat intriguingly, many of the CNS alterations, including changes in BDNF levels found in germ-free mice occur in a sex-specific manner with only male germ-free animals exhibiting the serotonergic alterations described above as well as the decrease in *Bdnf* expression (Clarke *et al.* 2013). Germ-free mice have been shown to have modest increases in hippocampal volume and the pyramidal neurons within the ventral hippocampus were shorter and less branched, and had deficits in the number of stubby and mushroom spines. On the other hand decreased branching was observed for dentate granule cells without any overt change at the spine density level in germ-free mice (Luczynski *et al.* 2016*b*). The birth of new neurons in the adult hippocampus, neurogenesis, plays a critical role in modulating learning and memory and in mediating the behavioural responses to stress and antidepressant drugs (O'Leary & Cryan, 2014). Recent data using germ-free animals have shown that neurogenesis is also regulated by the microbiome. Germ-free mice exhibit increased adult hippocampal neurogenesis in the dorsal hippocampus (Ogbonnaya *et al.* 2015). Post-weaning microbial colonisation of germ-free mice failed to reverse the changes in adult hippocampal neurogenesis, suggesting that a critical window exists in the pre-weaning period during which the microbiota exerts its influences on adult hippocampal neurogenesis (Ogbonnaya *et al.* 2015). Intriguingly antibiotic administration, which depletes the microbiota, to adult animals actually decreases neurogenesis. Moreover, this effect was reversed by exercise or administration of a probiotic cocktail (Mohle *et al.* 2016).

The effects of the microbiome on brain development are not specific to the hippocampus with data emerging that there are alterations in amygdala function as well. The amygdala is a critical brain area for social behaviour as well as being a key node for the gating of anxiety and fear-related behaviour (Ledoux, 2007; Stilling *et al.* 2015). Structural and functional changes in the amygdala are associated with a variety of neuropsychiatric disorders ranging from autism spectrum (Schumann & Amaral, 2006; Mosconi *et al.* 2009) to anxiety disorders (Ledoux, 2007; Janak & Tye, 2015). Germ-free mice have increased amygdala volume and have dendritic hypertrophy in the basolateral amygdala (BLA). Moreover, the pyramidal neurons of the BLA in germ-free mice have more thin, stubby and mushroom spines compared to mice with normal microbiota (Luczynski *et al.* 2016*b*). Using RNA-sequencing significant differences at the levels of differential gene expression, exon usage and RNA editing were found in the amygdala. The expression of immediate early response genes such as *Fos*, *Fosb*, *Egr*2 or *Nr*4*a*1 were increased in the amygdala of germ-free mice in concert with increased signalling of the transcription factor CREB in germ-free mice (Stilling *et al.* 2015). Moreover, differential expression and recoding of several genes involved in fundamental brain processes ranging from neuronal plasticity, metabolism, neurotransmission and morphology were identified. A significant downregulation was noted for immune system-related genes (Stilling *et al.* 2015), which is in line with the underdeveloped immune system and immature microglia reported in germ-free mice (Erny *et al.* 2015). This adds further evidence to a key role of the immune system in mediating the effects of the microbiota on brain physiology and behaviour. Indeed, the recently discovered lymphatic branches in the central nervous system may be one such mechanism (Louveau *et al.* 2015).

Another aspect of neurodevelopment shown to be critically regulated by the microbiome is prefrontal cortical myelination (Hoban *et al.* 2016). Germ-free mice have hypermyelination and increased expression of genes
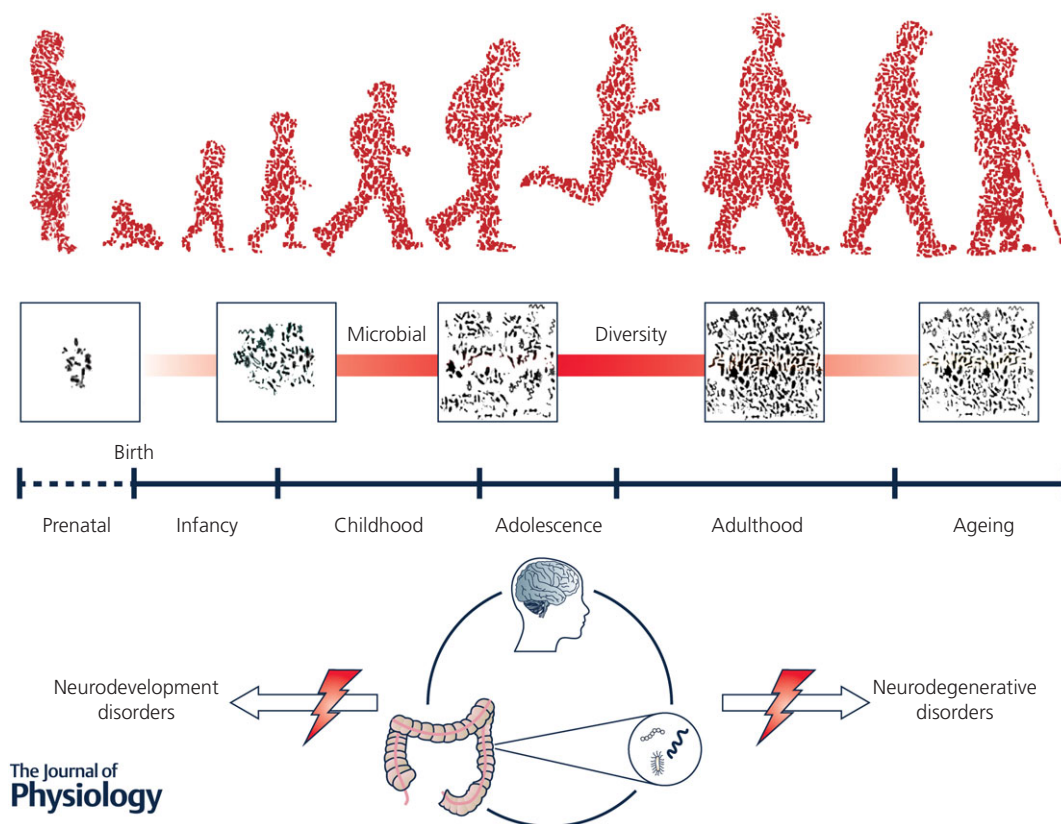

Figure 1. We are living in a microbial world throughout our lifespan
A growing body of evidence suggests that gut microbiota is essential to human health and is a key player in the bidirectional communication across the gut–brain axis. The microbiota dynamically changes across the lifespan, establishing its relationship with the host at critical windows during infancy, adolescence and ageing. At these time windows, there is an increased vulnerability to external insults, which may result in enhanced susceptibility to brain disorders. Early life disturbance of the developing gut microbiota has the potential to significantly impact on neurodevelopment and potentially lead to adverse mental health outcomes later in life. Similarly, the microbiota may contribute to the ageing process and the trajectory of neurodegenerative disorders.

involved in myelination and myelin plasticity processes in the prefrontal cortex but not other brain areas investigated (Hoban *et al.* 2016). Similar findings have also emerged in adult animals treated with antibiotics (Gacias *et al.* 2016).

It should be noted that although germ-free mice have been instrumental in advancing all aspects of microbiome research including microbiome-to-brain signalling (Grover & Kashyap, 2014; Luczynski *et al.* 2016*a*), there are many challenges in their use. This is especially true with regards to the marked alterations in the immune system and gastrointestinal tract and in terms of lacking any true clinical translation (Nguyen *et al.* 2015; Al-Asmakh & Zadjali, 2015; Arrieta *et al.* 2016). That said the preclinical use of germ-free mice is not to directly mimic the human condition but to provide a platform to explore the role of bacteria on host development and function and answer the question whether the microbiome is involved or not (Nguyen *et al.* 2015; Faith *et al.* 2014; Al-Asmakh & Zadjali, 2015; Arrieta *et al.* 2016). Nonetheless, germ-free mice have limited utility to address experimental questions regarding the impact of altered microbiota composition that first occurs later in life. Thus studies using antibiotic treatment have proven useful complementary alternatives. Antibiotic treatment has also been shown to affect other brain neurodevelopment processes. Indeed, antibiotic treatment in adult mice alters BDNF protein levels in both the amygdala and hippocampus (Bercik *et al.* 2011*a*). On the other hand administration of antibiotics early in life to male rats has been shown to increase visceral hypersensitivity in adulthood without affecting anxiety, cognitive, immune or stress-related responses (O'Mahony *et al.* 2014). The increased visceral hypersensitivity was paralleled by specific changes in the spinal expression of pain-associated genes (transient receptor potential cation channel subfamily V member 1, the α-2A adrenergic receptor and cholecystokinin B receptor) (O'Mahony *et al.* 2014). More recently we targeted the adolescent period in mice whereby many of the phenotypic changes in germ-free mice could be recapitulated by treating with antibiotics. Indeed, at a behavioural level, post-weaning depletion of the gut microbiota led to reduced anxiety coupled with cognitive deficits (Desbonnet *et al.* 2015). Moreover, at a neurochemical level in the adult brain altered tryptophan metabolism was observed coupled with significantly reduced BDNF, oxytocin and vasopressin expression in adolescent antibiotic-treated mice (Desbonnet *et al.* 2015).

Role for the microbiota in neurological conditions?

A growing body of evidence, originating with studies in irritable bowel syndrome, has implicated the microbiota–gut–brain axis in the pathogenesis of stress-related disorders (De Palma *et al.* 2014; Moloney *et al.* 2015). Recently, however, the gut microbiota

has been implicated in other brain disorders including autism, schizophrenia and Parkinson's disease. There is still considerable debate and much research needed to determine whether or not gut microbiota changes are core to the pathophysiology of such conditions or are merely epiphenomenal.

Autism. Autism spectrum disorder (ASD) is a neurodevelopmental disorder with a clear genetic basis whose prevalence is on the increase (Ziats *et al.* 2015). Autism is characterised by a constellation of symptoms including deficits in social behaviour, communication and interaction across multiple domains and repetitive, narrow repertoire of patterns of behaviour, interests, or activities (Chen *et al.* 2015). In general, symptoms emerge early in the developmental period and significantly impair social and occupational functioning. Although genetics is key in autism pathogenesis there is a very strong gene–environment interaction at play with over 50% of the neurobiology driven by non-heritable factors (Chen *et al.* 2015). Moreover, up to 70% of patients with the syndrome co-present with gastrointestinal symptoms and hence the view that a disruption of the gut–brain axis is involved (Mayer *et al.* 2014*b*).

Another facet contributing to social deficits in this condition is a lack of social recognition, a symptom which can be modelled in animals. Our group examined the behaviour of germ-free mice in the three-chamber test, a well-validated assay to assess social behaviour (Moy *et al.* 2004), where a germ-free mouse was placed in the centre chamber with a familiar mouse in one chamber and a novel mouse in the other chamber (Desbonnet *et al.* 2014). Interestingly, germ-free mice spent as much time with the familiar as with the novel mouse; this is in contrast to the behaviour of conventionally colonised mice who spend more time with the novel than the familiar mouse. Germ-free mice are more likely to spend time with an object or an empty chamber than with another mouse, a decidedly abnormal behaviour for a sociable animal. Colonisation of the germ-free mice does partially normalise their behavioural patterns especially in the context of sociability deficits and increased repetitive behaviours – hallmark traits of autism; however, social cognitive deficits remained despite colonisation (Desbonnet *et al.* 2014). These behavioural changes are also associated with significant alterations in underlying neurochemistry and gene expression (Stilling *et al.* 2015; Hoban *et al.* 2016). These findings of social deficits in germ-free mice have recently been replicated (Buffington *et al.* 2016) but opposite findings have also been reported (Arentsen *et al.* 2015).

Preclinical work demonstrated that the microbiota modulates behavioural and selective gastrointestinal abnormalities associated with autism and other neurodevelopmental disorders (Hsiao *et al.* 2013). The authors

used the maternal immune activation model induced by Polyinosinic-polycytidylic acid (poly-IC) injection during pregnancy and found altered gastrointestinal barrier defects and microbiota alterations. Dietary administration of the human commensal *Bacteroides fragilis*, given three times during adolescence, was sufficient to correct gut permeability as well as stereotyped and other abnormal behaviours. Interestingly, as in our germ-free studies following recolonisation (Desbonnet *et al.* 2014) social cognition deficits were insensitive to reversal by the bacteria. Furthermore, metabolomics approaches identified a number of bacteria-derived metabolites that may be involved in the autism-related behaviours and that were sensitive to manipulation by *Bacteroides fragilis*.

In humans, prenatal exposure to the mood stabiliser valproate is a major risk-factor for autism (Christensen *et al.* 2013). It is of interest that de Theije and colleagues have shown that the autism-like behavioural changes that occur in mouse models of valproate exposure are coincident with alterations in microbiota (de Theije *et al.* 2014). Sex differences were observed in these studies, which is in agreement with the clinical literature in autism (Young & Pfaff, 2014).

Clinically, a number of relatively underpowered studies investigating the faecal microbiota in patients with autism spectrum disorder have been reported (Mayer *et al.* 2014*b*; Rosenfeld, 2015). Tomova and colleagues examined the microbiota in Slovakian children and noted a significant decrease of the Bacteroidetes/Firmicutes ratio and increases in the amount of *Lactobacillus* spp. and a modest elevation in *Desulfovibrio* spp. in the faecal microbiota (Tomova *et al.* 2015). Administration of a probiotic diet was shown to normalise the Bacteroidetes/Firmicutes ratio and *Desulfovibrio* spp. levels in these children. In a recent study no significant difference in microbiota diversity or composition was detected between autistic children with their neurotypical siblings (Son *et al.* 2015). On analysis of the 16S rRNA sequencing data an increase in the low abundance *Chloroplast* genus was observed in ASD. However, the authors caution that such changes could reflect a relatively high ingestion of chia seeds by these children reinforcing the strong link between diet and microbiome analysis and the need for dietary information in any cross-sectional or intervention study of the microbiome in human samples. As recently summarised by Mayer and colleagues there is a paucity of large comprehensive studies of the microbiome in autism (Mayer *et al.* 2014*b*). Again the issue of chicken or egg emerges; are these changes induced by stereotyped diets seen in many individuals as a product of obsessional behaviour patterns? Also the heterogeneous nature of the disease needs to be taken into account and much more effort is needed to tease out the precise role of the microbiome in both the aetiology and treatment of the disorder.

Increasing attention is currently being paid to oxytocin, the hypothalamic peptide which has been shown to increase sociability. The oxytocin receptor knockout mouse shows considerable deficits in social behaviour (Chini *et al.* 2014), and intriguingly, a recent study demonstrated that a probiotic bacterium (*Lactobacillus reutri*) can influence hypothalamic posterior pituitary activity and increase oxytocin levels raising the possibility of influencing social behaviour by targeting the gut microbiota (Erdman & Poutahidis, 2014). Moreover, a recent study found that in a mouse model of maternal obesity (a risk factor for autism in humans) there were alterations in social behaviour, oxytocin cell numbers, synaptic plasticity and microbiota composition. Remarkably, *Lactobacillus reuteri* could reverse these changes (Buffington *et al.* 2016). Some preliminary studies in humans indicate that intra-nasally administered oxytocin may positively alter social behaviour patterns (Yatawara *et al.* 2016), and a series of large clinical trials are under-way to test oxytocin and related therapies for autism spectrum disorder (Shen, 2015). However, there is still considerable debate as to whether preclinical findings of an oxytocin-mediated increase in social behaviour will translate to the clinical setting, and if they do, which patients and which aspects of the syndrome are likely to benefit most.

Schizophrenia. Schizophrenia is another neuro-developmental disorder and one of the most debilitating illnesses affecting relatively young people, usually commencing in the late teens and early twenties (Miyamoto *et al.* 2012). There is a global prevalence of around 1% and the condition is characterised by altered thought processes (delusions and hallucinations) and frequently a deterioration in cognitive functioning. For most patients the condition is life-long, often with chronic psychosocial deterioration. Current treatments, which primarily target the dopaminergic system, are either ineffective or only partially effective in many patients. There is thus a major requirement for the emergence of alternative, more effective therapeutic targets.

The microbiota has come under the spotlight in relation to the condition. Certain drugs such as phencyclidine (PCP) are used to model schizophrenia-like syndromes (Moghaddam & Javitt, 2012). In rodents sub-chronic PCP treatment induces cognitive deficits and hyper-locomotor activity. Recently it was found that in rodents, sub-chronic PCP significantly altered the gut microbiota and that such changes correlated highly with memory performance (Pyndt Jorgensen *et al.* 2015). Interestingly, administration of the antibiotic ampicillin blocked the PCP-induced memory deficits. The authors speculate that the cognitive deficits seen in some patients with schizophrenia may be induced by changes in the gut microbiota. It is important to note that the atypical antipsychotic olanzapine, which targets the dopamine D4 and 5-HT2

receptors, and is one of the most widely prescribed antipsychotics, exerts significant impact on the gut microbiota (Davey *et al.* 2013). Indeed, recent studies from our group (Davey *et al.* 2012; 2013) and others (Morgan *et al.* 2014; Bahr *et al.* 2015) point to a key role of the microbiome in manifesting antipsychotic-induced weight gain. A growing body of literature is now focusing on the collateral impact of various medications outside of CNS drugs on microbiome composition (Lu *et al.* 2015; Spanogiannopoulos *et al.* 2016).

Reviewing the clinical literature we have argued (Dinan *et al.* 2014) strongly that genomic studies in schizophrenia should include a study of microbial DNA. In support of this a recent investigation has found that antibiotic therapy that alters the gut microbiota can be used to potentiate the action of antipsychotics in patients with schizophrenia (Khodaie-Ardakani *et al.* 2014). What we lack at this point is any detailed analysis of the gut microbiota in patients with the disorder.

Microbiota and ageing. Over 100 years ago the Pasteur Institute's Elie Metchnikoff received the Nobel Prize for his discovery of the macrophage (Cryan & Dinan, 2015). Later in his career he focused on the concept of longevity and proposed that people lived longer in parts of Bulgaria and Eastern Europe because of the high amount of fermented foods containing lactic acid bacteria that they eat (Mackowiak, 2013). With the advent of germ-free mice in the 1940s, which lived longer than their conventionalised controls (Gustafsson, 1946; Glimstedt, 1959), it became clear that there was a direct link between microbiota and senescence. More recently, with the ELDERMET study, the relationship between microbiota and functional outcomes in the elderly, especially in terms of frailty, are being realised (Claesson *et al.* 2011, 2012). Importantly, the microbiota in the elderly was shown to be strongly influenced by diet, opening up dietary-based intervention strategies in the elderly. This has given rise to the view that healthy ageing is associated with microbial diversity (Biagi *et al.* 2010; Lynch *et al.* 2015).

There has been much focus on the neurobiological mechanisms underlying ageing. It is clear that neuro-inflammatory processes play a key role in ageing, with a growing emphasis on the role of the brain's resident immune cells, the microglia (Jyothi *et al.* 2015). More recently, it has been shown that microglia activation is under constant regulation by the gut microbiome (Erny *et al.* 2015). These provocative findings suggest that it is possible to manipulate neuroimmune responses by targeting the gut microbiome. In particular, bacterial metabolites, especially short chain fatty acids, are crucial to these effects.

Another consequence of ageing and age-related disorders such as Alzheimer's disease is the progressive

leakiness of the blood–brain barrier (BBB). In a very provocative finding Braniste and colleagues have shown, using a variety of techniques, that the integrity of the BBB is dependent on appropriate microbiota composition in the gut (Braniste *et al.* 2014). Once again short chain fatty acids are key metabolites in mediating such effects.

It is clear that stress can have marked effects on microbiota (Moloney *et al.* 2014) and the impact of stress on the ageing brain can be particularly pernicious (Prenderville *et al.* 2015). Both ageing and stress can weaken gastrointestinal barrier function and drive a proinflammatory phenotype via the microbiota (Kelly *et al.* 2015). In addition both ageing and stress can also negatively impact BBB permeability (Esposito *et al.* 2002; Montagne *et al.* 2015). The consequences of both barriers being compromised has the potential to accelerate 'inflamm-aging' processes in the brain. Understanding the mechanisms underlying how the microbiome can influence such processes is now worthy of attention.

Parkinson's disease, Alzheimer's disease and cognitive impairment. Parkinson's disease is generally a disorder seen in the elderly and characterised by degeneration of the dopaminergic nigro-striatal pathway, with a characteristic pattern of abnormal movements. Studies indicate that the enteric nervous system is frequently involved due to the effects of α-synuclein (Miraglia *et al.* 2015). It is well established that alteration in bowel function, mainly in the form of constipation, can precede the onset of prototypical motor symptoms. Svensson and colleagues reviewed all Danish patients who underwent vagotomy over the period of 1977–1995 and compared them with a matched general population cohort (Svensson *et al.* 2015). They explored the risk of developing Parkinson's disease following a full truncal vagotomy or a selective vagotomy. The risk of developing Parkinson's disease was significantly decreased in patients who underwent a full truncal vagotomy compared to those who underwent a selective vagotomy. The latter had a risk similar to that of the general population. These data offer the suggestion that the vagus nerve may be critically involved in the pathogenesis of the disorder. This is particularly important given the key role of the vagus nerve in mediating microbiome-to-brain signalling (Bravo *et al.* 2011). However, it is worth noting that vagotomy also affects efferent vagal signalling, which has important anti-inflammatory effects (Olofsson *et al.* 2012). Moreover, vagotomy also slows down gastrointestinal motor function that can lead to bacterial overgrowth (Grace *et al.* 2013), which also may complicate interpretation of vagotomy experiments.

In the first study of its kind the gut microbiota has recently been sequenced in patients with Parkinson's disease (Scheperjans *et al.* 2015). The microbiota of 72 patients and 72 matched controls were pyrosequenced. There was a major reduction in the levels of Prevotellaceae

in the patients. There was a positive association between the levels of Enterobacteriaceae and the severity of postural instability and gait difficulty. The authors point out that their study does not address either the temporal or causal relationship between the gut microbiota and the core features of the disease. Another analysis of microbiota composition in Parkinson's disease (PD) pointed to a reduction in butyrate-producing bacteria (*Blautia*, *Coprococcus* and *Roseburia*) in faeces and *Faecalibacterium* in the mucosa. This was coincident with an increase in *Ralstonia* in mucosal samples more abundant in mucosa of PD than controls (Keshavarzian *et al.* 2015). While some have argued that microbiota transplantation might benefit patients there is certainly no conclusive evidence as yet. Neither are there any reports of controlled trials of probiotics. It is clear that much more research is needed to determine the relative role of the microbiome in Parkinson's disease (Dobbs *et al.* 2016; Felice *et al.* 2016).

Alzheimer's disease and vascular dementias are the most common causes of cognitive decline in ageing populations in Western countries. The hippocampus plays a major role in information processing and memory storage, with long-term potentiation (LTP) – the physiological process thought to underlie cognitive events in the hippocampus – disrupted in animal models of Alzheimer's disease (Lynch, 2004). Studies in rodents indicate that LTP begins to decline in middle age but most dramatically in ageing animals (Lynch, 2004).

VSL#3 is a widely studied probiotic mixture of eight different gram-positive bacterial strains. When aged animals were treated with this combination the microbiota showed a significant change, with increases in Actinobacteria and Bacteroidetes, both of which were reduced in vehicle-treated animals (Distrutti *et al.* 2014). The age-related attenuation of LTP was decreased in the VSL#3-treated animals. Furthermore, microglial activation was decreased, the pivotal trophic factor BDNF was increased, and a gene array found alterations in the expression of inflammation and neuronal plasticity-related genes. More recently we have shown that a bifidobacterium *B. longum* 1714 had a positive impact on cognition in the mouse (Savignac *et al.* 2015). These results are of interest but clearly require translation in humans.

Surprisingly, a detailed analysis of the microbiota in patients with Alzheimer's disease is lacking (Alam *et al.* 2014). However, in type 2 diabetes (T2D), which is a risk factor for Alzheimer's disease, there is alteration in the gut microbiota (Allin *et al.* 2015; He *et al.* 2015). This is seen as an important feature of this condition, but whether it is important in Alzheimer's disease or not is an open question.

More recently, preliminary data published in pre-print format has implicated the microbiota in the accumulation of amyloid plaques in a mouse model of Alzheimer's disease (Harach *et al.* 2015). In this study the authors generated a transgenic Alzheimer's disease mouse model under germ-free conditions and found a dramatic reduction of cerebral Aβ amyloid pathology when compared to control Alzheimer's disease animals, which had a normal intestinal microbiota, albeit one different to healthy, wild-type mice. Most intriguingly, colonisation of germ-free Alzheimer's disease mice with microbiota harvested from conventionally raised Alzheimer's disease mice dramatically increased cerebral Aβ pathology. Also in a similar vein, antibiotic treatment has been recently shown to limit Aβ pathology and neuroinflammation (Minter *et al.* 2016). Simultaneously, a growing body of research is investigating the microbial basis for triggering Aβ pathology (Kumar *et al.* 2016). Together, these data offer hope for the future generation of a novel microbiota-based approach to ameliorate symptoms of Alzheimer's disease.

Conclusions

Understanding the manner in which gut microbes influence gut–brain axis communication has been the subject of considerable research effort in recent times. It is now generally accepted that the gut microbiota influences psychological processes such as the stress response and cognition (Dinan *et al.* 2015). Whether changes in the microbiota are central to the pathophysiology of at least some psychiatric disorders is unproven, though widely speculated upon. Evidence is accumulating for a role for the gut microbiota in autism and age-related disorders such as Parkinson's and Alzheimer's diseases. Future work must investigate whether or not the exciting data that have largely emerged from animal work can be translated to humans, especially given the different diets and microbiota composition between species. Moreover, the exact mechanisms underlying the communication between microbiome and brain need to be further elucidated.

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Additional information

Competing interests

None declared.