Microreview

Microbes and the mind: emerging hallmarks of the gut microbiota–brain axis

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Summary

The concept of a gut microbiota–brain axis has emerged to describe the complex and continuous signalling between the gut microbiota and host nervous system. This review examines key microbial-derived neuromodulators and structural components that comprise the gut microbiota–brain axis. To conclude, we briefly identify current challenges in gut microbiota–brain research and suggest a framework to characterize these interactions. Here, we propose five emerging hallmarks of the gut microbiota–brain axis: (i) Indistinguishability, (ii) Emergence, (iii) Bidirectional Signalling, (iv) Critical Window Fluidity and (5) Neural Homeostasis.

Introduction

Trillions of microorganisms, collectively termed the gut microbiota, reside throughout the mammalian gastrointestinal (GI) tract, extending host digestive (Turnbaugh et al., 2007), metabolic (Nicholson et al., 2012), immune (Round and Mazmanian, 2009) and neural function (Sampson and Mazmanian, 2015). These microbes modulate the distant and complex centre of the nervous system—the brain. In turn, the central nervous system (CNS) exerts top-down regulation, shaping gut physiology and microbial composition via hypothalamus–pituitary axis (HPA) and inflammatory responses (Demaude et al., 2006; O’Mahony et al., 2009).

The neuromodulators

Short-chain fatty acids

Within the GI tract, microbes metabolize dietary fibre into short-chain fatty acids (SCFAs), mainly acetate, propionate and butyrate. An important energy source for the host, these bacterial metabolites also have biological activity. SCFAs act on G-protein coupled receptors (Brown et al., 2003; Le Poul et al., 2003) and function as histone deacetylase inhibitors (Waldecker et al., 2008). In addition, SCFAs can also interact with neurons in the CNS and enteric nervous system (ENS), regulating heart rate, oxygen consumption and GI motility (Cherbut et al., 1998; Kimura et al., 2011; Soret et al., 2010). SCFAs have been shown to initiate beneficial glucose metabolism through a
gut–brain neural circuit, a possible mechanism for the benefits of high-fibre diets (De Vadder et al., 2014). Furthermore, injecting SCFAs directly into the cerebrum has a measurable impact on behaviour in rodent models. Propionate impairs social behaviours and increases repetitive behaviour (MacFabe et al., 2011), while butyrate decreases depressive-like behaviour, with concurrent changes in histone acetylation and the expression of brain-derived neurotrophic factor (BDNF) (Schroeder et al., 2007). However, whether SCFAs from the gut can cross the blood–brain barrier (BBB) to directly impact brain function remains unknown. Injection of SCFAs into the CNS is a highly artificial system, and a major limitation of these studies is the inability to track SCFAs from the gut to the brain. Recently, Frost and colleagues bridged this gap by labelling dietary fibre with a carbon radioisotope to track its progress through the mouse body. Acetate produced from dietary fibre in the gut crossed the BBB and accumulated in the hypothalamus. The infusion of acetate into the brain activated hypothalamic neurons, modulated the expression of regulatory neuropeptides and ultimately suppressed appetite (Frost et al., 2014). Similar approaches combined with microbial analyses are needed to robustly demonstrate the impact of microbially produced SCFAs on brain function.

**Brain-derived neurotrophic factor**

BDNF, the most abundant neurotrophin in the human cortex, enhances neuroimmune responses and coordinates synaptic formation, plasticity and function (Gorski et al., 2003; Huang and Reichardt, 2003; Lu et al., 2013; Wu et al., 2013). In addition, this secretory protein regulates neuronal differentiation, proliferation and survival and has a critical role modulating memory and learning formation (Alonso et al., 2002; Jones et al., 1994; Koponen et al., 2004). Several studies have described the therapeutic potential of BDNF, as BDNF administration provided broad neuroprotective effects against Alzheimer’s and Parkinson’s disease in animal models (Nagahara et al., 2009; Singh et al., 2006).

In addition to genetic and epigenetic control of BDNF (Boule et al., 2012), prebiotics and diet regulate BDNF
levels (Savignac et al., 2013), which suggests a potential microbial-directed mechanism. Sudo et al. demonstrated the impact of the gut microbiota on behaviour and brain chemistry utilizing a germ-free (GF) mouse model. Male GF mice exhibited a significant decrease in hippocampal BDNF expression, accompanied by an elevated HPA stress response (Sudo et al., 2004). In an independent study, Diaz Heijitz et al. observed decreased anxiety-like behaviours in GF mice and reported significant decreases in BDNF mRNA expression within the hippocampus, amygdala and cingulate cortex, CNS structures involved in anxiety responses (Diaz Heijitz et al., 2011). In contrast, Neufeld et al. observed an increase in mRNA BDNF expression in female GF mice, indicating possible sex-dependent regulation of BDNF (Neufeld et al., 2011). In mice, strain-dependent mechanisms may also control BDNF expression and subsequent behavioural shifts. Balb/c mice display a timid behavioural phenotype compared with NIH Swiss mice. When researchers transplanted the gut microbiota of Balb/c mice into GF NIH Swiss mice, and vice versa, mice temporarily adopted the behavioural phenotype of the parental strain. GF Balb/c mice given an NIH Swiss microbiota exhibited decreased exploratory behaviours, while NIH Swiss mice exposed to the Balb/c microbiota displayed decreased exploratory behaviour and hippocampal BDNF levels. These findings indicate that even brief windows of microbial flux elicit neural and behavioural effects (Bercik et al., 2011).

In non-GF studies, alterations of the gut microbiota via infection and antibiotics also influence BDNF expression. Trichuris muris, a murine whipworm parasite, mimics Trichuris trichiura infections in humans (Hurst and Else, 2013). A T. muris infection alters gut physiology, eliciting increased GI inflammation and pro-inflammatory cytokines. T. muris-infected mice displayed increased anxiety-like behaviour associated with decreased BDNF mRNA expression within the hippocampus. Treatment with anti-inflammatory agents mitigated anxiety responses and reduced levels of pro-inflammatory cytokines, but did not impact BDNF expression. Treatment with the probiotic Bifidobacterium longum normalized behaviours and BDNF expression, but did not reduce cytokine levels, highlighting the varied pathways involved in gut microbiota–brain interactions (Bercik et al., 2010). Moreover, researchers observed shifts in hippocampal BDNF expression and mouse exploratory behaviour following broad-spectrum antibiotic treatment (Bercik et al., 2011). Further studies examining BDNF regulation via the gut microbiota are needed to establish mechanisms that bridge bacterial modulations and behavioural manifestations.

GABA

γ-Aminobutyric acid (GABA) is the primary inhibitory neurotransmitter in the CNS (Krnjevic, 1997). Like BDNF, GABA deficiencies have been linked with anxiety, depression and Alzheimer’s disease (Cryan and Kaufmann, 2005; Möhler, 2012; Ramos et al., 2006). Within the brain, GABA biosynthesis occurs via the glutamine–glutamate–GABA cycle (Bak et al., 2006; Schousboe and Waagepetersen, 2007). However, GABA is also found throughout the ENS and GI tract (Hyland and Cryan, 2010). In a recent ex vivo study, Barrett et al. demonstrated that Lactobacillus and Bifidobacterium strains isolated from the human gut microbiota synthesized GABA when grown on glutamate-containing media (Barrett et al., 2012). GF mice also exhibit decreased levels of GABA within the colon, suggesting in vivo production of GABA by the gut microbiota (Matsumoto et al., 2012). While intestinally synthesized GABA has not been shown to enter the brain, ‘gut GABA’ may modulate the CNS via ENS or vagal nerve activation.

In 2011, researchers examined the role of the gut microbiota on GABA expression within the brain. Mice treated with Lactobacillus rhamnosus, a probiotic with anti-inflammatory properties, exhibited altered GABA receptor expression in the brain accompanied by reduced anxiety-and depressive-like behaviours (Bravo et al., 2011). Again, the precise mechanisms driving shifts in behaviour and brain chemistry remain unknown.

Serotonin

Serotonin (5-hydroxytryptamine, 5-HT) regulates diverse biological processes, including respiration, GI secretion and peristalsis, cardiovascular responses, and behavioural and neurological functions (Berger et al., 2009; O’Mahony et al., 2009; Spoont, 1992). Alterations in serotonin neurotransmission and expression are linked with multiple psychiatric disorders, notably depression (López-Figueroa et al., 2004; Müller and Schwarz, 2007). The vast majority of serotonin, an estimated 95%, is found within the GI tract. Here, 90% of serotonin is localized within epithelial enterochromaffin cells (ECs) and the remaining 10% remains within the ENS (Kim and Camilleri, 2000). And while serotonin biosynthesis in the brain remains largely independent of the GI tract (Bertacini, 1960), serotonin biosynthesis within the GI tract involves specific microbiota–gut interactions (Reigstad et al., 2015; Yano et al., 2015).

Although commensal microbes can produce neuroactive molecules (Barrett et al., 2012; Patterson et al., 2014), recent work revealed a microbe–host mechanism of serotonin production (Reigstad et al., 2015; Yano et al., 2015). Spore-forming bacteria within the gut microbiota release metabolites, including SCFAs, signalling EC
production of serotonin (Yano et al., 2015). These microbially stimulated ECs increase expression of tryptophan hydroxylase 1 (Tph1), the enzyme catalysing the rate-limiting step of serotonin biosynthesis from tryptophan (Jéquier et al., 1967; Yano et al., 2015).

The vast majority GI tryptophan is not utilized for serotonin production, but rather metabolized via the kynurenine pathway (Schwarcz et al., 2012). Alteration of kynurenine:tryptophan ratios has been linked with depression and anxiety (Dantzer et al., 2008a; O’Connor et al., 2009). The gut microbiota may modulate this ratio directly, via production of reactive oxygen species which inhibit kynurenine metabolism (Valladares et al., 2013), or indirectly, via regulation of pro-inflammatory cytokines involved in kynurenine synthesis (Moreau et al., 2005; Müller and Schwarz, 2007). Treatment with B. infantis, an anti-inflammatory probiotic, reduced the kynurenine:tryptophan ratio in rats, although no shifts in stress behaviours were detected (Desbonnet et al., 2008).

Marked alterations in tryptophan and serotonin expression in GF models also support gut microbial regulation of tryptophan metabolism and serotonin signalling. GF mice have increased levels of plasma tryptophan. Colonization of GF mice normalized anxiety-like behaviours and plasma tryptophan levels, but failed to normalize serotonin levels in the hippocampus, suggesting a critical window of microbiota-dependent serotonin regulation in the brain (Clarke et al., 2013). In addition, GF mice display decreased levels of GI serotonin compared with microbe-exposed counterparts (Reigstad et al., 2015; Wikoff et al., 2009; Yano et al., 2015) with colonization of spore-forming bacteria restoring peripheral serotonin levels (Yano et al., 2015). In contrast, one study reported no difference in levels of intestinal serotonin following antibiotic treatment, perhaps reflecting the resistance of spore-forming bacteria to antimicrobial attack (Bercik et al., 2011). For an extensive review of tryptophan metabolism and serotonin signalling throughout the gut microbiota–brain axis, see O’Mahony et al. (2015).

While the effects of spore-forming bacteria on mood and behaviour remain unexamined, concurrent studies revealed antidepressant properties of specific gut microbes (Desbonnet et al., 2010). Whether spore-forming bacteria belong in the emerging class of “psychobiotics” is yet to be determined (Dinan et al., 2013).

The structures

Gastrointestinal epithelium

The epithelial layer of the GI tract is a semi-permeable barrier that allows the passage of nutrients and immune signals into circulation, while preventing the movement of microorganisms and inflammatory factors (De Santis et al., 2015). Permeability of the GI epithelium has been linked with a number of neurological disorders, including autism spectrum disorder (ASD) (de Magistris et al., 2010), Parkinson’s disease (Forsyth et al., 2011), and major depression (Mass et al., 2008). It has been suggested that increased bacterial translocation across a leaky GI epithelium leads to neuroinflammation and behavioural symptoms. This association, however, remains highly controversial (Julio-Pieper et al., 2014). For detailed reviews of the immune mechanisms involved in GI epithelial integrity and the gut–brain axis, see McCusker and Kelley (2013), Sampson and Mazmanian (2015), and Steinmeyer et al. (2015).

Lipopolysaccharide (LPS) induces the expression of pro-inflammatory cytokines by immune and non-immune cells, both systemically and in the brain (Breder et al., 1994), and induces depressive-like behaviour in rodents (Fischer et al., 2015; Frenois et al., 2007). Increased levels of pro-inflammatory cytokines in the blood can also induce ‘sickness behaviour’, characterized by depressive-like symptoms that include decreased motor activity, social withdrawal and lack of interest in the environment (Dantzer et al., 2008b). Together, these studies suggest that a barrier breach resulting in bacterial translocation from the gut lumen to the blood could have significant impact on neural function. Furthermore, there is increasing evidence to suggest that this occurs during neurological disorders in humans (Kelly et al., 2015).

Major depression in humans has been associated with increased levels of microbial components in the serum (Kéri et al., 2014; Maes et al., 2012; Mass et al., 2008), as well as increased expression of toll-like receptor 4 (TLR4), an LPS sensor, in peripheral blood mononuclear cells (Kéri et al., 2014), suggestive of bacterial translocation across the GI epithelium. Psychological stress in rodents can induce intestinal permeability, bacterial translocation and neuroinflammation (Ait-Belghnaoui et al., 2014; Gárate et al., 2013; Moussaoui et al., 2014; Silva et al., 2014). Some studies suggest that these symptoms may be partially attenuated by antibiotics (Gárate et al., 2013), probiotics (Ait-Belghnaoui et al., 2014; Silva et al., 2014; Zareie et al., 2006) and the loss of TLR4 (Gárate et al., 2013).

Autism spectrum disorder also shows comorbidity with GI dysfunction in humans (Petra et al., 2015). In support of the gut permeability hypothesis, Mazmanian and colleagues have demonstrated that a mouse model of ASD exhibits GI barrier dysfunction. Treatment with the gut commensal Bacteroides fragilis improved both permeability and behavioural defects in these mice. Moreover, the symptoms of ASD could be recapitulated with a microbially produced metabolite (Hsiao et al., 2013). Taken together, these studies support a barrier breach model in which bacteria or their metabolites cross the leaky GI epithelium inducing neural changes. However, associations in humans remain purely correlative, and
studies are frequently confounded by small sample size and lack of appropriate control groups. Going forward, study design must be carefully assessed in order to draw meaningful conclusions regarding GI barrier breach and the gut microbiota–brain axis.

Vagus nerve

The vagus nerve, the tenth cranial nerve, extends from the brain into the muscular and mucosal layers of the gut, regulating control of satiation (Forsythe et al., 2014; Phillips and Powley, 1998) and GI secretion and peristalsis (Browning and Travaglì, 2003; Forsythe et al., 2014). Primarily composed of sensory fibres, the vagus nerve serves as the primary afferent pathway conveying immune, microbial and nutrient information from the gut to the CNS (Forsythe et al., 2014; Wang et al., 2002). In contrast, stimulation of vagal efferent fibres elicits a systemic anti-inflammatory response from the CNS, attenuating the release of pro-inflammatory cytokines (Borovikova et al., 2000). These dual roles suggest an important interface for bidirectional gut microbiota–brain interactions.

Perhaps the most studied role of the vagus nerve in gut microbiota–brain interaction is within the context of behaviour. The Polyvagal Theory proposes that the myelinated branch of the vagus nerve, occurring solely within mammals, is the basis for the emergence of non-endocrine social behaviour (Montiel-Castro et al., 2013; Porges, 2009) and experimental studies have revealed a vagal role in behaviour. *Citrobacter rodentium* infection, a murine model of inflammation, results in increased anxiety-like behaviours independent of pro-inflammatory cytokine expression. High levels of c-Fos protein-positive neurons within the ganglia of *C. rodentium*-infected mice indicated afferent vagal signalling from the microbiota to the brain (Lyte et al., 2006). In an independent study, Bravo et al. observed that changes in GABA signalling and behaviour following probiotic exposure required a functionally intact vagus nerve (Bravo et al., 2011). However, vagal integrity did not impact behavioural shifts driven by antibiotic treatment or cytokine-driven inflammation (Bercik et al., 2010; Bercik et al., 2011). Although the vagus nerve provides one mechanism of gut–brain interactions, the gut microbiota clearly utilizes both vagus-dependent and vagus-independent signalling mechanisms.

The vagus nerve has also been suggested as a possible route of microbial-initiated neurodegenerative disease. In a 20 year clinical study, Svensson et al. reported that full truncal vagotomy, severance of the vagal trunk at the base of the abdomen, was linked with a decreased risk of Parkinson’s disease. These findings support the ‘dual hit’ hypothesis of PD aetiology, which proposes that a microbial infection within the GI initiates Parkinson’s disease through invasion of the brain, likely via the vagus nerve (Hawkes et al., 2009; Svensson et al., 2015). The promising role of the vagus as a conduit of gut microbiota–brain communication and neurodegenerative pathogenesis requires further examination.

Blood–brain barrier

The BBB serves as the main gatekeeper of the brain, regulating the passage of oxygen and nutrients from the circulatory system and guarding the nervous system from toxins and pathogens. This barrier maintains brain homeostasis, regulating transport of peptides (Banks, 2008), neurotransmitter precursors (Pardridge, 1977) and cytokines into the brain (Banks et al., 2001). Like the GI epithelium, the BBB is composed of epithelial cells linked by tight junction proteins (Persidsky et al., 2006). Weakened BBB integrity, or ‘leaky BBB’, allows unchecked entry of potentially toxic molecules into the brain. Indeed, breach of the BBB has been linked with the pathogenesis of many inflammatory and neurodegenerative diseases, including stroke (Kahles et al., 2007), traumatic head injury (Barzó et al., 1996), multiple sclerosis (Kermode et al., 1990) and Alzheimer’s and Parkinson’s disease (Kortekaas et al., 2005; Ryu and McLarnon, 2009).

While the exact aetiology of BBB deterioration is likely multifactorial, recent work highlights potential microbial-induced mechanisms. Cellular components of Gram-positive bacteria, including lipoprotein A, elicit strong pro-inflammatory cytokine responses, weakening the BBB in vitro models (Boveri et al., 2006). In 2013, researchers reported that rats fed a high-fat diet exhibit weakened BBB integrity and decreased hippocampal-dependent cognitive functioning (Davidson et al., 2013).

As diet is a major factor influencing composition of the gut microbiota, it is tempting to infer potential microbial-dependent mechanisms. In a seminal study, Braniste et al. reported that GF mice exhibit increased BBB permeability. Exposure of pathogen-free gut microbes increased expression of tight junction proteins in GF adult mice, strengthening BBB integrity. The increased BBB permeability observed in GF mice began during fetal development and continued throughout adulthood, suggesting an early developmental window for maternal microbiota regulation of BBB formation (Braniste et al., 2014). Because SCFAs promote barrier integrity and tight junction assembly at the GI epithelium (Peng et al., 2009), researchers posit that SCFAs produced by the gut microbiota of the mother and later offspring likely contributed to BBB formation and maintenance (Braniste et al., 2014).

Both the function and structural make-up of the BBB have led to multiple comparisons with the GI epithelium (Daneman and Rescigno, 2009). Indeed, the complexity and modulatory capacity of the gut microbiota suggest that this ‘second brain’ is not so different from the first.

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Emerging hallmarks of the Gut-microbiota brain axis: future work and challenges

Nearly two millennia have passed since Galen recorded intricate viscera–brain connections in *de Anatomicis Administrationibus*. The 21st century re-examination of gut–brain interactions following the emergence of gut microbiota–brain research has led to a paradigm shift in both neuroscience and microbiology (Mayer *et al.*, 2014), providing an opportunity to readdress major challenges in the microbiome field.

The impact of the gut microbiota on numerous aspects of host health and homeostasis has been established. Now, researchers must move beyond correlative studies linking microbial communities with diseases to causative-based research. By its very name, the gut microbiota–brain axis suggests a research approach beyond microbiome or brain profiling with emphasis on the underlying mechanisms involved in gut–brain interactions. Another challenge in microbiome studies, and, by extension, gut microbiota–brain research, is the need for improved ‘omics’ tools. Advanced techniques to add conceptual information and analyse metagenomic, proteomic and metabolomic microbiome datasets are crucial for a deeper understanding of the microbiota. However, the worth of such analyses depends upon the robustness of the model system/s. Despite the popularity of GF models, vast immune (Mazmanian *et al.*, 2005), metabolic (Tremaroli and Bäckhed, 2012) and neurological (Neufeld *et al.*, 2011) differences may limit the utility of GF animals to accurately model complex, multi-system changes following microbial exposure. Model robustness must also be addressed within conventionally raised models, as vendor, in-breeding, and strain contribute to variations in the rodent gut microbiota (Ericsson *et al.*, 2015; Friswell *et al.*, 2010; Hildebrand *et al.*, 2013; Hufeldt *et al.*, 2010). In addition, behavioural differences between murine strains (Bercik *et al.*, 2011; Moy *et al.*, 2007) and sex-dependent regulation of specific neuromodulators (Clarke *et al.*, 2013; Neufeld *et al.*, 2011) impact analysis of model-based, gut microbiota–brain research. For a detailed review of challenges, see Ravel *et al.* (2014).

Lastly, we suggest that the highly varied and complex gut microbiota–brain interactions be codified into a set of shared characteristics. These conceptual characteristics will provide a useful framework to explore the gut microbiota–brain axis and examine microbial-driven neurological disease. Here, we propose five such hallmarks characterizing gut microbiota-host interactions (Fig. 2). Following each characteristic, we have included open questions and research directions that emerge from this conceptual framework.

*Indistinguishability*

In 2007, the Human Microbiome Project characterized the human as a ‘supraorganism’—a metaorganism comprised of interdependent human and microbial components...
(Turnbaugh et al., 2007). A growing number of microbiome researchers recognize the gut microbiota as a vital component of the human metaorganism (Biagi et al., 2011; Bosch and McFall-Ngai, 2011). Thus, the gut microbiota–brain axis does not reflect control of the gut microbiota by the host or vice versa, but rather synergetic extensions of the host nervous system. Within this framework, the boundary separating microorganism from host blurs, highlighting our indistinguishability—a radical blurring of self and (microbial) non-self (T. Rees, personal communication). Indistinguishability raises many gut microbiota–brain research questions, including: What intermediate host or microbial elements are involved in translating the presence of the microbiota to the brain? What distinguishes a pathogenic (non-self) microbe from the commensal microbiota? How do environmental pressures (such as stress, diet, and antibiotics) impact interactions within the supraorganism?

**Emergence**

While many gut microbiome–brain studies have focused on the influence of one bacterium on brain function (Bercik et al., 2010; Bravo et al., 2011; Desbonnet et al., 2008) there is a recent shift to examine the impact of a microbiome on the host nervous system (Bercik et al., 2011; Braniste et al., 2014; Diaz Heijtz et al., 2011). Recently, our lab developed and characterized a novel mouse model of environmental enteropathy (EE), a chronic GI inflammatory disease that significantly contributes to malnutrition (Brown et al., 2015; Korpe and Petri, 2012). To replicate EE features, mice were fed a low-protein diet and exposed to both *Escherichia coli* and *Bacteroidetes* species. Exposure to *E. coli* or *Bacteroidetes* alone did not produce EE. Rather, the interactions of these commensal gut microbes in a specific context produced a pathological response. Likewise, gut microbes exhibits functional emergent properties, collectively influencing host brain and behaviour (Ley et al., 2006; Qureshi and Mehler, 2013). We suggest that while specific microbes contribute to pathogenic states, collective gut microbial interactions drive neural health and disease via the gut microbiota–brain axis. More work on emergent properties of the gut microbiota is needed. Potential research questions examining emergence include: What is the role of microbiome–microbe interactions in brain function? Which specific microbial interactions positively or negatively influence immune activation or barrier permeability, affecting behavioural shifts? Similarly, what combinations of prebiotics/probiotics exhibit promising therapeutic potential to treat neurological conditions?

**Bidirectional signalling**

Both top-down (brain-gut) and bottom-up (gut-brain) signals travel along the gut microbiota–brain axis. To briefly illustrate, microbes stimulate the production of BDNF, which elicits behavioural resilience during stress conditions (Bercik et al., 2011; Sudo et al., 2004). During stress conditions, the brain also modulates gut microbiota composition, likely through activation of the HPA (Galley et al., 2014). The hierarchy of bottom-up or top-down interkingdom signalling on neurological health remains debatable. While factors including host physiology and environment impact these interactions, the signalling mechanism originates from (i) a gut microbe (or, more likely, the collective interactions of the gut microbiota) and (ii) the host nervous system. Research questions addressing interkingdom bidirectional signalling include: Does the ‘origin’ of signal (microbiota versus brain) affect hierarchy of gut microbiota–brain signalling? Which top-down signals modulate the gut microbiota? Are the dual signals involved in the gut microbiota–brain axis direct or indirect?

**Critical window fluidity**

Typically stable throughout adulthood, the composition of the gut microbiota dynamically changes during ‘critical windows’ in host development, notably the perinatal stage (Borre et al., 2014). Environmental factors, which include the gut microbiota, influence early life development, often resulting in lifelong structural and functional changes (Seckl and Meaney, 2004). During birth, infants are rapidly colonized by microorganisms primarily originating from the mother. Even before birth, the maternal microbiota impacts intrauterine brain development (Braniste et al., 2014) and maternal microbial infections during pregnancy are linked with subsequent psychiatric and neurodevelopmental disorders in offspring (Boks, 2008; Lee et al., 2015). Moreover, early life alterations of the gut microbiota enhance risk of inflammatory, autoimmune, and psychiatric diseases (Arrieta et al., 2015; O’Mahony et al., 2009; Russell et al., 2012). In a GF study, early exposure to microorganisms reduced anxiety-like behaviours. In contrast, researchers observed no reduction of anxiety-like behaviour when adult GF mice were exposed to microbes, indicating an early critical window for microbial–brain modulation (Diaz Heijtz et al., 2011). However, these windows of microbial flux are not confined to developmental periods. Acute, albeit typically temporary, alterations of the gut microbiota composition are elicited by diet, infection and antibiotics (Cryan and Dinan, 2012; David et al., 2014). We suggest that robust microbe-to-mind modulations occur at these fluid critical windows, and recommend further examination of both acute and developmental periods, including early childhood, adolescence and ageing. Potential research questions include: When are the critical developmental periods in which the brain is more susceptible to microbial influence? Conversely,
are there developmental periods in which the microbial community may be more heavily influenced by neural signals? Which dual signals are most important during critical developmental periods or acute alterations of the gut microbiota? Can microbial interventions (probiotics, prebiotics, faecal transplants) at critical developmental periods reduce susceptibility to neurological disorders?

Neural homeostasis

Gut microbes produce or induce production of neuromodulators, maintaining brain chemistry and cognition. In turn, the host nervous system modulates GI motility and barrier homeostasis, sustaining the microbial community (Cryan and Dinan, 2012; Rhee et al., 2009). Through these bidirectional signals, the gut microbiota–brain axis maintains metorganismal homeostasis of the nervous system. Both the absence or alterations of the gut microbiota impair neural homeostasis, resulting in behavioural and neurochemical shifts (Bercik et al., 2011; Sudo et al., 2004). To cite one example, researchers recently demonstrated that the gut microbiota maintains homeostasis of microglia, the innate immune cells of the CNS. GF mice display microglial immaturity, a phenotype also observed following antibiotic-induced eradication of the microbiota. In addition, GF mice exhibit reduced microglial immune responses. Exposure to either a complex microbiota or microbial metabolites largely restored microglial morphology and function, partially rescuing neural homeostasis (Erny et al., 2015). Research questions that arise from the examination of neural homeostasis include: What are the mechanisms involved in microbial-driven neural homeostasis? Are there critical gut microbes or metabolites involved in maintenance of neural homeostasis? What are the functional differences of the peripheral (gut, ENS) and central (brain) neurotransmitters?

Interestingly, the term homeostasis emerged in the 1920s as a prerequisite for the evolution of the mammalian nervous system. The physiologist Walter Cannon coined the term to describe the ability of mammals to maintain a constant milieu intérieur (internal environment), expanding a concept developed by Claude Bernard a century earlier. Bernard summed up this concept, writing, ‘The stability of the milieu intérieur is the condition for the free and independent life’ (Gross, 1998). Perhaps today, Cannon and Bernard would remark that the intrinsic homeostatic function of gut microbiota–brain interactions characterize the condition of the free and interdependent life.

Rather than provide a definition of microbe–mind interactions, these hallmarks serve as an organizational framework to examine and conceptualize characteristics of the complex gut–microbiota brain axis. These mechanisms and even the full extent of gut microbiota–brain interactions remain undiscovered. Continued interdisciplinary dialogue and research are required to further develop this conceptual framework and our knowledge of the gut microbiota–brain axis.

Astonishing discoveries await.

Acknowledgements

The authors would like to thank members of the Finlay Lab and the Canadian Institute for Advanced Research-Humans and Microbiome Project (CIFAR-HMB) for thought-provoking discussions exploring the human–microbiota connection. In particular, the authors thank EM Brown, NC Marshall and T Rees for critical revision of this manuscript. BBF is the UBC Peter Wall Distinguished Professor and a CIFAR Senior Fellow. The Finlay Lab is supported by operating grants from the Canadian Institute for Health Research (CIHR) and CIFAR-HMB. KCB is a CIFAR-HMB scholar and KEH is supported by a CIHR Masters Award, R Howard Webster Foundation Fellowship, Shaughnessy Hospital Volunteer Society Fellowship and Elwyn Gregg Memorial Fellowship. The authors declare no conflict of interest.

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