Microbiota and neurodevelopmental windows: implications for brain disorders

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Gut microbiota is essential to human health, playing a major role in the bidirectional communication between the gastrointestinal tract and the central nervous system. The microbiota undergoes a vigorous process of development throughout the lifespan and establishes its symbiotic rapport with the host early in life. Early life perturbations of the developing gut microbiota can impact neurodevelopment and potentially lead to adverse mental health outcomes later in life. This review compares the parallel early development of the intestinal microbiota and the nervous system. The concept of parallel and interacting microbial–neural critical windows opens new avenues for developing novel microbiota-modulating based therapeutic interventions in early life to combat neurodevelopmental deficits and brain disorders.

Microbiota–gut–brain axis

Microbes within and on our bodies are a thriving dynamic population forming a symbiotic superorganism. The collective comprises a myriad of bacteria, of approximately 10^{14} cells, containing 100 times the number of genes of the human genome [1]. Despite the evolution of this microbiome (see Glossary) for 500 million years [2,3], it is only recently that advances in sequencing technology have allowed us to appreciate the full nature of the complexities of host–microbe relationships. The largest microbial component of the human microbiome is located in the large intestine of the gastrointestinal (GI) tract. It is now clear that the gut microbiota plays a key role in the life and health of the host by protecting against pathogens, metabolizing dietary nutrients and drugs, and influencing the absorption and distribution of dietary fat [2]. However, the influence of the microbiota extends beyond the GI tract, playing a major role in the bidirectional communication

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Glossary

Alzheimer's disease: a progressive age-associated neurodegenerative disorder characterized by cognitive decline and build-up of protein 'plaques' and 'tangles' in the brain.
Astrocytes: the most abundant glial cell of the human brain, providing support for the blood–brain barrier, provision of nutrients to the nervous tissue, and a role in the repair and scarring process of the CNS following traumatic injuries.
Attention deficity hyperactivity disorder (ADHD): a psychiatric disorder usually occurring in childhood characterized by significant attention problems, hyperactivity, or impulsivity.
Autism: a neurodevelopmental disorder characterized by the presence of stereotypical behavior and communication and social interaction deficits, with a male gender bias.
Brain–gut axis: a complex network of communication between the gut and the brain, which modulates the GI tract and CNS, playing an important role in maintaining homeostasis.
Cortical neurogenesis: the generation of new neurons in the cerebral cortex.
Dysbiosis: a microbial imbalance on or within the body, often localized to the gut.
Gliogenesis: the generation of new glial cells.
Irritable bowel syndrome: a disorder of the brain–gut axis. In addition to GI symptoms, irritable bowel syndrome is also associated with frequent comorbidities of depression and anxiety.
Hippocampal neurogenesis: a process by which neurons are generated in the hippocampus.
Leaky gut: an increase in the permeability of the intestinal mucosa, allowing bacteria and toxins to leak into the bloodstream (Box 2).
Microbiome: the collective genomes of all the microorganisms in a microbiota.
Microbiota: the entire microbial population residing in particular parts of the body, such as the intestine or skin.
Mood disorders: a constellation of mental disorders encompassing major depression and bipolar disorder (combining episodes of both mania and depression).
Neurodevelopment: the development of the CNS system, occurring mostly in prenatal and early life.
Neurulation: a key neurodevelopmental event that begins the genesis of the nervous system by 'folding' the embryonic nervous system to form the neural tube.
Schizophrenia: a mental disorder characterized by positive (hallucinations and delusions) and negative (anhedonia, social withdrawal) symptoms, which typically emerge in adolescence and early adulthood.
Short chain fatty acids (SCFAs): bacterial products or metabolites from the fermentation of dietary carbohydrates in the gut, which have immunomodulatory properties and can interact with nerve cells by stimulating the sympathetic nervous system.
Synaptic pruning: a process where synapses are eliminated during neurodevelopment and/or aging.
Systems matching: a process that refines neuronal numbers to match the requirements of the neural circuit which they become part of.
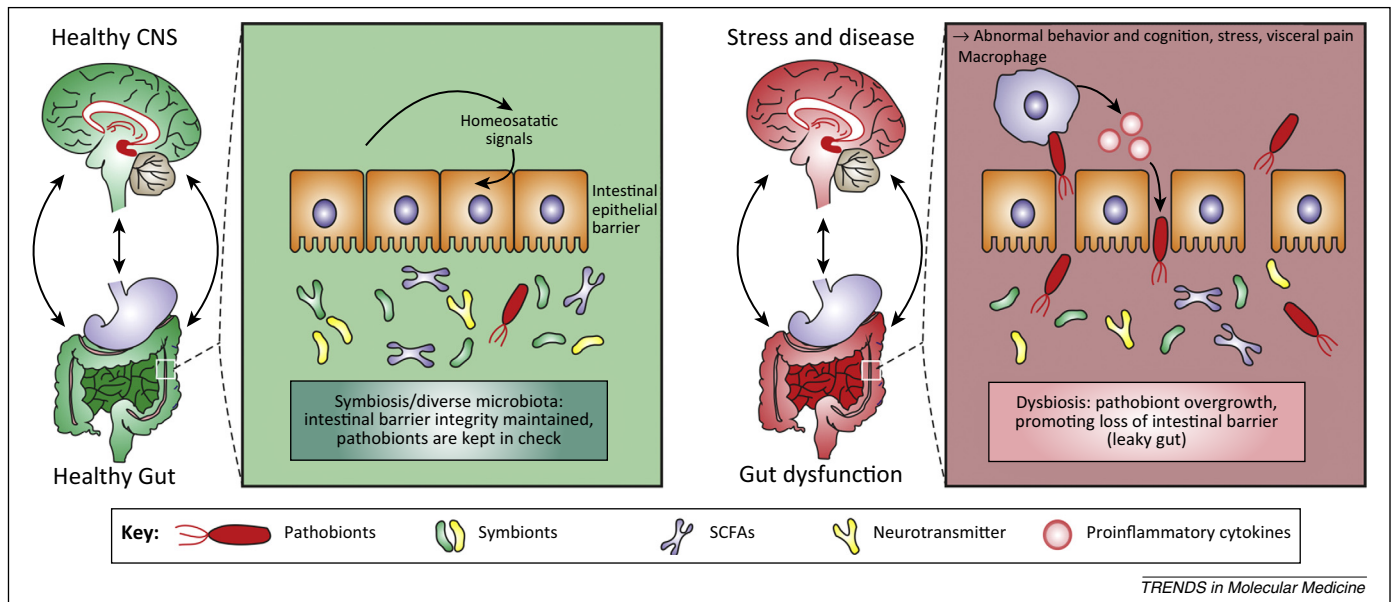
Figure 1. Microbiota–gut–brain axis communication in health and disease. (Left) Under healthy conditions, the predominance of symbiotic bacteria, an intact intestinal barrier, a healthy innate immunity controlling pathobiont overgrowth inside the intestinal tract and healthy gut function support the symbiotic relationship between CNS function and gut microbiota. (Right) Under pathological stress and/or disease conditions, 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. A change in the balance of symbionts and pathobionts favoring pathobiont overgrowth results in dysbiosis, which can induce inflammation. During inflammatory responses, macrophages contribute to pathogenesis through inappropriate responses to enteric microbial stimuli, inefficient clearance of microbes from host tissues, and impaired proinflammatory and anti-inflammatory responses, and loss of barrier function (leaky gut; see Box 2). This promotes the increased translocation of pathogenic bacterial components from the intestinal mucosa to the systemic circulation, where they activate innate immunity, characterized by production of proinflammatory cytokines, resulting in systemic inflammation and abnormal gut function. These mechanisms potentially lead to impaired CNS function such as altered neurochemistry, cognition, behavior, stress response, and visceral pain. Abbreviations: CNS, central nervous system; SCFAs, short chain fatty acids.

between the GI tract and the central nervous system (CNS) [4] (Figure 1).

The concept of the brain–gut axis emerged in the 19th and early 20th centuries from the pioneering observations of Beaumont, Darwin, and Cannon in tandem with the now classical physiological studies of Ivan Pavlov [4,5]. More recently, given the realization of the importance of the microbiota in modulating health, the brain–gut axis has been extended to the microbiota–gut–brain axis [6–8], which represents a complex network of communication between the gut, the intestinal microbiota, and the brain, modulating immune, GI, and CNS functions [6,9]. It encompasses the CNS, the sympathetic and parasympathetic branches of the autonomic nervous system, as well as the enteric nervous system and the neuroendocrine and neuroimmune systems [10]. In healthy individuals, the normal dominant microbiota is relatively stable and forms a mutually beneficial rapport with the host. However, perturbations in the delicate synergetic host–microbiota relationship may have serious consequences and has the potential to exacerbate brain, digestive, and metabolic disorders [9–14] (Figure 1). For example, bidirectional communication between the microbiota and the CNS influences stress reactivity, pain perception, neurochemistry, and several brain–gut axis disorders [4,7,15]. Despite several investigations focusing on the exploration of the bidirectional communication between the microbiota and the CNS, more is known about gut microbiota modifying CNS (Box 1), whereas the mechanisms by which the CNS can modify gut microbial communities remain to be fully elucidated.

In addition to neural, endocrine, and metabolic pathways, immunological mechanisms may be an additional mechanism in signaling with the potential to affect neurodevelopment. Leaky gut (Figure 1 and Box 2) as the result of dysbiosis offers an alternative mechanism of inducing an immune response, and the phenotypic changes of immune cells induced by gut microbes may modulate the neurodevelopment–microbiota interaction.

The dynamics of the various endogenous and exogenous factors, which may have a profound effect on the microbiota composition leading to dysbiosis and impacting multiple human pathologies, from metabolic syndromes to mental disorders are slowly being unraveled [16]. The microbiota undergoes a vigorous process of maturation and development throughout lifespan and establishes its mutually beneficial cohabitation with the host during life. The composition of the gut microbiota during critical periods of CNS development is affected by a broad range of factors. Perturbation of any of these factors can lead to host stress or disease (Figure 1).

Shaping of the microbiota occurs in parallel with neurodevelopment and they have similar critical developmental windows (Figure 2) sensitive to damage. Recently, the microbiota–gut–brain axis emerged as a key player in neurodevelopmental phases, indicating that early-life events during initial colonization and microbiota development can determine general and mental health in later life [17,18]. Importantly, childhood and adolescence are the most dynamic periods of change in relation to microbiota and brain development. Thus, disruptions during such critical periods of dynamic microbiota–host interaction have the potential to profoundly alter brain–gut signaling, affect health throughout life, and increase the risk of (or lead to) neurodevelopmental disorders.

Box 1. Potential mechanisms by which the microbiota affects CNS function

Altered microbial composition. Exogenously administered potential probiotic bacteria or infectious agents can affect the composition of the gut microbiota in multiple ways [77]. For example, they can compete for dietary ingredients, bioconvert sugars into fermentation products with inhibitory properties, produce growth substrates for other bacteria, compete for binding sites on the enteric wall, improve gut barrier function, reduce inflammation, and stimulate innate immune responses.

Immune activation. Microbiota and probiotic agents can have direct effects on the immune system [91]. The immune system also exerts bidirectional communication with the CNS [92], making it a prime target for transducing the effects of bacteria on the CNS. In addition, indirect effects of the gut microbiota and probiotics on the innate immune system can result in alterations in the circulating levels of proinflammatory and anti-inflammatory cytokines that directly affect brain function.

Neural pathways. The vagus nerve, the major nerve of the parasympathetic division of the autonomic nervous system, regulates several vital body functions [93,94]. Microbiota can elicit signals via the vagal nerve to the brain and vice versa [95–97]. For example, the behavioral effects mediated by two separate probiotic strains in rodents were dependent on intact vagal nerve activation [98].

Tryptophan metabolism. Serotonin is a biogenic amine that functions as a neurotransmitter in the body and depends on the availability of its precursor, tryptophan. Dysregulation of the kynurenine arm of the tryptophan metabolic pathway is involved in many disorders of both the brain and GI tract. This initial step in the kynurenine cascade is catalyzed by either indoleamine-2,3-dioxygenase or the largely hepatic-based tryptophan 2,3-dioxygenase. The activity of both enzymes can be induced by inflammatory mediators and by corticosteroids [99]. There is some evidence to suggest that a probiotic can alter concentrations of kynurenine [100].

Gut hormonal response. The gut communicates to the brain via hormonal signaling pathways that involve the release of gut peptides with modulatory functions from enteroendocrine cells [101,102]. Studies in germ-free mice suggest that the gut microbiota mediates and regulates the release of gut peptides [103].

Bacterial metabolites. Bacterial products or metabolites from gut commensals, such as SCFAs, may translocate from the intestinal mucosa to the systemic circulation, where they could interfere with immune regulation and CNS function. SCFAs are produced via the fermentation of dietary carbohydrates and have immunomodulatory properties [104]. SCFAs can interact with nerve cells by stimulating the sympathetic nervous system [105].

The aim of this review is to juxtapose the parallel early development of the intestinal microbiota and the nervous system taking into account environmental and maternal influences that can affect microbe to brain signaling and thus behavior throughout life.

Developmental windows: gut microbiota and neurodevelopment

The prenatal and postnatal periods in mammalian development are critical developmental windows that are characterized by rapid changes in neuronal and microbial organization. During these periods environmental factors could have a long-term impact on brain and behavior, resulting in brain disorders (Figure 2). Brain development requires a delicate and complex balance of genetic and environmental factors both during prenatal and postnatal periods. Disruption of these elements can alter developmental trajectories and may lead to the onset of neurodevelopmental and other brain disorders later in life [19–21]. Recently, several preclinical studies using germ-free mice

highlighted the ability of early life microbiota to influence neurodevelopment, with long-lasting effects on neural function [17,22,112]. During development, the nervous system is assembled and sculpted by a series of temporally regulated developmental processes that shape the functional neural circuitry that is critical for normal cognitive, motor, and emotional development. This is a complex process consisting of an orchestrated series of neurodevelopmental events including, but not limited to, neurogenesis (the birth of new neurons), axonal and dendritic growth, synaptogenesis, and refinement of these synaptic connections, which generate the required numbers of neurons and the appropriate synaptic density to match the requirements of the neural circuit they become part of, a process known as 'systems matching' [23,24]. These processes begin *in utero* and are later refined and modified during early postnatal development. Disturbance during these processes can have profound structural and functional consequences for brain development in affected offspring [19–21,25].

Prenatal period
Microbiota development
Despite a common dogma that the intrauterine environment and fetus are sterile until delivery, some evidence demostrates bacterial presence in the intrauterine environment, suggesting that these bacteria may influence the microbiota of the infant before birth [26–30]. The presence of bacterial species in the fetus (such as *Escherichia coli*, *Enterococcus faecium*, and *Staphylococcus epidermidis*) could result from the translocation of the mother's gut bacteria via the bloodstream and placenta [28]. Specifically, *Enterococcus*, *Streptococcus*, *Staphylococcus*, and *Propionibacterium* species have been isolated from umbilical cord blood, suggesting the potential for translocation. Further support of this notion comes from the animal study demonstrating that E. *faecium* strains orally fed to the dams were later detected in the amniotic fluid [31]. *Lactobacillus* and *Bifidobacterium* DNA were detected in the placenta of vaginally and cesarean section delivered infants, without cultivation of any viable cells, suggesting translocation form the mother's gut, (Figure 3) [29]. More recently the presence of a defined placenta microbiome has been identified with a signature similar to that in the mother's oral cavity. The functional role of this microbiome on the developing brain now needs to be characterized [32]. Establishment of pioneer gut microbiota is a crucial stage in neonatal development, overlapping with this critical period of brain development [33] (Figure 4).

Brain development
The first major neurodevelopmental event critical to normal brain development is formation of the neural tube, a process event known as neurulation. In humans, this occurs by 3–4 weeks of gestation [25,34,35], following which cortical neurogenesis occurs predominantly during *in utero* gestation, but can continue up to 2.5 years of age [25,36], outside the neurogenic niches that persist in defined areas of the postnatal brain. Furthermore, hippocampal neurogenesis peaks around 8–9 weeks and this can persist well into the postnatal period [25,37,38]. Gliogenesis (the development of glial cells) also begins during the *in utero* period and mature

Box 2. Leaky gut: at the core of brain–gut axis disorders?

The intestinal epithelium, the largest mucosal surface in the human body, provides an interface between the environment and the host, and is a key element in maintaining the equilibrium and overall health of the organism. In a healthy state, the intestinal epithelium and its associated tight-junction proteins (these include occludin, junctional adhesion molecule, and claudin family members that interact with cytoplasmic linker proteins such as zonula occludens-1) together with the mucus layer act as a physical barrier to bacteria and foreign antigens.

Under certain pathological conditions such as infections or stress, the integrity of the epithelial barrier is compromised and it becomes leaky, allowing for the translocation of pathobionts (pathological bacteria) across the mucosal lining to sites where direct interaction with immune cells and the enteric nervous system can occur [106]. This leads to activation of an immune response characterized by increased production of peripheral proinflammatory mediators and eventually the CNS (Figure 1).

Leaky gut or impaired intestinal permeability has been linked not only to the GI dysfunction but also to psychiatric disorders, such as depression and chronic fatigue syndrome [107–109]. For example, patients with major depression have been found to show increased peripheral blood antibody titers to lipopolysaccharides derived from Gram-negative enterobacteria as compared with normal volunteers [108]. Microbial-based strategies that enhance barrier function may be useful for such disorders. Indeed, in preclinical studies, administration of probiotics such as *Lactobacillus farciminis*, *Bacteroides fragilis*, and *Lactobacillus salivarius* can reverse the leakiness induced by acute stress, maternal infection, and hydrogen peroxide, respectively [86,110,111]. It is currently unclear whether the intestinal permeability is a cause or consequence of such brain disorders and future research efforts in this regard will be important for the field. Nonetheless, a better understanding of the role and the mechanisms of a leaky gut in the pathogenesis of brain–gut axis disorders will allow the advent of novel therapies aimed at re-establishing appropriate intestinal barrier function.

astrocytes are present in the brain by 15 weeks of gestation. Although these vary in density in different anatomical locations, they continue to differentiate throughout the fetal stage and well into the postnatal period [39]. This peak period of gliogenesis coincides with a large increase in neuronal complexity through elaboration of the dendritic fields [40], coupled with a robust increase in synaptogenesis only after astrocytes appear within the brain [41], suggesting mechanisms exist to ensure that appropriate neuronal–glial interactions are established.

Importantly, the developing brain has been shown to be susceptible to both internal and external environmental cues during prenatal life. Maternal diet, infection, prenatal stress, and microbial pathogen infections during the prenatal period have been associated with neurodevelopmental disorders such as autism, attention deficit hyperactivity disorder (ADHD), and schizophrenia [42–45]. Experimental studies in rodents provide further support for this notion, demonstrating that exposure to microbial pathogens during similar developmental periods results in behavioral abnormalities, including anxiety-like behavior and impaired cognitive function [42,46,47]. Maternal health plays a key role in microbiota development and neurodevelopment [48], therefore characterizing the composition of the microbiota during pregnancy and its contribution to the development of the newborn's microbiota, and potentially brain development is an important step in developing microbiota-modulating interventions (Figure 3).

Postnatal period
Microbiota development

During and shortly after birth, infants are exposed to microbes mainly originating from the mother. Growing evidence suggests that it is the inoculation and subsequent development of the intestinal microbiota in early life that is crucial for healthy development, especially neurodevelopment. The most dramatic changes in the composition of the intestinal microbiota take place postnatally. A plethora of factors influence the composition of the infant gut microbiota and potential functional outcomes (Figure 4). The initial microbial gut colonization is dependent on the birth delivery mode. Whereas vaginally born infants are colonized by fecal and vaginal bacteria from the mother, infants born by cesarean delivery are exposed to a different bacterial milieu closely related to that of the human skin and hospital environment [33,49]. Although reasons for these correlations are difficult to tease apart, it has been linked to the crucial role of the early life environment in the development of a healthy microbiome. Infants delivered by cesarean delivery are more likely to suffer from allergies, asthma, GI dysfunction, obesity, and diabetes later in life [50], yet it is currently unclear if it is the actual birth mode or the medical indication for this intervention that most influences brain development and behavior.

In addition to the birth delivery mode, gestational age is thought to contribute to the microbial composition of the host. For example, the microbiota of preterm infants lacks two of the main bacterial genera usually present in full-term infants [51]. Breastfeeding, however, enriches the microbiota of preterm infants with the missing microbial species, enhancing the ability of the infant microbiome to utilize human milk oligosaccharides [52]. In addition to the maternal role in the developing infant's microbiome [53], genetic and environmental factors play a role in defining the adult core microbiome. For example, twin studies revealed higher similarities in the microbiota composition between monozygotic and dizygotic twins in comparison to other family members, suggesting a significance of environmental factors over genetics [54,55], and that microbial ecologies tend to cluster in family members [56]. The contribution of genetic background and environmental factors to the microbiota of the host and subsequent functional outcomes remain to be fully elucidated. During the first days of life, gut microbiota of the infant is of low diversity and unstable, and stabilizes during breast or formula feeding. The precise composition of the developing microbiota population is dependent on whether the infant is breast or formula fed [57]. The microbiota of the formula-fed infants appears to be more diverse than breastfed infants, whose microbiota has a more stable colonized pattern [58,113,114]. However, breastfed infants demonstrate better neurodevelopmental outcomes and higher scores on intelligence tests [59], but whether these neurodevelopmental outcomes are a reflection of the microbiota composition remains to be elucidated. The next great changes in the composition of the intestinal microbiota come with the introduction of solid food and weaning, since diet plays a crucial role in modulating microbiota composition.
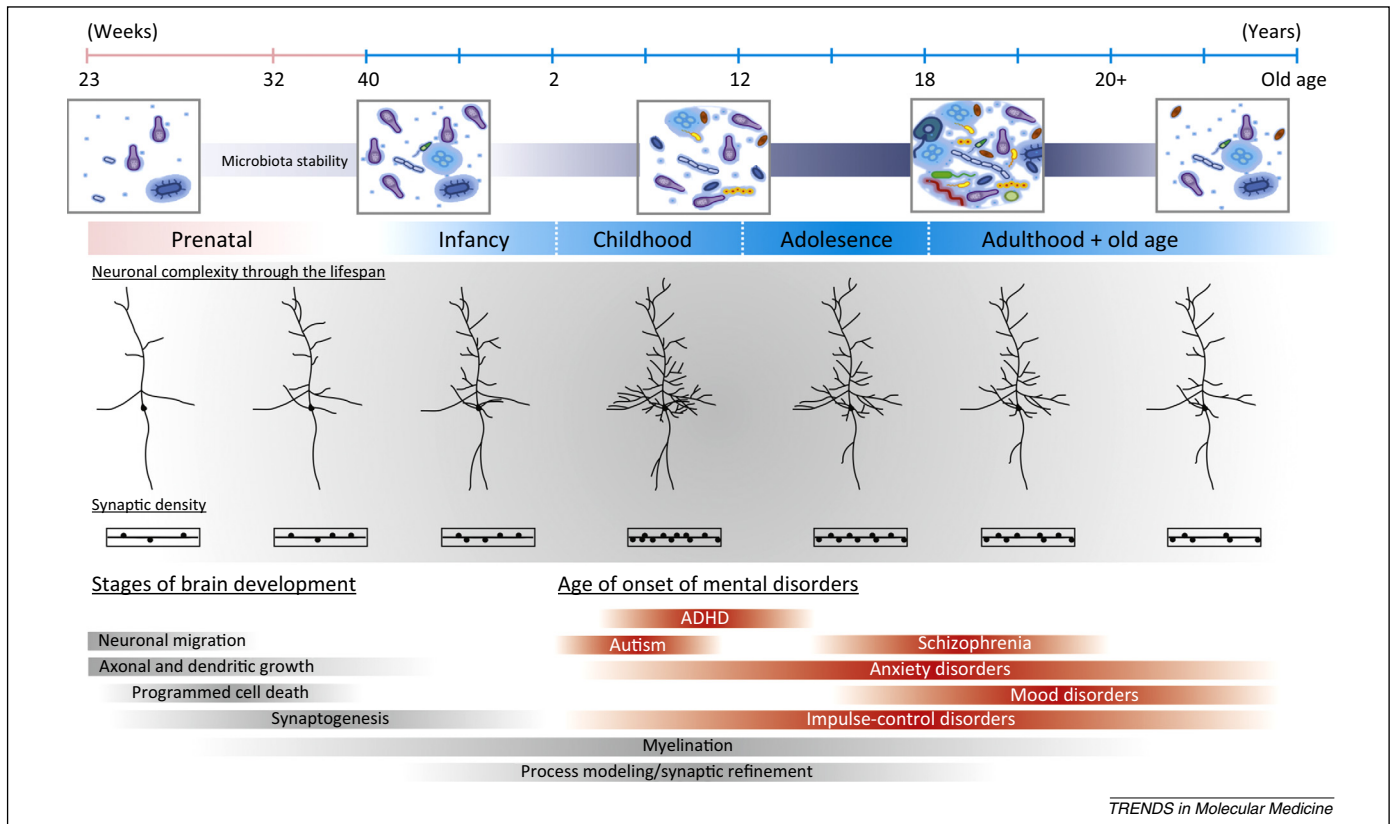
Figure 2. Temporal profile of neurodevelopmental sequences in relation to the age of onset of mental disorders and degree of microbiota stability/diversity throughout life. Gut microbiota is essential to human health and is a key player in the bidirectional communication between the gastrointestinal tract and the central nervous system. The microbiota dynamically changes throughout lifespan, establishing its symbiotic rapport with the host with critical windows during infancy, adolescence, and aging. During these windows, the organism is vulnerable to external stressors, which may result in mental disorders. Early life perturbations of the developing gut microbiota can impact neurodevelopment and potentially lead to adverse mental health outcomes later in life. The concept of parallel and interacting microbial–neural critical windows opens new avenues for developing novel microbiota-modulating based therapeutic interventions in early life to combat neurodevelopmental deficits and brain disorders. Abbreviation: ADHD, attention deficit hyperactivity disorder.

Neurodevelopment

The postnatal period is critical for brain development. For most vertebrates, the majority of organ and tissue development occurs during embryogenesis, and postnatal changes are primarily concerned with growth. However, the CNS is different in that a considerable amount of morphological development, cell differentiation, and acquisition of function takes place during postnatal development. Synaptogenesis begins in earnest in the human brain after approximately post-birth (after the appearance of astrocytes) and synaptic density increases rapidly after birth to reach maximum levels by approximately 2 years of age, at which point there are 50% more synapses than are found in the adult brain [36,60,61]. After this stage, the brain undergoes a process of synaptic refinement and elimination to reduce the number of synapses in a region-specific manner to adult levels by mid-adolescence [60,62,63]. Recent evidence demonstrates that developmental cortical remodeling, including the substantial elimination of synapses, continues well beyond adolescence and throughout the third decade of life, before stabilizing at adult levels [61]. This process of synaptic remodeling is not accompanied by any major neuronal loss, suggesting that this process of refinement reflects a strengthening and consolidation of newly formed neural circuits during childhood, which continues well into adulthood. This is an extraordinarily protracted phase of developmental

reorganization, which may reflect the extended phase of the development of cognitive abilities and the behavioral transition from developmental mode to adult status in humans [61]. What is clear is that this has profound implications for our understanding of how environmental exposures at specific stages of the lifespan may impact neural plasticity to modify neurobehavioral development, and what implications this may have for the risk of neurodevelopmental and neuropsychiatric disorders.

Childhood and adolescence period

Microbiota development

The gut microbiota, following initial colonization during infancy and birth, continues to develop throughout childhood and adolescence. Gradual changes in microbiota composition occur during early childhood, with a general reduction in the number of aerobes and facultative anaerobes and an increase in the populations of anaerobic species [64]. Although it is generally assumed that children's gut microbiota resembles that of an adult [65], recent studies demonstrate a less diverse microbiota with sufficiently different microbial gut communities in adolescent children in comparison to adults [66–68]. Furthermore, adolescence is associated with hormonal changes and fluctuations; however, not much is known about the effects of puberty and hormonal changes on microbiota development. It is currently unclear whether hormonal
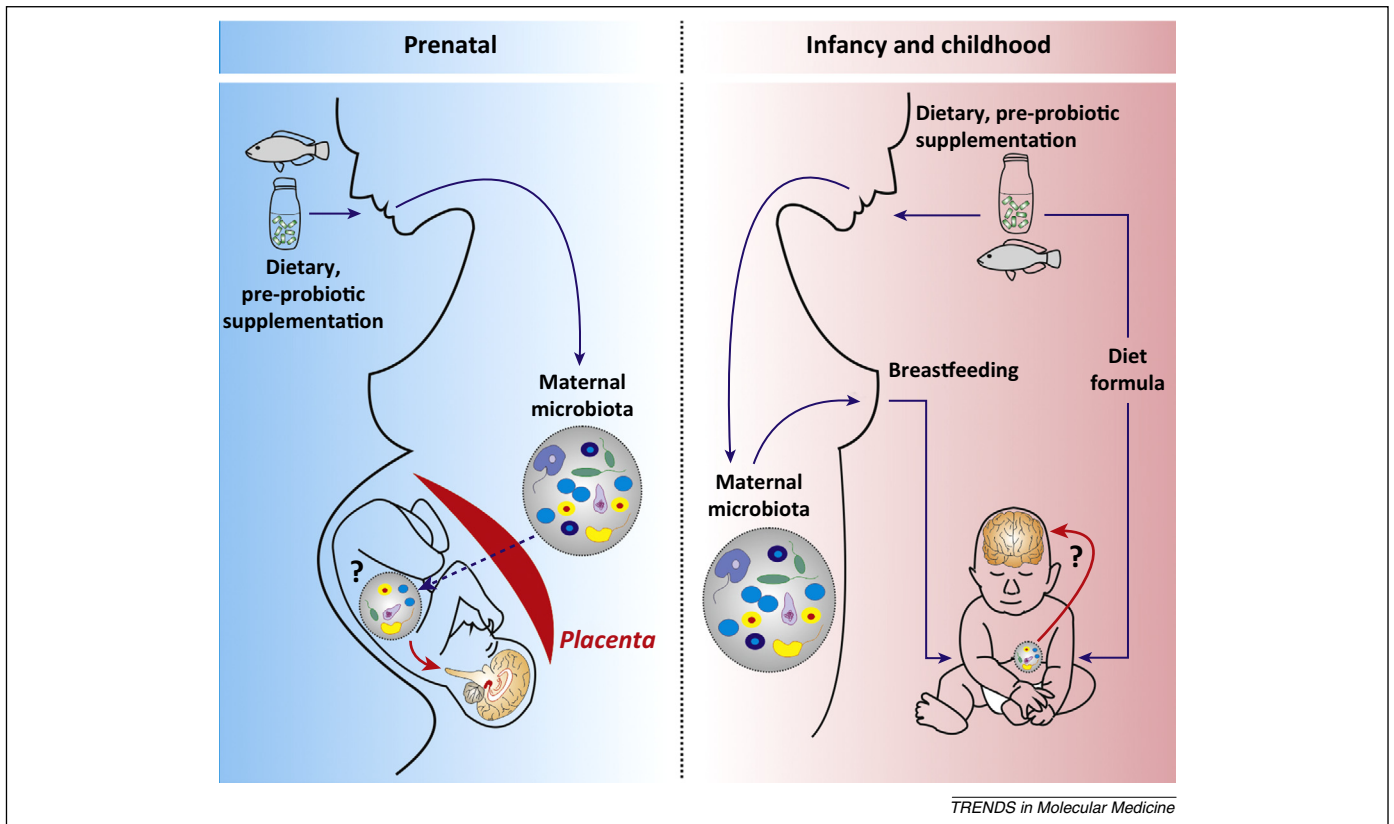
Figure 3. Windows of opportunity to modulate the microbiome of the infant prenatally and postnatally. Microbiota–gut–brain communication during prenatal and postnatal development is shown. Although still controversial, some evidence suggests that the microbiota of the infant before birth is not sterile, but may be influenced by the maternal immune state and nutrition. Prenatal and postnatal development undergoes vigorous neurodevelopmental phases and it is possible that it may be indirectly influenced by the fetal microbial population (via microbiota of the mother). This opens avenues for the development of novel dietary and microbe-modulating therapies, which may directly and indirectly alter the composition of the microbiota and neurodevelopment of the infant.

changes during adolescence have differential effects on the microbiota of females and males that may result in differential susceptibility of men and women to various disorders. For example, autism and schizophrenia have a higher occurrence in males [69], whereas mood disorders and inflammatory bowel syndrome are more prevalent in females [70,71]. Outstanding questions regarding the functional consequences of microbiota dysbiosis during this critical time period will be important challenges to be addressed in coming years.

It appears that instability and immaturity of gut microbiota during childhood and adolescence could be susceptible to environmental insults, such as the use of antibiotics, stress, harmful environment, poor diet, and infection, which could result in dysbiosis and potentially have a negative impact on general and mental health, leading to development of brain disorders later in life.

Neurodevelopment
Importantly, similar to gut microbiota development, brain maturation undergoes a crucial developmental phase during childhood and adolescence. The adolescence period is considered the most critical for development and onset of various brain disorders (Figure 2) [72]. Early adolescence is a key stage during neurodevelopment with various structural, neurochemical, and molecular changes occurring in response to genetic and environmental signals [72]. These include synaptic pruning, where the elimination of the

extra synapses occurs, resulting in decreased levels of cortical gray matter as the brain matures. Coinciding with this is the formation of new neuronal connections producing a phase of high plasticity throughout much of the brain. A consequence of this major neuronal rewiring during adolescence is a high level of vulnerability to pathological insults ranging from stress to drugs, to abuse, and to dietary deficiencies [72]. This developmental period is also the peak time for the onset of numerous psychiatric disorders including schizophrenia, substance abuse, and mood disorders [72] (Figure 2). Thus, the vulnerability of the adolescent brain to pathological insult in combination with instability and immaturity of gut microbiota during adolescence makes the brain exceptionally susceptible to aberrant changes that forecast the onset of brain disorders during this time period.

Adulthood and aging
As adulthood approaches, the gut microbiota stabilizes and becomes more diverse [65]. The adult gut microbiota is individual-specific and remains relatively stable over time [65], and can resist detrimental environmental elements such as use of antibiotics and stress by restoring its diverse and stable 'normal' core microbiota [73]. However, it is worth noting that recent evidence challenges the idea of the gut microbiota as stable during adulthood [74], suggesting that gut microbes can be rapidly altered by short-term changes in diet. At this point it is unclear whether
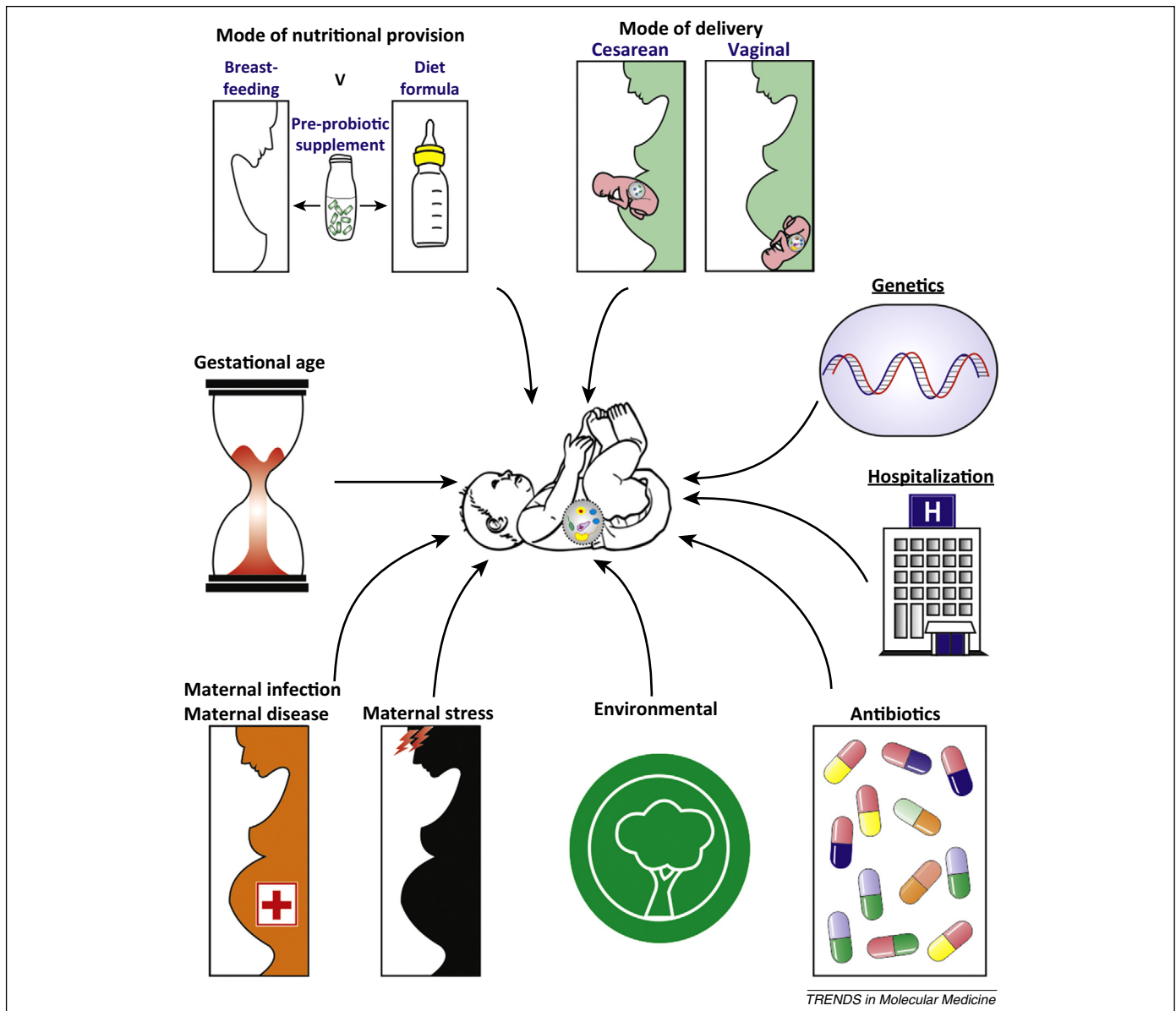
Figure 4. Factors influencing the development of the infant microbiota. Several factors play a role in shaping of the bacterial landscape in the development of the infant microbiota. In addition to mode of birth, mode of early nutrition, environment, other factors such as gestational age, genetics, and hospitalization, also influence the microbial composition of the infant. Infections (both maternal and infant) and antibiotic usage influence the trajectory of the developing microbiota as does the selective transient enrichment by probiotics and prebiotics. Taken together, such a plethora of factors with the ability to modulate the microbiota development suggest the importance of environmental influence superimposed over genetics in the establishment of a core microbiome.

CNS health could also be rapidly affected by such changes. Similar to the maturation and stabilization of the gut microbiota, continuous brain maturation occurs during this period of adulthood.

Regressive (e.g., synaptic pruning) and progressive (e.g., myelination) cellular events continue to occur in adulthood. Although the brain reaches its maximal weight by approximately 20 years of age [75], white matter volume continues to increase up until approximately the mid-forties [76], which coincides with the peak of myelination observed at approximately 50 years of age [76]. Although adulthood does not appear to be a critical or vulnerable phase, it remains a period during which alterations in the microbiota can influence brain and behavior. Therefore, maintaining a healthy gut microbiota and mental health is an important aspect in possible prevention or attenuation brain disorders associated with aging.

Although the aging period is not part of the neurodevelopmental category, it is still a critical window for both CNS and gut function. Aging can have a detrimental effect on the composition of the gut microbiota, which in turn might influence health outcomes at this stage of life [77]. The gut microbiome evolves throughout the lifespan, but microbiota diversity and stability decline with aging [78,79]. It has recently been shown that the microbial composition of aged individuals correlated with, and was influenced by, their residential community, dietary regimen, and health status of the individual [80]. In addition to a range of medications used by the elderly, impaired digestive and motility functions, and thus malabsorption of nutrients, and a weakened immune system contribute to compromised diversity and stability of the gut microbiota composition [77,79,81]. Decreased stability and diversity of the gut microbiota in the elderly is accompanied by reduced

brain volume and cognitive function. Indeed, brain weight begins to decline around approximately 55 years of age [75]. Volumetric imaging studies in aging populations have shown gray and white matter volume loss in an age-dependent manner, with peak loss occurring in most dorsal brain regions between the ages of 50 and 70 with a more gradual decline thereafter [82,83]. These age-associated changes in brain morphology are in parallel with the disrupted immune system, increased oxidative stress, and accumulation of amyloid plaques in the brain, all of which are reflected in weakened cognitive and behavioral function, and may manifest in various age-related memory impairments and disorders such as Alzheimer's disease.

Interestingly, findings from the ELDERMET (http://eldermet.ucc.ie) research consortium focused on prospectively characterizing the gut microbiota of elderly subjects, suggest the importance of nutrition on microbiota composition in the elderly [80]. These findings prompt the notion of modulating the microbiota of the elderly by dietary/microbiota-modifying interventions to restore microbiota diversity and thus improve general and possibly mental health, especially in such a critical window as aging.

Brain disorders and the microbiota–gut–brain axis: autism and beyond

Maternal infection and stress during pregnancy have been shown to increase the risk for neurodevelopmental disorders such as schizophrenia and autism in offspring (or distinct cognitive and behavioral pathological symptoms in later life). This association appears to be critically dependent on the precise prenatal timing of the insult. Neurodevelopmental disorders are characterized by impaired brain development and behavioral, cognitive, and/or physical abnormalities. Several share behavioral abnormalities in sociability, communication, and/or compulsive activity. Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterized by the presence of stereotypical behavior, communication, and social interaction deficits [84]. Although ASD etiology remains unknown, genetic and environmental factors are thought to play a role in its etiopathogenesis [85]. GI abnormalities associated with ASDs have been linked to alterations in microbiota composition and function [18,86–89]. Despite these correlational studies, clinical data interpretation is compromised by high rates of antibiotic use and marked dietary variations in ASD patients, which makes it difficult to draw definite conclusions about ASD-related microbiota changes [4]. Studies in germ-free mice demonstrated robust and reproducible social deficits and increases in repetitive behaviors similar to that observed in ASD [90], suggesting that the microbiota is a critical factor in the development of social behavior and the etiology of ASDs. Most recently, studies demonstrated that autism-like behavioral and GI phenotypes are associated with altered microbiota in two separate mouse models of ASDs [18,86]. Both clinical and preclinical studies provide promising evidence indicating an important role for the gut microbiota in the pathogenesis of ASDs, creating opportunities for developing novel therapeutic strategies in managing neurodevelopmental disorders via microbiome-based treatment. Indeed, *Bacteroides fragilis* given in early adolescence has been shown to ameliorate some, but not all, of the behavioral dysfunctions in

> **Box 3. Outstanding questions**
>
> - Does the microbiota during adolescence play a role in brain function and behavior?
> - What are the underlying mechanisms as to how bacteria in the gut signal to the brain? Does it change across the lifespan?
> - What is the reason for sex-specific effects in neurodevelopment and microbiota development and their consequences?
> - Can microbiota-based therapies be used in humans for treating mental disorders?

a mouse model of autism [86]. Moreover, whether other neurodevelopmental disorders such as schizophrenia and ADHD are associated with microbiota changes remains to be investigated either in animal models or human populations, and such studies are now warranted.

Concluding remarks and future perspectives

It is becoming clear that perturbations in microbiota can contribute to neurodevelopmental and psychiatric disorders onset later in life. Knowing that the microbiota can significantly interfere with the human cognitive and immune systems, the initiation of symbiosis, especially during prenatal, early postnatal, and adolescence phases appears to be a crucial step for optimizing brain development overall and mental health later in life. Although there seem to be critical windows in neurological and microbiota development, the underlying mechanisms of these effects remain to be elucidated (Box 3). Unraveling the mechanisms that trigger these sequelae will improve our knowledge of the etiology of neurodevelopmental psychiatric disorders, enable identification of biomarkers of dysfunction, and allow the identification of new windows of opportunity for the development of novel therapeutic interventions.

In conclusion, the concept of parallel and interacting microbial–neural critical windows opens new avenues for developing novel microbiota-modulating based therapeutic strategies in early life to combat neurodevelopmental deficits and brain disorders. Unlike a genetic background, the gut microbiota may be modified in throughout life and possibly pregnancy. Early preweaning and adolescence periods appear to be critical periods for modifying enteric microbiota with the potential to prevent the development of abnormal behaviors. Consequently, it is becoming clear that understanding the early interaction between the intestinal microbiota and the host opens novel avenues for nutritional/therapeutic interventions in at-risk populations, particularly for infants and young children.

References

1 Eckburg, P.B. *et al.* (2005) Diversity of the human intestinal microbial flora. *Science* 308, 1635–1638
2 Cho, I. and Blaser, M.J. (2012) The human microbiome: at the interface of health and disease. *Nat. Rev. Genet.* 13, 260–270
3 Stilling, R.M. *et al.* (2014) Microbial genes, brain & behaviour – epigenetic regulation of the gut–brain axis. *Genes Brain Behav.* 13, 69–86
4 Cryan, J.F. and Dinan, T.G. (2012) Mind-altering microorganisms: the impact of the gut microbiota on brain and behaviour. *Nat. Rev. Neurosci.* 13, 701–712
5 Aziz, Q. *et al.* (2013) Gut microbiota and gastrointestinal health: current concepts and future directions. *Neurogastroenterol. Motil.* 25, 4–15
6 Rhee, S.H. *et al.* (2009) Principles and clinical implications of the brain–gut–enteric microbiota axis. *Nat. Rev. Gastroenterol. Hepatol.* 6, 306–314

7 Collins, S.M. *et al.* (2012) The interplay between the intestinal microbiota and the brain. *Nat. Rev. Microbiol.* 10, 735–742

8 O'Mahony, S.M. *et al.* (2011) Maternal separation as a model of brain–gut axis dysfunction. *Psychopharmacology (Berl.)* 214, 71–88

9 Mayer, E.A. (2011) Gut feelings: the emerging biology of gut–brain communication. *Nat. Rev. Neurosci.* 12, 453–466

10 Grenham, S. *et al.* (2011) Brain–gut–microbe communication in health and disease. *Front. Physiol.* 2, 94

11 Cryan, J.F. and O'Mahony, S.M. (2011) The microbiome–gut–brain axis: from bowel to behavior. *Neurogastroenterol. Motil.* 23, 187–192

12 Aziz, Q. and Thompson, D.G. (1998) Brain–gut axis in health and disease. *Gastroenterology* 114, 559–578

13 Bonaz, B.L. and Bernstein, C.N. (2013) Brain–gut interactions in inflammatory bowel disease. *Gastroenterology* 144, 36–49

14 Davari, S. *et al.* (2013) Probiotics treatment improves diabetes-induced impairment of synaptic activity and cognitive function: behavioral and electrophysiological proofs for microbiome–gut–brain axis. *Neuroscience* 240, 287–296

15 Foster, J.A. (2013) Gut feelings: bacteria and the brain. *Cerebrum* 2013, 9

16 Moloney, R.D. *et al.* (2014) The microbiome: stress, health and disease. *Mamm. Genome* 25, 49–74

17 Diaz Heijtz, R. *et al.* (2011) Normal gut microbiota modulates brain development and behavior. *Proc. Natl. Acad. Sci. U.S.A.* 108, 3047–3052

18 de Theije, C.G. *et al.* (2014) Altered gut microbiota and activity in a murine model of autism spectrum disorders. *Brain Behav. Immun.* 37, 197–206

19 Ben-Ari, Y. (2013) Neuropaediatric and neuroarchaeology: understanding development to correct brain disorders. *Acta Paediatr.* 102, 331–334

20 Rapoport, J.L. *et al.* (2012) Neurodevelopmental model of schizophrenia: update 2012. *Mol. Psychiatry.* 17, 1228–1238

21 Thompson, B.L. *et al.* (2009) Prenatal exposure to drugs: effects on brain development and implications for policy and education. *Nat. Rev. Neurosci.* 10, 303–312

22 Sudo, N. *et al.* (2004) Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. *J. Physiol.* 558, 263–275

23 Rager, G. (1978) Systems-matching by degeneration II. Interpretation of the generation and degeneration of retinal ganglion cells in the chicken by a mathematical model. *Exp. Brain Res.* 33, 79–90

24 Herrup, K. and Sunter, K. (1987) Numerical matching during cerebellar development: quantitative analysis of granule cell death in staggerer mouse chimeras. *J. Neurosci.* 7, 829–836

25 Workman, A.D. *et al.* (2013) Modeling transformations of neurodevelopmental sequences across mammalian species. *J. Neurosci.* 33, 7368–7383

26 Funkhouser, L.J. and Bordenstein, S.R. (2013) Mom knows best: the universality of maternal microbial transmission. *PLoS Biol.* 11, e1001631

27 DiGiulio, D.B. *et al.* (2008) Microbial prevalence, diversity and abundance in amniotic fluid during preterm labor: a molecular and culture-based investigation. *PLoS ONE* 3, e3056

28 Jimenez, E. *et al.* (2008) Is meconium from healthy newborns actually sterile? *Res. Microbiol.* 159, 187–193

29 Satokari, R. *et al.* (2009) Bifidobacterium and Lactobacillus DNA in the human placenta. *Lett. Appl. Microbiol.* 48, 8–12

30 Rautava, S. *et al.* (2012) Probiotics modulate host–microbe interaction in the placenta and fetal gut: a randomized, double-blind, placebo-controlled trial. *Neonatology* 102, 178–184

31 Jimenez, E. *et al.* (2005) Isolation of commensal bacteria from umblicial cord blood of healthy neonates born by cesarean section. *Curr. Microbiol.* 51, 270–274

32 Aagaard, K. *et al.* (2014) The placenta harbors a unique microbiome. *Sci. Transl. Med.* 6, 237ra65

33 Dominguez-Bello, M.G. *et al.* (2010) Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc. Natl. Acad. Sci. U.S.A.* 107, 11971–11975

34 DeSesso, J.M. *et al.* (1999) Apparent lability of neural tube closure in laboratory animals and humans. *Am. J. Med. Genet.* 87, 143–162

35 Rice, D. and Barone, S., Jr (2000) Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ. Health Perspect.* 108 (Suppl. 3), 511–533

36 Herschkowitz, N. *et al.* (1997) Neurobiological bases of behavioral development in the first year. *Neuropediatrics* 28, 296–306

37 Clancy, B. *et al.* (2007) Web-based method for translating neurodevelopment from laboratory species to humans. *Neuroinformatics* 5, 79–94

38 Rakic, P. and Nowakowski, R.S. (1981) The time of origin of neurons in the hippocampal region of the rhesus monkey. *J. Comp. Neurol.* 196, 99–128

39 Roessmann, U. and Gambetti, P. (1986) Astrocytes in the developing human brain, An immunohistochemical study. *Acta Neuropathol.* 70, 308–313

40 Wise, S.P. and Jones, E.G. (1976) The organization and postnatal development of the commissural projection of the rat somatic sensory cortex. *J. Comp. Neurol.* 168, 313–343

41 Barker, A.J. and Ullian, E.M. (2010) Astrocytes and synaptic plasticity. *Neuroscientist* 16, 40–50

42 Marques, A.H. *et al.* (2013) The influence of maternal prenatal and early childhood nutrition and maternal prenatal stress on offspring immune system development and neurodevelopmental disorders. *Front. Neurosci.* 7, 120

43 Bale, T.L. *et al.* (2010) Early life programming and neurodevelopmental disorders. *Biol. Psychiatry* 68, 314–319

44 Mittal, V.A. *et al.* (2008) Gene–environment interaction and covariation in schizophrenia: the role of obstetric complications. *Schizophr. Bull.* 34, 1083–1094

45 Finegold, S.M. (2011) State of the art; microbiology in health and disease, Intestinal bacterial flora in autism. *Anaerobe* 17, 367–368

46 Goehler, L.E. *et al.* (2008) *Campylobacter jejuni* infection increases anxiety-like behavior in the holeboard: possible anatomical substrates for viscerosensory modulation of exploratory behavior. *Brain Behav. Immun.* 22, 354–366

47 Bilbo, S.D. *et al.* (2005) Neonatal infection-induced memory impairment after lipopolysaccharide in adulthood is prevented via caspase-1 inhibition. *J. Neurosci.* 25, 8000–8009

48 Donnet-Hughes, A. *et al.* (2010) Potential role of the intestinal microbiota of the mother in neonatal immune education. *Proc. Nutr. Soc.* 69, 407–415

49 Biasucci, G. *et al.* (2010) Mode of delivery affects the bacterial community in the newborn gut. *Early Hum. Dev.* 86 (Suppl. 1), 13–15

50 Jakobsson, H.E. *et al.* (2014) Decreased gut microbiota diversity, delayed Bacteroidetes colonisation and reduced Th1 responses in infants delivered by caesarean section. *Gut* 63, 559–566

51 Barrett, E. *et al.* (2013) The individual-specific and diverse nature of the preterm infant microbiota. *Arch. Dis. Child. Fetal Neonatal Ed.* 98, F334–F340

52 Costello, E.K. *et al.* (2012) The application of ecological theory toward an understanding of the human microbiome. *Science* 336, 1255–1262

53 Parfrey, L.W. and Knight, R. (2012) Spatial and temporal variability of the human microbiota. *Clin. Microbiol. Infect.* 18 (Suppl. 4), 8–11

54 Turnbaugh, P.J. *et al.* (2009) A core gut microbiome in obese and lean twins. *Nature* 457, 480–484

55 Lozupone, C.A. *et al.* (2012) Diversity, stability and resilience of the human gut microbiota. *Nature* 489, 220–230

56 Maynard, C.L. *et al.* (2012) Reciprocal interactions of the intestinal microbiota and immune system. *Nature* 489, 231–241

57 Thum, C. *et al.* (2012) Can nutritional modulation of maternal intestinal microbiota influence the development of the infant gastrointestinal tract? *J. Nutr.* 142, 1921–1928

58 Schwartz, S. *et al.* (2012) A metagenomic study of diet-dependent interaction between gut microbiota and host in infants reveals differences in immune response. *Genome Biol.* 13, r32

59 Kramer, M.S. *et al.* (2008) Breastfeeding and child cognitive development: new evidence from a large randomized trial. *Arch. Gen. Psychiatry* 65, 578–584

60 Huttenlocher, P.R. (1979) Synaptic density in human frontal cortex – developmental changes and effects of aging. *Brain Res.* 163, 195–205

61 Petanjek, Z. *et al.* (2011) Extraordinary neoteny of synaptic spines in the human prefrontal cortex. *Proc. Natl. Acad. Sci. U.S.A.* 108, 13281–13286

62 Glantz, L.A. *et al.* (2007) Synaptophysin and postsynaptic density protein 95 in the human prefrontal cortex from mid-gestation into early adulthood. *Neuroscience* 149, 582–591

63 Huttenlocher, P.R. and Dabholkar, A.S. (1997) Regional differences in synaptogenesis in human cerebral cortex. *J. Comp. Neurol.* 387, 167–178

64 Hopkins, M.J. *et al.* (2002) Variation in human intestinal microbiota with age. *Dig. Liver Dis.* 34 (Suppl. 2), S12–S18

65 Palmer, C. *et al.* (2007) Development of the human infant intestinal microbiota. *PLoS Biol.* 5, e177

66 Agans, R. *et al.* (2011) Distal gut microbiota of adolescent children is different from that of adults. *FEMS Microbiol. Ecol.* 77, 404–412

67 Guarino, A. *et al.* (2012) Composition and roles of intestinal microbiota in children. *J. Matern. Fetal Neonat. Med.* 25 (Suppl. 1), 63–66

68 Ringel-Kulka, T. *et al.* (2013) Intestinal microbiota in healthy U.S. young children and adults – a high throughput microarray analysis. *PLoS ONE* 8, e64315

69 Jacquemont, S. *et al.* (2014) A higher mutational burden in females supports a "female protective model" in neurodevelopmental disorders. *Am. J. Hum. Genet.* 94, 415–425

70 McHenry, J. *et al.* (2014) Sex differences in anxiety and depression: role of testosterone. *Front. Neuroendocrinol.* 35, 42–57

71 Loftus, E.V., Jr and Sandborn, W.J. (2002) Epidemiology of inflammatory bowel disease. *Gastroenterol. Clin. North Am.* 31, 1–20

72 Paus, T. *et al.* (2008) Why do many psychiatric disorders emerge during adolescence? *Nat. Rev. Neurosci.* 9, 947–957

73 Rajilic-Stojanovic, M. *et al.* (2013) Long-term monitoring of the human intestinal microbiota composition. *Environ. Microbiol.* 15, 1146–1159

74 David, L.A. *et al.* (2014) Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 505, 559–563

75 Dekaban, A.S. (1978) Changes in brain weights during the span of human life: relation of brain weights to body heights and body weights. *Ann. Neurol.* 4, 345–356

76 Sowell, E.R. *et al.* (2003) Mapping cortical change across the human life span. *Nat. Neurosci.* 6, 309–315

77 O'Toole, P.W. and Claesson, M.J. (2010) Gut microbiota: changes throughout the lifespan from infancy to elderly. *Int. Dairy J.* 20, 281–291

78 Claesson, M.J. *et al.* (2011) Composition, variability, and temporal stability of the intestinal microbiota of the elderly. *Proc. Natl. Acad. Sci. U.S.A.* 108 (Suppl. 1), 4586–4591

79 Biagi, E. *et al.* (2010) Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. *PLoS ONE* 5, e10667

80 Claesson, M.J. *et al.* (2012) Gut microbiota composition correlates with diet and health in the elderly. *Nature* 488, 178–184

81 Biagi, E. *et al.* (2013) Ageing and gut microbes: perspectives for health maintenance and longevity. *Pharmacol. Res.* 69, 11–20

82 Bartzokis, G. *et al.* (2003) White matter structural integrity in healthy aging adults and patients with Alzheimer disease: a magnetic resonance imaging study. *Arch. Neurol.* 60, 393–398

83 Ge, Y. *et al.* (2002) Age-related total gray matter and white matter changes in normal adult brain, Part I: volumetric MR imaging analysis. *Am. J. Neuroradiol.* 23, 1327–1333

84 Happe, F. *et al.* (2006) Executive function deficits in autism spectrum disorders and attention-deficit/hyperactivity disorder: examining profiles across domains and ages. *Brain Cogn.* 61, 25–39

85 Grabrucker, A.M. (2012) Environmental factors in autism. *Front. Psychiatry* 3, 118

86 Hsiao, E.Y. *et al.* (2013) Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell* 155, 1451–1463

87 Douglas-Escobar, M. *et al.* (2013) Effect of intestinal microbial ecology on the developing brain. *JAMA Pediatr.* 167, 374–379

88 Mulle, J.G. *et al.* (2013) The gut microbiome: a new frontier in autism research. *Curr. Psychiatry Rep.* 15, 337

89 Macfabe, D.F. (2012) Short-chain fatty acid fermentation products of the gut microbiome: implications in autism spectrum disorders. *Microb. Ecol. Health Dis.* 23, 19260

90 Desbonnet, L. *et al.* (2014) Microbiota is essential for social development in the mouse. *Mol. Psychiatry* 19, 146–148

91 Power, S.E. *et al.* (2014) Intestinal microbiota, diet and health. *Br. J. Nutr.* 111, 387–402

92 Dantzer, R. (2009) Cytokine, sickness behavior, and depression. *Immunol. Allergy Clin. North Am.* 29, 247–264

93 Goehler, L.E. *et al.* (2005) Activation in vagal afferents and central autonomic pathways: early responses to intestinal infection with *Campylobacter jejuni. Brain Behav. Immun.* 19, 334–344

94 Forsythe, P. *et al.* (2010) Mood and gut feelings. *Brain Behav. Immun.* 24, 9–16

95 Wang, X. *et al.* (2002) Evidences for vagus nerve in maintenance of immune balance and transmission of immune information from gut to brain in STM-infected rats. *World J. Gastroenterol.* 8, 540–545

96 Borovikova, L.V. *et al.* (2000) Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. *Nature* 405, 458–462

97 Lyte, M. *et al.* (2006) Induction of anxiety-like behavior in mice during the initial stages of infection with the agent of murine colonic hyperplasia *Citrobacter rodentium. Physiol. Behav.* 89, 350–357

98 Perez-Burgos, A. *et al.* (2013) Psychoactive bacteria *Lactobacillus rhamnosus* (JB-1) elicits rapid frequency facilitation in vagal afferents. *Am. J. Physiol. Gastrointest. Liver Physiol.* 304, G211–G220

99 Ruddick, J.P. *et al.* (2006) Tryptophan metabolism in the central nervous system: medical implications. *Expert Rev. Mol. Med.* 8, 1–27

100 Desbonnet, L. *et al.* (2008) The probiotic *Bifidobacteria infantis*: an assessment of potential antidepressant properties in the rat. *J. Psychiatr. Res.* 43, 164–174

101 Cameron, J. and Doucet, E. (2007) Getting to the bottom of feeding behaviour: who's on top? *Appl. Physiol. Nutr. Metab.* 32, 177–189

102 Wren, A.M. and Bloom, S.R. (2007) Gut hormones and appetite control. *Gastroenterology* 132, 2116–2130

103 Schele, E. *et al.* (2013) The gut microbiota reduces leptin sensitivity and the expression of the obesity-suppressing neuropeptides proglucagon (Gcg) and brain-derived neurotrophic factor (Bdnf) in the central nervous system. *Endocrinology* 154, 3643–3651

104 Macfarlane, S. and Macfarlane, G.T. (2003) Regulation of short-chain fatty acid production. *Proc. Nutr. Soc.* 62, 67–72

105 Kimura, I. *et al.* (2013) The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat. Commun.* 4, 1829

106 Gareau, M.G. *et al.* (2008) Pathophysiological mechanisms of stress-induced intestinal damage. *Curr. Mol. Med.* 8, 274–281

107 Maes, M. *et al.* (2012) Increased IgA and IgM responses against gut commensals in chronic depression: further evidence for increased bacterial translocation or leaky gut. *J. Affect. Disord.* 141, 55–62

108 Maes, M. *et al.* (2008) The gut–brain barrier in major depression: intestinal mucosal dysfunction with an increased translocation of LPS from gram negative enterobacteria (leaky gut) plays a role in the inflammatory pathophysiology of depression. *Neuro Endocrinol. Lett.* 29, 117–124

109 Maes, M. and Leunis, J.C. (2008) Normalization of leaky gut in chronic fatigue syndrome (CFS) is accompanied by a clinical improvement: effects of age, duration of illness and the translocation of LPS from gram-negative bacteria. *Neuro Endocrinol Lett.* 29, 902–910

110 Ait-Belgnaoui, A. *et al.* (2012) Prevention of gut leakiness by a probiotic treatment leads to attenuated HPA response to an acute psychological stress in rats. *Psychoneuroendocrinology* 37, 1885–1895

111 Miyauchi, E. *et al.* (2012) Mechanism of protection of transepithelial barrier function by *Lactobacillus salivarius*: strain dependence and attenuation by bacteriocin production. *Am. J. Physiol. Gastrointest. Liver Physiol.* 303, G1029–G1041

112 Clarke, G. *et al.* (2013) The microbiome–gut–brain axis during early life regulates the hippocampal serotonergic system in a sex-dependent manner. *Mol. Psychiatry* 18, 666–673

113 Fan, W. *et al.* (2014) Impact of diet in shaping gut microbiota revealed by a comparative study in infants during the six months of life. *J. Microbiol. Biotechnol.* 24, 133–143

114 Roger, L.C. *et al.* (2010) Examination of faecal Bifidobacterium populations in breast- and formula-fed infants during the first 18 months of life. *Microbiology* 156, 3329–3341