Trends in Molecular Medicine

CellPress REVIEWS

Feature Review

Bacterial Peptidoglycans from Microbiota in Neurodevelopment and Behavior

Ayoze Gonzalez-Santana[1] and Rochellys Diaz Heijtz[1,2,*]

It is increasingly recognized that the gut microbiota profoundly influences many aspects of host development and physiology, including the modulation of brain development and behavior. However, the precise molecular mechanisms and signaling pathways involved in communication between the microbiota and the developing brain remain to be fully elucidated. Germline-encoded pattern-recognition receptors (PRRs) that recognize conserved microbial molecular signatures such as bacterial surface molecules (e.g., peptidoglycans, PGNs) have emerged as potential key regulators of gut microbiota–brain interactions. We highlight current evidence supporting multiple and essential roles for PGNs and their sensing molecules beyond innate immunity, extending to neurodevelopment and behavior. In addition, the possible implications of the PGN signaling pathway for the pathogenesis of neurodevelopmental disorders such as autism spectrum disorder (ASD) are considered.

Highlights

The gut microbiota has emerged as a key regulator of brain development and behavior. However, the mechanisms mediating the interactions between the microbiota and the developing brain are still poorly understood.

The central activation of PRRs by bacteria-derived products such as PGN (a major component of the bacterial cell wall) could represent a potential pathway affecting the developing brain and behavior.

PGN-sensing molecules are abundantly expressed in the placenta and developing brain during specific time-windows of perinatal development, and have been implicated in the regulation of social behavior and anxiety, as well as in behavioral responses to stress.

PGN and its sensing molecules represent an attractive biological pathway for the development of novel biomarkers and therapeutic strategies for neurodevelopmental and psychiatric disorders.

The Gut Microbiota: A Key Regulator of Neurodevelopment and Behavior

The mammalian gastrointestinal (GI) tract harbors a dynamic and complex community of microorganisms, including bacteria, archaea, fungi, and viruses – known collectively as the gut microbiota. Microbial colonization is an evolution-driven process that has provided mutual benefits to both microbes and the mammalian host. The first major exposure to a complex microbiota occurs during birth via vertical mother-to-infant microbial transmission (i.e., when infants are exposed to their maternal vaginal, fecal, and perineal microbes), and is a pivotal early life event that determines the initial assembly of the infant gut microbiota [1] (Box 1). The bacterial population is currently the best-characterized. Although the ratio between bacteria to human cells is close to one [2], the total number of bacterial genes in the collective human microbiome is estimated to be 2–20 million, exceeding the human genome by at least a factor of 100 [3,4]. Many of these bacterial genes encode enzymes that perform metabolic functions that are not achievable by the host and produce metabolites that can impact on host health and disease. Over the past decades, studies have demonstrated that gut bacteria interact and perform numerous crucial functions for their host, including dietary energy extraction, the production of vitamins, protection against invading gut pathogens [5,6], as well as promoting the development and function of the immune system [1,7]. Recent investigations have revealed that the gut microbiota exerts a broader range of effects on host physiology and development beyond the GI tract, including the modulation of brain development and behavior [8,9].

In a landmark study, Sudo and colleagues in 2004 showed that postnatal bacterial colonization affects early-life programming of the hypothalamic–pituitary–adrenal (HPA) axis, the central stress response system [10]. More specifically, they took advantage of germ-free (GF) mice (devoid of bacteria throughout life) to assess how absence of the microbiota affects the HPA axis response to various stress modalities. In adult GF mice, exposure to a mild restraint stress induced an exaggerated release of adrenocorticotropic hormone (ACTH) and corticosterone compared with control mice with a normal composition of microbiota and no specific pathogens (known as

[1]Department of Neuroscience, Karolinska Institutet, Biomedicum, 171 77 Stockholm, Sweden
[2]Institut National de la Santé et de la Recherche Médicale (INSERM) Unité 1239, University of Rouen Normandy, 76130 Rouen, France

*Correspondence: rochellys.heijtz@ki.se (R. Diaz Heijtz).

Box 1. Factors Influencing the Development of the Infant Microbiota

During early postnatal life, the development of the infant gut microbiota follows successive waves of microbial exposure and colonization that are influenced by age-associated events such as dietary transitions (e.g., cessation of breastfeeding and the introduction of solid foods into the diet), leading to a more complex and diverse adult-like ecosystem around the first 2–3 years of age [109]. However, the exact age at which the toddler gut microbiota comes to resemble the adult pattern may vary depending on the prenatal and neonatal history.

Several factors play an important role in shaping the development of the infant microbiota and potential functional outcomes; these include the mode of delivery (cesarean delivery vs vaginal delivery), breastfeeding practices (breast milk vs formula feeding), and antibiotic use [8,9]. In addition, other factors such as length of gestation (i.e., full-term vs pre-term birth), nutritional status (under- and overnutrition), hospitalization, and genetics can have a profound impact on neonatal microbial colonization trajectories. The fact that the assembly and maturation of the infant gut microbiota co-occurs in parallel with key neurodevelopmental process (e.g., myelination, synaptogenesis, synaptic refinement, and acquisition of brain functions) has led to realization that the early postnatal period represents a critical time-window for gut microbiota–brain interactions, and also a key determinant of health in later life [8,9].

specific pathogen-free mice, SPF). The exaggerated HPA stress responses of GF mice were fully reversed through colonization with *Bifidobacterium infantis*. Several years later, Diaz Heijtz and colleagues demonstrated that the absence of microbiota profoundly affects motor activity and anxiety-like behavior, as well as the expression of four canonical pathways (i.e., Krebs cycle, synaptic long-term potentiation, steroid hormone metabolism, and cAMP-mediated signaling) and the turnover of neurotransmitters including dopamine and serotonin [11]. Importantly, microbiota reconstitution of GF mice in early life partially normalized their behavioral phenotypes, whereas colonization of adult GF mice had no effect. Together, these observations led to the concept of a sensitive period, early in life, in which microbial colonization of the gut can influence brain development, function, and behavior. Around the same time other groups independently published similar findings ([12,13]; online Milestone 18: the microbiota–gut–brain axis, in *Nature Milestones in Human Microbiota Research*: www.nature.com/immersive/d42859-019-00041-z/index.html). Since then, several studies have demonstrated that the gut microbiota modulates a wide range of neurodevelopmental processes, including blood–brain barrier (BBB) formation and integrity [14], microglial maturation and function [15,16], and myelination [17–19], as well as several complex behaviors [20,21]. One important concept that has emerged from these preclinical studies is that some effects of the gut microbiota on the brain are sex-specific [22]. Other studies showed that gut bacteria influence behavioral abnormalities and associated brain pathology in animal models of neurodevelopmental and psychiatric disorders [23–25]. These preclinical and other clinical findings lent support to the hypothesis that gut microbiota may also influence the development of the human brain, and that its disruption could contribute to the pathogenesis of these disorders [25,26].

It is now increasingly recognized that neurodevelopmental disorders such as ASD are often comorbid with GI problems. For example, children with ASD have been reported to be 6–8-fold more likely to suffer from one or more chronic GI problems than are typically developing children [27]. These GI issues include frequent abdominal pain, diarrhea, constipation, gaseousness, and painful stooling. Moreover, some studies have found a strong positive association between autism severity and GI dysfunction [28,29]. These associations of ASD with increased prevalence of GI issues and reports of altered gut bacterial composition in this population motivate further explorations into the precise molecular mechanisms mediating crosstalk between the gut microbiota and the developing brain [26,30]. The gut microbiota contains trillions of bacteria which are source of a diverse peptidoglycome, bacterial PGN motifs that are shed from the cell wall as bacteria divide [31,32]. In this review we highlight the emerging evidence supporting novel roles for bacterial PGN (a unique and essential component of the bacterial cell wall that is absent from eukaryotic cells) in neurodevelopment and

behavior, after a general overview of key biological signaling pathways implicated in the microbiota–gut–brain axis. Finally, we discuss how alterations in the PGN signaling pathways early in life could impact on neurodevelopmental trajectories and increase the risk of neurodevelopmental disorders.

Biological Signaling Pathways in the Microbiota–Gut–Brain Axis

It has long been known that a complex bidirectional communication network exits between the gut and the central nervous system (CNS), referred to as the gut–brain axis. This network includes the sympathetic and parasympathetic branches of the autonomic nervous system, the enteric nervous system, and neuroendocrine and neuroimmune pathways. In recent decades the gut microbiota has been recognized as an important 'third component' of the gut–brain axis, thus leading to the new concept of the microbiota–gut–brain axis. However, the precise molecular mechanisms mediating interactions between gut microbes and the brain remain to be clearly defined. Emerging evidence suggests the involvement of multiple potential direct and indirect pathways (Figure 1), including signals carried by neuronal circuits (e.g., bidirectional vagus nerve-to-brain communication, and the enteric nervous system), activation of immune responses (e.g., cytokine and chemokine release within the gut or elsewhere that subsequently influence the brain), gut hormone signaling, tryptophan metabolism, and the production of microbial metabolites that indirectly or directly influence the brain, including bacterial fermentation metabolic byproducts such as short-chain fatty acids (SCFAs; propionate, butyrate, and acetate) [33].

Undoubtedly, one of the most direct routes for the microbiota to communicate with the brain is through the vagus nerve [34]. Studies have shown that several probiotics (beneficial bacteria that produce health outcomes such as *Bifidobacterium* and *Lactobacillus* families, which do not possess proinflammatory lipopolysaccharide chains) utilize the vagus nerve signaling to communicate with the brain and influence behavior [35]. For example, the beneficial effects of *Lactobacillus rhamnosus* JB1 on stress-induced anxiety- and depression-related behavior are abolished by vagotomy [36]. Similarly, the vagus nerve is a central gut–brain communication pathway by which *Lactobacillus reuteri* MM4-1A rescues social deficits in several mouse models of ASD, including a genetic model (*Shank3B* mutant mice) [37], three environmental models (valproic acid, GF, and maternal high-fat diet) [37,38], and an idiopathic model of ASD (BTBR T^{+} Itpr3^{tf}/J mice) [37]. However, the precise mechanisms by which *L. reuteri* interacts with the vagus nerve are not fully understood. It has recently been discovered that sensory cells from the GI tract with a primary endocrine function (enteroendocrine cells; referred to as neuropods) possess axon-like basal processes that form synapses with vagal afferents [39]. Therefore, it will be interesting to explore whether the actions of probiotics such as *L. reuteri* involve signaling through the neuropods. A recent optogenetic study showed that activation of gut-innervating vagal sensory ganglion neurons can also modulate reward-related behavior (e.g., self-stimulation behavior and conditioned place preference) and dopamine release from the substantial nigra in mice, indicating that the vagal gut-to-brain axis is also an important component of the brain reward system [40].

In recent years there has been keen interest in the role of SCFAs because they exert many beneficial effects on host physiology (e.g., they enhance the integrity of the intestinal epithelial barrier and protect against inflammation) [41]. Because SCFAs can interact with the gut–brain axis through several different mechanisms, including modulating host chromatin structure and gene transcription as well as activating G protein-coupled receptors, exposure to these bacterial metabolites during critical time-windows of development might have important implications for early-life programming of the brain and behavior. There is evidence that acetate can cross the BBB at physiological levels [42], but it is unclear to what extent propionate and butyrate cross into the brain. Nevertheless, some studies have demonstrated that butyrate can restore the

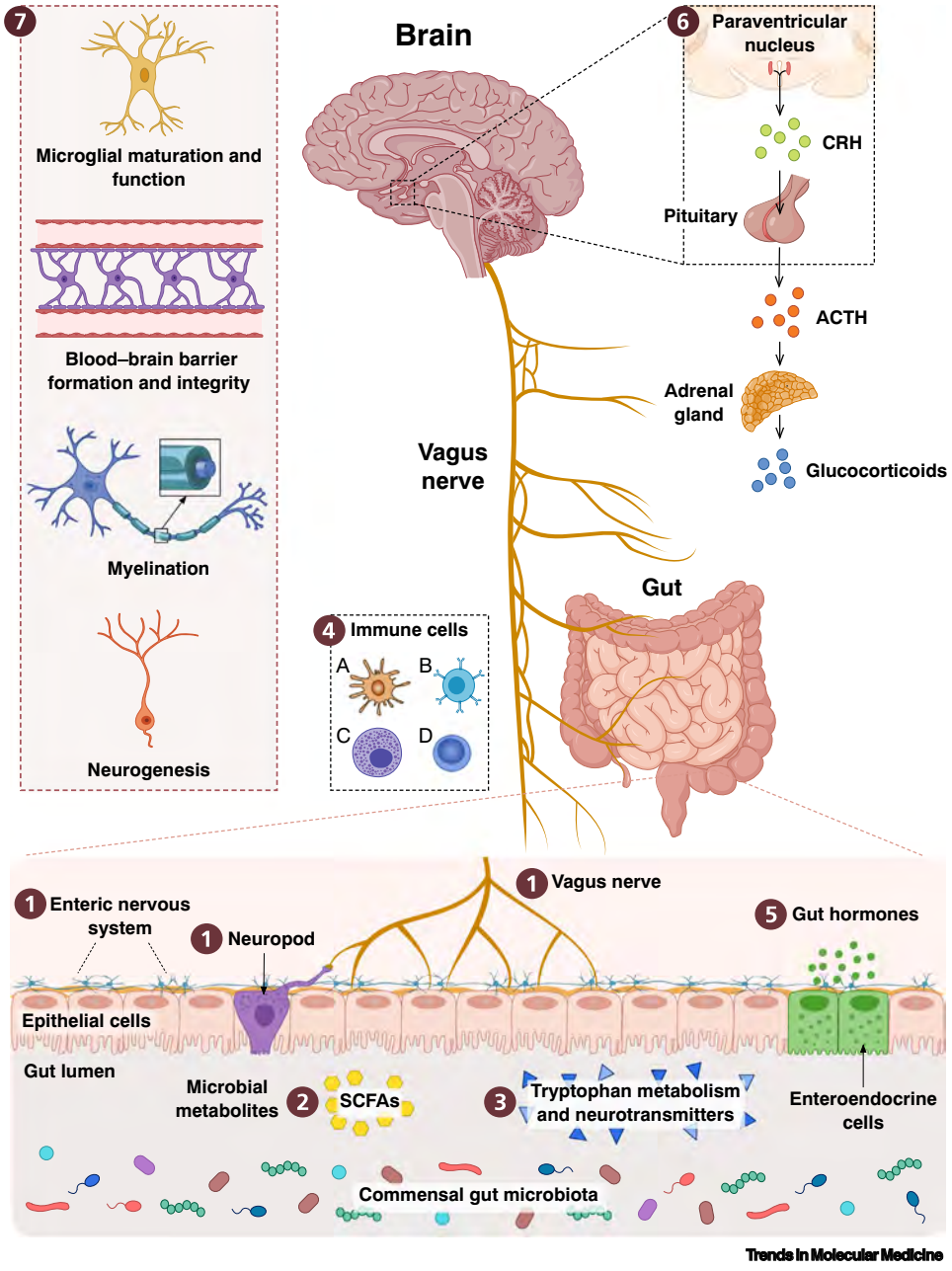

Figure 1. Biological Signaling Pathways and Molecules Involved in the Microbiota–Gut–Brain Axis. There are multiple direct and indirect pathways through which gut microbiota may influence the brain, including signals carried by neuronal circuits [e.g., bidirectional vagus nerve-to-brain communication, the enteric nervous system, and neuropods (1)], the production of bacterial fermentation metabolic byproducts such as short-chain fatty acids [SCFAs; propionate, butyrate, and acetate (2)], tryptophan metabolites and neurotransmitters (3), the release of cytokines by immune cells [(A) dendritic cell, (B) B cell, (C) mast cell, (D) T cell (4)], and gut hormone signaling (5). Some of these molecules can activate the vagus nerve or reach the brain via the systemic circulation, and directly affect brain functions. Activation of the hypothalamic–pituitary–adrenal (HPA) axis is characterized by the release of corticotropin-releasing hormone (CRH) from the paraventricular nucleus, which then stimulates the release of adrenocorticotropic hormone (ACTH) from the anterior pituitary gland. ACTH then acts on the adrenal cortex to stimulate the production and release of glucocorticoids (corticosterone in rodents and cortisol in humans) which can have a major impact on gut physiology (e.g., modulating the intestinal epithelial barrier and immune responses) and gut microbiota composition (6). The gut microbiota has been shown to influence various neurodevelopmental processes including microglial maturation and function, blood–brain barrier formation and integrity, myelination, and neurogenesis (7).

integrity of the BBB and that a mix of SCFAs can reverse aberrant microglia phenotypes in adult GF mice [14], indicating that SCFAs may be one of the signaling pathways by which bacteria modulate the brain. Several commensal bacteria have also been shown to produce neurotransmitters such as dopamine, serotonin, and metabolites that enter circulation and exert a range of consequences outside the GI tract [43]. With regard to the brain, a new study has shown that direct metabolites of the gut microbiome (e.g., imidazole propionate) or products of the combinatorial metabolism between the microbiome and host (e.g., 3-indoxyl-sulfate, trimethylamine-*N*-oxide, and phenylacetylglycine) are present in the forebrains of healthy mice as early as the neonatal period and remain into adulthood [44]. However, the role of these metabolites in neurodevelopment remains poorly understood. Recently, studies have raised the possibility that bacteria-derived products such as PGN might directly affect the developing brain and behavior through central activation of pattern-recognition receptors (PRRs) of the innate immune system [45].

PRRs as Potential Key Regulators of Gut Microbiota–Brain Interactions

There is growing recognition that PRRs have been adapted to mediate roles far beyond innate immunity, including host homeostasis and development [46]. PRRs are a series of germline-encoded host sensors that recognize highly conserved microbial molecular signatures or 'motifs' such as bacterial surface molecules (e.g., PGN), often referred to as microbe- or pathogen-associated molecular patterns (MAMPs or PAMPs). Important PRRs of the innate immune system include membrane-bound Toll-like receptors (TLRs) [47,48], cytosolic NOD-like receptors (nucleotide-binding oligomerization domain-like receptors; NOD1 and NOD2) [49,50], and PGN recognition proteins (PGRPs, PGLYRP1–4) [51,52]. Traditionally, the expression and function of PRRs have been investigated in the context of pathogenic microorganisms. However, the microbial motifs that activate PRRs are not restricted to pathogens because they include structural molecules such as lipopolysaccharide, a component of the outer membrane of Gram-negative bacteria, and PGN that is common to almost all bacterial cell walls [46] (Figure 2).

There is evidence from both invertebrates and vertebrates that motifs derived from commensal bacteria interact with PRRs to promote developmental processes and maintain homeostasis of the host [53]. For example, bacterial motifs have been implicated in the regression of a juvenile-specific epithelium that facilitates the colonization of the Hawaiian squid by the marine symbiotic bacterium *Vibrio fischeri*, which provides luminescence to the squid (Figure 3A) [54]. Moreover, the maturation of mammalian gut-associated lymphoid tissues (i.e., isolated lymphoid follicles, ILFs) is triggered by exposure of intestinal epithelial cells to PGN motifs derived from commensal Gram-negative bacteria during postnatal life, thus leading to the activation of the cytoplasmic PRR NOD1 and secretion of factors that stimulate the proliferation of isolated ILFs (Figure 3B) [7,55]. Furthermore, accumulating data indicate that PGN motifs from commensal bacteria can also diffuse through the epithelial barrier in the intestine and reach the systemic circulation. A seminal study by Clarke and colleagues showed that the gut microbiota is a source of PGN that systemically primes the innate immune system [56]. Specifically, these authors showed translocation of *meso*-diaminopimelic acid (*meso*-DAP)-type PGN from nonpathogenic Gram-negative bacteria in the intestinal mucosa to neutrophils in the bone marrow of mice. Activation of the cytoplasmic PRR NOD1 by *meso*-DAP-type PGN was sufficient to prime and restore neutrophil function in the bone marrow of mice with a manipulated microbiota (i.e., GF or antibiotic-treated mice), revealing a previously undescribed role for PGN in priming systemic innate immunity in the absence of infection (Figure 3C). Other groups have recently detected traces of various PGN fragments in fetal bovine serum (the most widely used serum supplement for *in vitro* cell culture) and in serum of healthy mice [57], suggesting that a homeostatic function of PGN signaling may have been previously underappreciated.

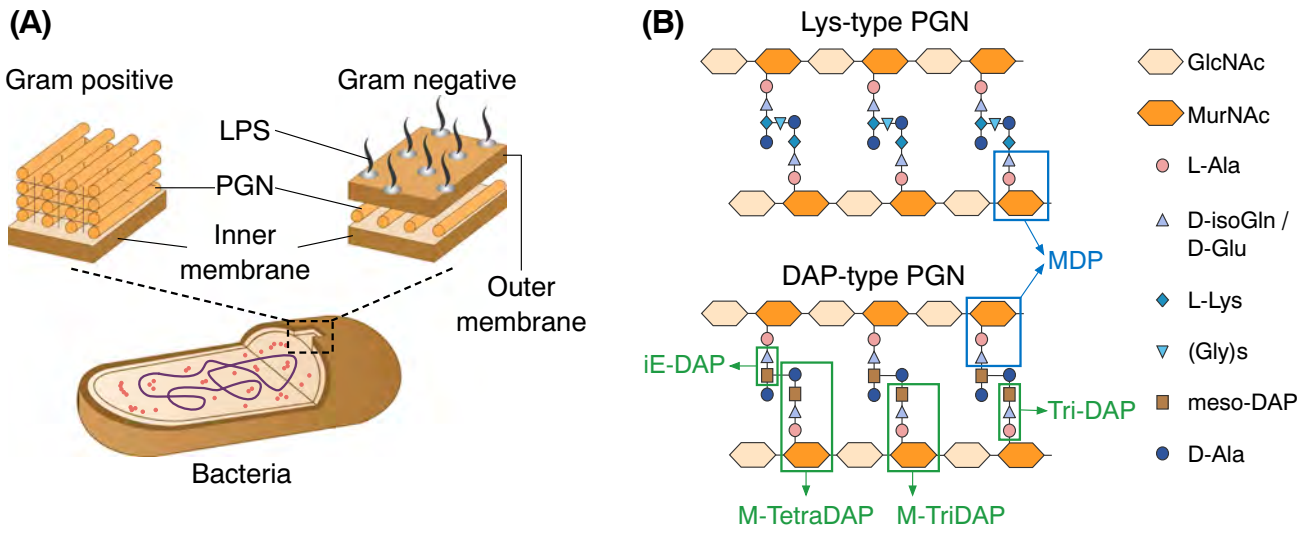

Figure 2. Bacterial Peptidoglycan (PGN) Location and Structure. (A) Gram-positive bacteria display a multilayered PGN cell wall which contains teichoic acids and often other polysaccharides, proteins, and lipoproteins (not shown). By contrast, Gram-negative bacteria have a relatively thin PGN cell wall that is surrounded by an outer membrane containing lipopolysaccharides (LPSs). (B) PGN is a polymer consisting of β-(1–4)-linked *N*-acetylglucosamine (GlcNAc) and *N*-acetylmuramic acid (MurNAc) in which the lactyl groups of MurNAc are replaced by stem peptides, typically containing four alternating L- and D-amino acids. The stem peptides from adjacent strands are often crosslinked, either directly or through short peptides. Shown are two examples of the general types of PGN: lysine-type PGN that is characteristic of the cell walls of most Gram-positive bacteria, and diaminopimelic acid (DAP)-type PGN that is present in Gram-negative bacteria and a subset of Gram-positive bacteria. PGN motifs are recognized via a series of pattern-recognition receptors (PRRs) such as NOD-like receptors. For example, an intact MurNAc moiety is essential for NOD2 recognition, which is strongly activated by muramyl dipeptide (MDP). By contrast, the presence of the glycan moiety is not required for NOD1 recognition because iE-DAP or Tri-DAP are sufficient for the activation of this PRR. However, NOD1 is also activated to *meso*-DAP-type PGN containing muramyl tripeptides (M-TriDAP) or tetrapeptides (M-TetraDAP). In addition, M-TriDAP modified by amidation of the *meso*-DAP residue has been shown to activate NOD2 but is not recognized by NOD1 [31].

A new antibody-based detection assay for the PGN muramyl-L-alanyl-D-isoglutamine (MDP) was described recently. The authors showed that this PGN monomer is ubiquitously present in serum or plasma of healthy humans, rodents, and non-human primates [58]. It should be noted that a wide concentration range of MDP levels were found in healthy individuals from European or Asian ancestry. Approximately 75% of all individuals had a range between 0.16–1 μg/ml, 22.5% between 1–4 μg/ml, and the extreme ends of the spectrum either had very high levels (4–5 μg/ml) or were below the level of detection (Figure 2d in [58]). However, patients suffering from autoimmune diseases (i.e., rheumatoid arthritis and systemic lupus erythematosus) had higher levels of circulating MDP than healthy controls, raising the possibility that disturbance of equilibria between PGN motifs and their sensing PRRs could contribute to host pathologic states. This finding is consistent with earlier work from Laman and colleagues showing the presence of antibodies specific for PGN in the cerebral spinal fluid (CSF) of patients with active multiple sclerosis (MS), an inflammatory demyelinating disease of the CNS [59]. Further details about the roles of PGN as a driver of chronic brain inflammation in MS and experimental autoimmune encephalomyelitis models are given in a recent review by the latter authors [60].

Bacterial PGN Motifs Can Be Translocated into the Brain and Sensed by Innate-Immune PRRs

The notion that bacterial products derived from microbiota could influence brain functions is not new [61,62]. In fact, several decades ago it was shown that some MDP-type PGNs induce prolonged (6–12 h) increases in slow-wave sleep (SWS) in rabbits, rats, and monkeys, and enhanced the amplitude of electroencephalogram (EEG) slow waves (0.5–4.0 Hz) during bouts of SWS. Of note, the somnogenic properties of muramyl peptides are dependent upon their

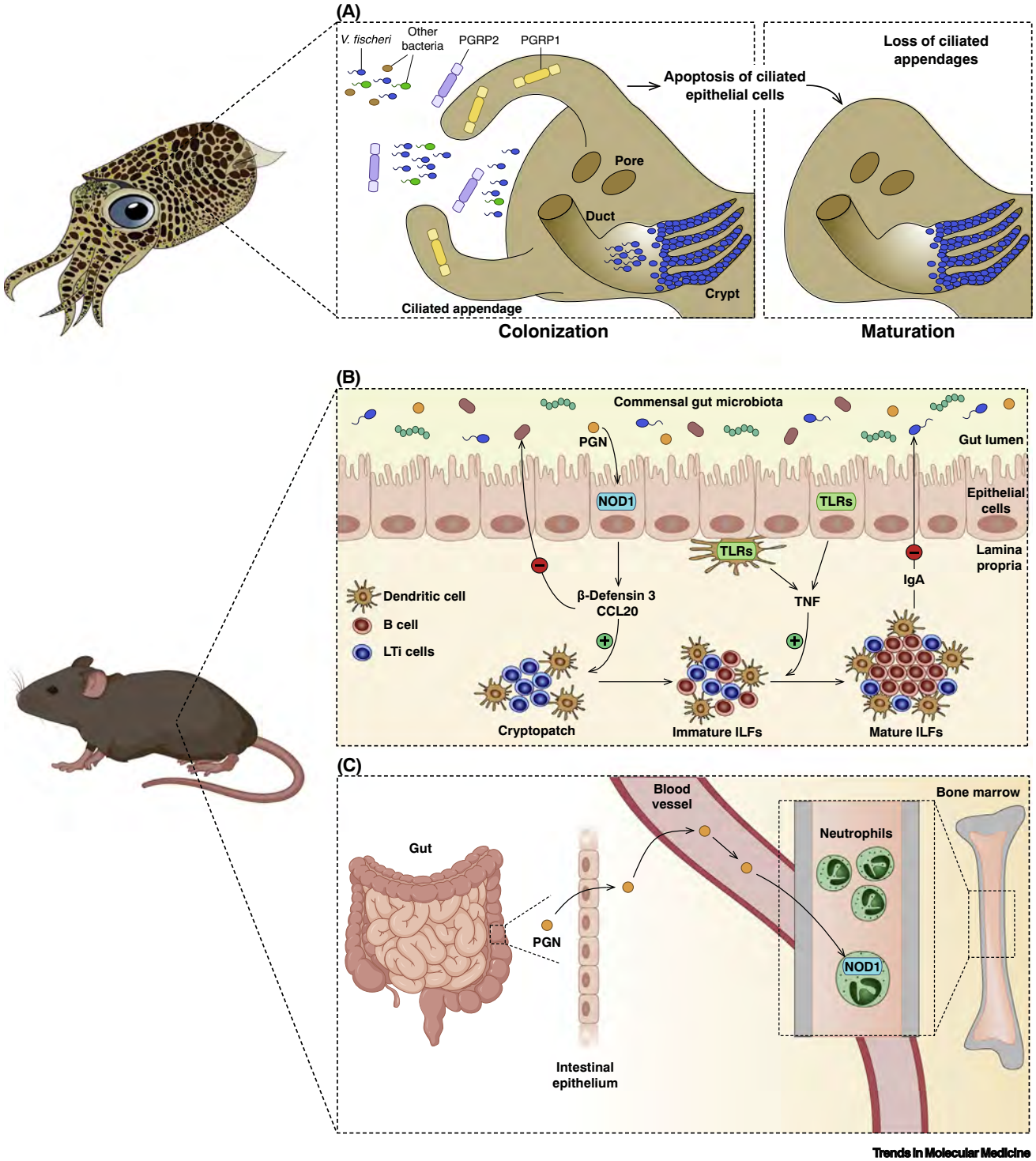

Figure 3. Evidence from Invertebrate and Vertebrate Models Supporting a Role of Peptidoglycan (PGN) in Crosstalk between Microbial Symbionts and Their Hosts. (A) Beneficial symbiotic relationship between the squid *Euprymna scolopes* and the luminescent bacteria *Vibrio fischeri*. Epithelial cells of the light organ of *E. scolopes* secrete mucus-containing peptidoglycan recognition protein 2 (PGRP2) that selects *V. fischeri* from other bacterial species present in the water. *V. fischeri*

(Figure legend continued at the bottom of the next page.)

precise biochemical structure [63,64], and can be distinguished from other activities of muramyl peptides such as induction of fever and adjuvant activity [61]. Moreover, PGN fragments were detected in the CSF and brain of sleep-deprived animals and human urine [65,66], implicating PGN in the pathophysiology of sleep disorders. New scientific discoveries showing a previously unappreciated complexity of the human microbiome and its wide-ranging impact on host health and disease have triggered re-evaluation of the possible role of microbial molecules and metabolites on host tissues, including the brain. Recently, Arentsen and colleagues showed that PGN fragments are present in the serum of healthy juvenile mice (i.e., SPF mice, [45]; see also Figure 2e in [58]). PGN levels in the serum of juvenile GF mice were found to be very low or at the limits of detection, confirming that the microbiota is the main source of PGN in the circulation. The same authors showed that PGN fragments are present in normal developing mouse brain, and these increase in parallel with postnatal bacterial colonization processes [45] (Figure 4A). It was also demonstrated that two families of PRRs that specifically detect PGN (i.e., PGN-recognition proteins, PGRPs; and NOD-like receptors) and the PGN transporter PEPT1 are highly expressed in the developing brain during distinct windows of postnatal mouse development. These findings raise the possibility that PGN motifs (e.g., muramyl dipeptides and mesoDAP-type PGN) may differentially affect brain development depending upon the postnatal age. At least two PGN-sensing molecules (PGLYRP2 and NOD1) are highly expressed in neurons in several brain regions including the prefrontal cortex, hippocampus, and cerebellum, thus indicating potential direct effects of PGN on neurons [45] (Figure 4B). Interestingly, the peak expression of NOD1 receptors coincides with the period of synaptogenesis in rodents. These molecules are also expressed in astrocytes and microglia (albeit at lower levels than in neurons). In addition, oligodendrocyte development and maturation may be affected by PGN because they express PGN-sensing molecules such as NOD1, according to an open-access database containing transcriptomic datasets from oligodendrocytes from different developmental stages (https://ki.se/en/mbb/oligointernode). It is worth pointing out that the expression of PGN-sensing molecules (PGRP1–4, NOD1, NOD2, TLR2, PEPT1) within the developing brain is highly sensitive to manipulation of the gut microbiota (i.e., GF conditions and perinatal antibiotic exposure) [45]. Moreover, there are brain region-dependent and sex-dependent differences in the expression of PGN-sensing molecules during postnatal brain development. Interestingly, these differences appear to be more pronounced in the prefrontal cortex, a key region implicated in a broad range of neurodevelopmental and psychiatric disorders [67]. Taken together, these findings suggest that there is a highly dynamic and sensitive period during which postnatal microbial gut colonization can influence developmental brain trajectories in an age-, region-, and sex-specific manner via PGN signaling.

Emerging Roles of PGN-Sensing Molecules in Brain Development, Function, and Behavior

A growing body of literature demonstrates that classical peripheral immune molecules can have important roles in the CNS under normal physiological conditions. For instance, MHC class I and the paired immunoglobulin-like receptor B (PIRB, an MHC I-binding receptor) have been implicated in synaptic plasticity, learning, and memory [68–70]. Moreover, some members of the TLR family

enters through the pores into the ducts of the light organ and colonizes the deep crypts, where they induce apoptosis and morphogenesis of the light organ (figure modified, with permission, from [52]). (B) Development of isolated lymphoid follicles (ILFs) by commensal gut microbiota. NOD1 receptors in intestinal epithelial cells are activated by PGN released by the gut microbiota and induce the expression of CCL20 and β-defensin 3. These factors are agonists of the CC chemokine receptor 6 (CCR6) that is expressed on intestinal B cells and lymphoid tissue inducer (LTi) cells in cryptopatches. Activated CCR6 cause the recruitment of $CCR6^{+}$ B cells to form immature ILFs. The development of immature ILFs into mature follicles requires tumor necrosis factor-α (TNF-α) produced by dendritic cells. B cells from mature ILFs produce IgA that modulates the composition of the gut microbiota (figure modified, with permission, from [108]). (C) The gut microbiota primes bone marrow-derived neutrophils. *Meso*-DAP-type PGN released by Gram-negative bacteria in the gut can be translocated into the systemic circulation and reach neutrophils in the bone marrow. Activation of the NOD1 receptor in neutrophils increases their capacity to react against microorganisms (figure modified, with permission, from [56]).

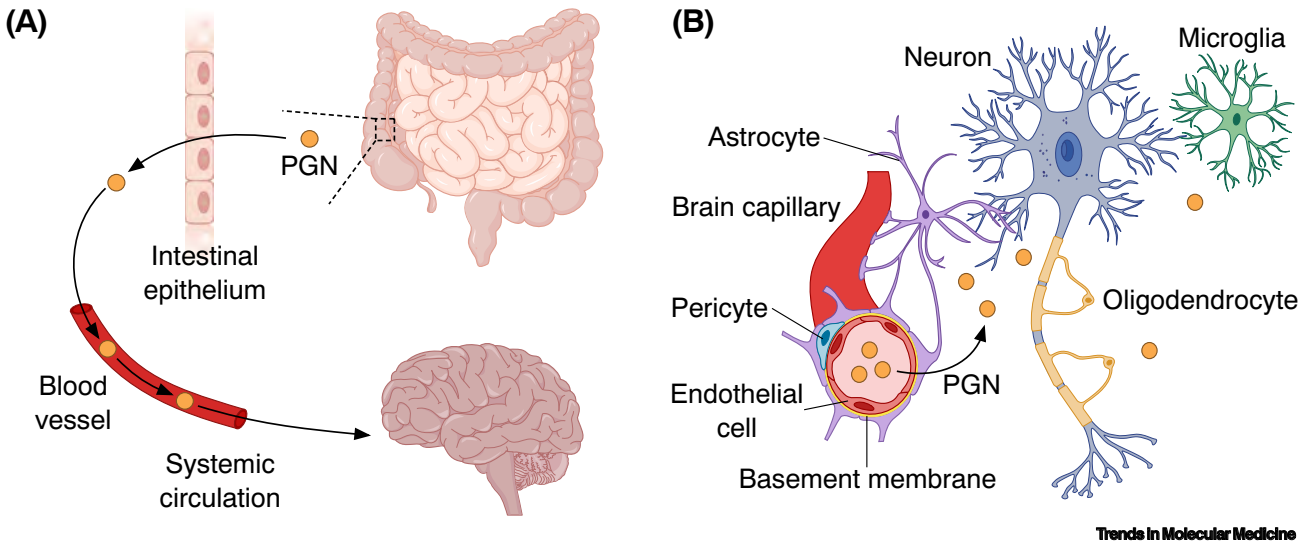

Figure 4. Translocation of Bacterial Peptidoglycan (PGN) from the Gut Microbiota to the Brain. (A) PGN motifs from the commensal gut microbiota disseminate systemically and can reach the brain. (B) Schematic representation of the blood–brain barrier that is composed of endothelial cells of the brain capillary wall, astrocyte end-feet surrounding the capillary, and pericytes embedded in the capillary basement membrane. PGN motifs can cross the blood–brain barrier and are sensed by pattern recognition receptors (PRRs) expressed in brain cells (neurons, oligodendrocytes, and microglia). See main text for further details.

have been shown to modulate key neurodevelopmental processes (e.g., neurite outgrowth, neuronal proliferation/differentiation, and neuronal cell death) and behavior [71–77]. Emerging evidence also points to novel roles for PGN-sensing molecules (i.e., PGRPs and NOD-like receptors) in the CNS [78]. The first known function of a PGN-sensing molecule in the CNS came from the *Drosophila* model system [79]. The authors demonstrated that a member of the PGRP family (PGRP-LC) is expressed in presynaptic terminals of *Drosophila* motor neurons. This PGN-sensing molecule plays a key role in the induction and sustained expression of presynaptic homeostatic plasticity; it therefore controls the homeostatic modulation of the readily releasable pool of synaptic vesicles following inhibition of postsynaptic glutamate receptor function.

In mammals, there are four PGRPs, including a 'long' isoform (PGLYRP2 or PGRP-L) that is a *N*-acetylmuramoyl-L-alanine amidase that hydrolyzes the amide bond between N-acetylmuramic acid (MurNAc) and L-Ala of bacterial PGN (which is unique among the mammalian PGRPs) [52]. In mice, PGLYRP2 is highly expressed in the brain (e.g., prefrontal cortex, striatum, hippocampus, and cerebellum) during the first few days of life [45]. In line with this, human PGLYRP2 protein has also been detected in postmortem cerebral cortex tissue of a young child, but not in most adult cortical tissues (i.e., moderate to strong labeling in the neuropil and glial cells, respectively, in the Human Protein Atlas; www.proteinatlas.org/) [80]. In a recent study it was demonstrated that PGLYRP2 knockout (KO) mice have altered expression of the ASD risk gene *c-Met*, as well as of BDNF (brain-derived neurotrophic factor) [45], that are both implicated in the formation and modulation of brain circuits [81,82]. Moreover, juvenile PGLYRP2 KO mice display a significant increase in social interaction levels toward an unfamiliar stimuli mouse, without any alterations in anxiety-like behavior or motor activity [45]. Interestingly, this phenotype was more pronounced in male offspring. In another study, the same authors demonstrated that absence of PGLYRP2 leads to major sex-dependent alterations in motor and anxiety-like behavior later in adulthood (i.e., 15 month-old mice) [83]. In this case, PGLYRP2-deficient female mice, but not males, show better motor performance. However, they display increased levels of anxiety-like behavior, suggesting that the modulatory effects of PGLYRP2 in the brain are highly dependent upon multiple host

factors including age, sex, and the type of neuronal circuit. Peptidoglycan recognition protein 1 (PGLYRP1, also known as tag7 protein or PGRP-S) is another member of the mammalian PGRP family that is expressed in the brain. Unlike PGLYRP2, the expression of PGLYRP1 peaks around the second week of postnatal life and continues to be expressed into adulthood [45]. Previous work has shown that *Pgrp1* mRNA levels increase in brain stem and hypothalamus after sleep deprivation in rodents [84], suggesting a possible role for this PGN-sensing molecule in homeostatic regulation of sleep. However, studies using more advanced transgenic mouse models (e.g., conditional brain-specific knockdown of *Pglyrp2*) will be fundamental to unraveling the specific role of PGRPs in the CNS.

Recently, Pusceddu and colleagues provided the first experimental evidence implicating NOD-like receptors in the regulation of the stress response, serotonin (5-HT) signaling, and behavior [85]. Specifically, these authors showed that mice deficient in both *Nod1* and *Nod2* (NodDKO mice) exhibit signs of stress-induced anxiety-like behavior, cognitive impairment, and depressive-like behavior under conditions of a hyperactive HPA axis, but not under baseline conditions. Surprisingly, no major sex differences were found. Moreover, these authors identified the central 5-HT system as a potential neural substrate mediating the behavioral phenotype of NodDKO mice. In fact, NodDKO mice had lower levels of 5-HT in both the hippocampus and brainstem under basal conditions. In addition, the expression profile of several key components of the 5-HT signaling pathways, such as tryptophan hydroxylase 2 (the rate-limiting enzyme for 5-HT synthesis), the 5-HT receptor 1a (which regulates 5-HT release), and the 5-HT transporter, were impaired after exposure to stress. Importantly, these authors showed that the stress-induced behavioral impairments observed in NodDKO mice could be restored by chronic treatment with a 5-HT reuptake inhibitor (fluoxetine). Another important finding of this study was that intestinal epithelial cell (IEC)-specific ablation of *Nod1* (*Vil–cre Nod1*$^{fl/fl}$), but not of *Nod2*, resulted in stress-induced impairments in cognitive function and anxiety-like behavior. The latter observations highlight a novel role for the IEC NOD1 receptors in behavioral responses to stress and identify this PGN-sensing molecule as potential new target for the treatment of stress-associated gut–brain disorders. However, the specific roles of NOD-like receptors within the brain remain to be further clarified. Previous studies have shown that murine microglia express robust levels of NOD2 mRNA and protein, but little or no NOD1 [86,87]. On the other hand, NOD1 protein is highly expressed in developing neurons across multiple brain regions including the prefrontal cortex, hippocampus, and cerebellum (supplementary Figure 3 in [45]). Both NOD1 and NOD2 are moderately expressed in astrocytes [45,87]. These findings raise the possibility that NOD1 and NOD2 may have distinct functions in glia and neurons of the adult and developing brain.

Potential Role of PGN in the Pathophysiology of Neurodevelopmental Disorders

It is now widely recognized that the maternal/fetal environment plays a crucial role in the development of the fetal brain and long-term neurodevelopmental trajectories, including susceptibility to mental health disorders in childhood and adulthood [88]. Indeed, epidemiological studies have shown an association between maternal infection/inflammation during pregnancy and increased risk of neurodevelopmental and psychiatric disorders such as ASD [89–92]. These findings are supported by experimental mouse studies demonstrating that maternal immune activation during pregnancy (MIA; through exposure to microbial pathogens or proinflammatory PAMPs) induces ASD-like behavioral traits in their offspring [93,94]. Recent work by Choi, Huh, and colleagues has demonstrated that MIA promotes abnormal cortical development and ASD-like behavioral phenotypes in mouse offspring through the activation of the maternal T helper 17 (Th17) cell IL-17a pathway [95]. These authors discovered that MIA-induced phenotypes in mouse offspring require commensal maternal gut bacteria (i.e., segmented filamentous bacteria, SFB) that promote Th17 cell differentiation [96]. For instance, pregnant C57BL/6 mice, which are colonized

Clinician's Corner

Traditionally, the translocation of bacterial products such as peptidoglycans (PGNs) into the brain has mainly been considered in the context of infection and inflammation. However, the recent realization of the size and complexity of the human microbiome and its wide-ranging impact on host physiology and development has triggered a re-evaluation of the possible role of bacteria-derived products and metabolites from non-pathogenic microbiota in modulating host tissue function, including brain development and behavior.

PGNs from commensal microbiota are ubiquitously present in the circulation and can cross the BBB. PGN-sensing molecules are highly expressed in the normal developing brain and their expression is highly sensitive to manipulations of the gut microbiota, including perinatal antibiotic exposure. Work from transgenic animal models implicates PGN-sensing molecules in the control of social behavior and anxiety.

PGN-sensing molecules are expressed in the human placenta and represent an important mediator for microbiota–host communication at the maternal–fetal interface. Studies in rodents suggest that circulating PGN fragments from maternal gut microbiota can influence the fetal brain and subsequent cognitive development.

From a clinical point of view, detection of PGN levels during the perinatal period could represent a new strategy to screen and identify infants and children at risk of neurodevelopmental and psychiatric disorders such as autism spectrum disorder.

At the genetic level, there is a need to explore potential associations between genetic polymorphisms in PGN-sensing molecules (e.g., PGN recognition proteins and NOD-like receptors) and neurodevelopmental disorders.

with SFB and contain higher numbers of gut Th17 cells, were more susceptible to MIA-induced behavioral and morphological pathology. Conversely, pregnant C57BL/6 mice which are not colonized by SFB and lack gut Th17 cells were not susceptible. These findings suggest that the composition of the maternal gut microbiota is an important contributing risk factor for MIA-induced behavioral abnormalities in offspring. Indeed, mounting evidence identifies the maternal gut microbiota as a common denominator by which a broad range of environmental risk factors such as diet (e.g., high-fat diet), infections, antibiotic exposure, and stress can affect both maternal health and fetal development [9,97,98]. The developing fetus receives oxygen, nutrients, and other bioactive compounds from the maternal circulation, and therefore alterations in the composition and metabolic activity of the maternal gut microbiota could profoundly influence prenatal brain developmental processes (e.g., neurogenesis, migration, and differentiation) and subsequent neurodevelopmental outcomes. In line with this notion, a new study in mice demonstrated that, during normal pregnancy, SCFAs from the commensal maternal gut microbiota can reach the developing embryo and promote the differentiation of sympathetic neurons via activation of GPR41 [99]. Moreover, studies demonstrating disrupted BBB maturation and an altered *microglial transcriptome profile* in GF mice during prenatal life underscore the importance of the commensal maternal microbiome during fetal brain development [14,16].

It has recently been proposed that MAMPs such as PGN could be an important mediator of host–microbiome interactions at the maternal–fetal interface because PGN reaches the systemic circulation not only during bacterial infections but also during normal physiological conditions [78]. In line with this notion, a recent study showed that components of the bacterial cell wall (i.e., the PGN–teichoic acid complex of *Streptococcus pneumoniae*, a major pathogen causing infections such as meningitis) can traverse the placental barrier through the platelet-activating factor receptor (PAFr) and affect the developing mouse fetal brain [100]. This PGN–teichoic acid complex induces the transcription factor FOXG1 in the fetal neocortex by activating TLR2, resulting in a 50% greater density of neurons in the cortical plate. This abnormal neuroproliferation was associated with abnormal cognitive development. Intriguingly, a TLR2-dependent response to this PAMP did not induce inflammation in the fetal brain. It was recently reported that NOD1, NOD2, and TLR2, as well as the putative PGN transporters PEPT2 and PAFr, are abundantly expressed in the human placenta [78]. These findings suggest that bacterial PGN motifs from the maternal commensal gut microbiota can also influence the developing brain, and that disruption of components of this signaling pathway could lead to abnormal motor, social, and cognitive development (Figure 5).

In humans, accelerated brain growth in infancy has been associated with a broad range of developmental delays in motor, language, and cognitive functions [101,102]. For instance, neurodevelopmental disorders, including ASD, have been associated with atypical brain connectivity patterns involving higher-order association neocortical regions [103–105]. A large proportion of high-confidence ASD-associated risk genes are pleiotropic, and affect proliferation/differentiation and/or synapse development in the neocortex, amygdala, hippocampus, striatum, and cerebellum, supporting the notion that ASD begins during prenatal life [106]. It will therefore be of great interest to investigate the potential link between maternal circulating PGN levels during pregnancy and sociocognitive development of infants, as well as potential novel associations between genetic variants in PGN-sensing molecules and ASD [107].

Concluding Remarks

Recent discoveries showing an unprecedented role for the commensal gut microbiota in the regulation of brain development and behavior have triggered a paradigm shift in our conceptualization of the origin of human brain disorders. The current focus of this new microbiome-gut–brain axis field is to delineate the precise molecular mechanisms and signaling pathways employed by

Outstanding Questions

What specific types of PGN motifs can cross the BBB under physiological conditions? How are they transported into brain cells?

How do the structure and function of circulating PGN motifs relate to the composition of the gut microbiota across development?

What is the specific function of PGN-sensing molecules in neurons and glial cells in the developing and adult brain under physiological conditions?

Do maternal circulating PGN levels during pregnancy correlate with the motor, social, and/or cognitive development of offspring?

Could a set of PGN-related markers be used to identify infants and children at risk of atypical brain development?

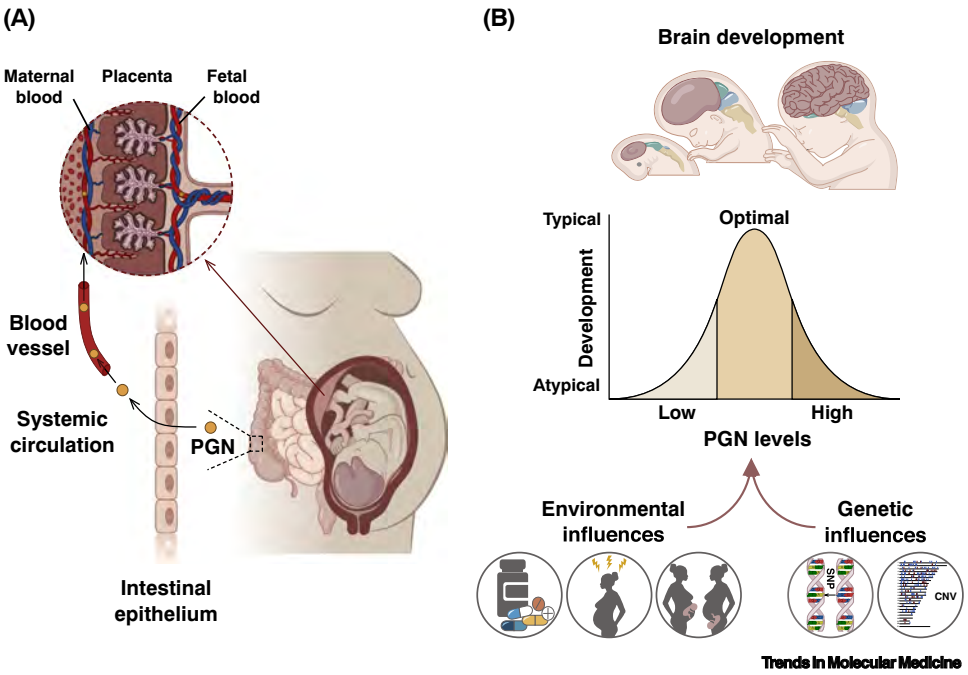

Figure 5. Schematic Representation of Proposed Routes of Peptidoglycan (PGN) Dissemination from the Maternal Microbiota to the Fetal Brain. (A) PGN fragments derived from commensal maternal gut microbiota translocate from the gut lumen into the bloodstream and reach the placenta. PGNs can transverse the placenta and enter the fetal brain where they can affect key neurodevelopmental processes (e.g., proliferation, differentiation, synaptogenesis, and myelination) via activation of PGN-sensing molecules of the innate immune system. (B) PGN signaling can be affected by host genetics (e.g., SNPs; and copy-number variations, CNVs; in genes coding for PGN-sensing molecules and/or transporters) and external factors such as antibiotic exposure, maternal stress, and mode of delivery. Optimal low PGN levels are needed for typical brain development. However, PGN levels that are insufficient or too high give rise to atypical brain development.

commensal gut bacteria to influence the brain. PRRs that recognize bacterial surface molecules, such as PGN, have emerged as potential novel mediators of microbiota–gut–brain axis signaling. The gut microbiota contains trillions of commensal bacteria producing a diverse peptidoglycome that can disseminate systemically and reach peripheral organs such as the brain. PRRs that specifically recognize PGN (i.e., PGN-sensing molecules, NOD-like receptors, and PGN recognition proteins), and the PGN transporter PEPT1 are highly expressed in the developing brain during specific windows of postnatal development. Emerging evidence suggests that PGN-sensing molecules have crucial roles in the regulation of social behavior and anxiety, and in behavioral responses to stress. Despite recent advances, many issues remain to be addressed, including the characterization of the specific types of PGN that can cross the BBB under physiological conditions, and how they are transported into brain cells (neurons, astrocytes, microglia, and oligodendrocytes) (see Outstanding Questions). It is worth acknowledging that the laboratory of Gomperts Boneca at the Institut Pasteur in Paris has been advancing some of these questions by combining different PGN labeling methods with advanced imaging and genetic approaches (abstracts from the Great Wall Symposium, September 25–27, 2019). From a clinical perspective, it would be of great importance to determine whether serum or plasma levels of PGN motifs during pregnancy and/or early postnatal life could help to identify infants and children at risk of atypical social and cognitive development. It would be equally important to identify potential genetic risk variants in genes that encode PGN-sensing molecules that are associated with atypical neurodevelopmental trajectories. This could be accomplished in a multicenter, prospective, longitudinal study of infants at high or low risk of atypical brain development such as ASD.

In parallel, the potential effects of PGN motifs on early aspects of human brain prenatal development could be investigated by taking advantage of human induced pluripotent stem cell technology, and this would allow the generation of personalized neurons or glial cells. This information will provide key mechanistic insights into how PGN can influence brain development, function, and behavior, as well as novel potential therapeutic targets for the treatment of neurodevelopmental and psychiatric disorders that are often associated with comorbid GI problems and altered gut microbiota composition.

Acknowledgments

The authors apologize to colleagues whose work could not be cited owing to space constraints and the special focus on recent work. Work from the laboratory of R.D.H. cited in this review has been supported by grants from the Swedish Research Council, the Swedish Brain Foundation, the Olle Engkvist Byggmästare Foundation, and the Frimurare Barnhus Foundation. All figures were created in part using BioRender (https://biorender.com).

References

1. Dominguez-Bello, M.G. *et al.* (2019) Role of the microbiome in human development. *Gut* 68, 1108–1114
2. Sender, R. *et al.* (2016) Are we really vastly outnumbered? Revisiting the ratio of bacterial to host cells in humans. *Cell* 164, 337–340
3. Tierney, B.T. *et al.* (2019) The landscape of genetic content in the gut and oral human microbiome. *Cell Host Microbe* 26, 283–295
4. Qin, J. *et al.* (2010) A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* 464, 59–65
5. Cho, I. and Blaser, M.J. (2012) The human microbiome: at the interface of health and disease. *Nat. Rev. Genet.* 13, 260–270
6. Rowland, I. *et al.* (2018) Gut microbiota functions: metabolism of nutrients and other food components. *Eur. J. Nutr.* 57, 1–24
7. Al Nabhani, Z. and Eberl, G. (2020) Imprinting of the immune system by the microbiota early in life. *Mucosal Immunol.* 13, 183–189
8. Borre, Y.E. *et al.* (2014) Microbiota and neurodevelopmental windows: implications for brain disorders. *Trends Mol. Med.* 20, 509–518
9. Diaz Heijtz, R. (2016) Fetal, neonatal, and infant microbiome: perturbations and subsequent effects on brain development and behavior. *Semin. Fetal Neonatal Med.* 21, 410–417
10. Sudo, N. *et al.* (2004) Postnatal microbial colonization programs the hypothalamic–pituitary–adrenal system for stress response in mice. *J. Physiol.* 558, 263–275
11. Diaz Heijtz, R. *et al.* (2011) Normal gut microbiota modulates brain development and behavior. *Proc. Natl. Acad. Sci. U. S. A.* 108, 3047–3052
12. Neufeld, K.M. *et al.* (2011) Reduced anxiety-like behavior and central neurochemical change in germ-free mice. *Neurogastroenterol. Motil.* 23, 255–264
13. 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
14. Braniste, V. *et al.* (2014) The gut microbiota influences blood–brain barrier permeability in mice. *Sci. Transl. Med.* 6, 263ra158
15. Erny, D. *et al.* (2015) Host microbiota constantly control maturation and function of microglia in the CNS. *Nat. Neurosci.* 18, 965–977
16. Thion, M.S. *et al.* (2018) Microbiome influences prenatal and adult microglia in a sex-specific manner. *Cell* 172, 500–516
17. Gacias, M. *et al.* (2016) Microbiota-driven transcriptional changes in prefrontal cortex override genetic differences in social behavior. *Elife* 5, e13442
18. Hoban, A.E. *et al.* (2016) Regulation of prefrontal cortex myelination by the microbiota. *Transl. Psychiatry* 6, e774
19. Radulescu, C.I. *et al.* (2019) Manipulation of microbiota reveals altered callosal myelination and white matter plasticity in a model of Huntington disease. *Neurobiol. Dis.* 127, 65–75
20. Lynch, J.B. and Hsiao, E.Y. (2019) Microbiomes as sources of emergent host phenotypes. *Science* 365, 1405–1409
21. Sherwin, E. *et al.* (2019) Microbiota and the social brain. *Science* 366, eaar2016
22. Jaggar, M. *et al.* (2020) You've got male: sex and the microbiota–gut–brain axis across the lifespan. *Front. Neuroendocrinol.* 56, 100815
23. Needham, B.D. *et al.* (2018) Searching for the gut microbial contributing factors to social behavior in rodent models of autism spectrum disorder. *Dev. Neurobiol.* 78, 474–499
24. Hsiao, E.Y. *et al.* (2013) Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell* 155, 1451–1463
25. Lum, G.R. *et al.* (2020) Emerging roles for the intestinal microbiome in epilepsy. *Neurobiol. Dis.* 135, 104576
26. Vuong, H.E. and Hsiao, E.Y. (2017) Emerging roles for the gut microbiome in autism spectrum disorder. *Biol. Psychiatry* 81, 411–423
27. Chaidez, V. *et al.* (2014) Gastrointestinal problems in children with autism, developmental delays or typical development. *J. Autism Dev. Disord.* 44, 1117–1127
28. Adams, J.B. *et al.* (2011) Gastrointestinal flora and gastrointestinal status in children with autism – comparisons to typical children and correlation with autism severity. *BMC Gastroenterol.* 11, 22
29. Wang, L.W. *et al.* (2011) The prevalence of gastrointestinal problems in children across the United States with autism spectrum disorders from families with multiple affected members. *J. Dev. Behav. Pediatr.* 32, 351–360
30. Srikantha, P. and Mohajeri, M.H. (2019) The possible role of the microbiota–gut–brain axis in autism spectrum disorder. *Int. J. Mol. Sci.* 20 pii: E2115
31. Wheeler, R. *et al.* (2014) The biology of bacterial peptidoglycans and their impact on host immunity and physiology. *Cell. Microbiol.* 16, 1014–1023
32. Irazoki, O. *et al.* (2019) Peptidoglycan muropeptides: release, perception, and functions as signaling molecules. *Front. Microbiol.* 10, 500
33. Cryan, J.F. *et al.* (2019) The microbiota–gut–brain axis. *Physiol. Rev.* 99, 1877–2013
34. Fulling, C. *et al.* (2019) Gut microbe to brain signaling: what happens in vagus. *Neuron* 101, 998–1002
35. Sarkar, A. *et al.* (2018) The microbiome in psychology and cognitive neuroscience. *Trends Cogn. Sci.* 22, 611–636
36. Sarkar, A. *et al.* (2016) Psychobiotics and the manipulation of bacteria–gut–brain signals. *Trends Neurosci.* 39, 763–781
37. Sgritta, M. *et al.* (2019) Mechanisms underlying microbial-mediated changes in social behavior in mouse models of autism spectrum disorder. *Neuron* 101, 246–259 e6
38. Buffington, S.A. *et al.* (2016) Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. *Cell* 165, 1762–1775
39. Kaelberer, M.M. *et al.* (2018) A gut–brain neural circuit for nutrient sensory transduction. *Science* 361, eaat5236

40. Han, W. *et al.* (2018) A neural circuit for gut-induced reward. *Cell* 175, 665–678.e23
41. Silva, Y.P. *et al.* (2020) The role of short-chain fatty acids from gut microbiota in gut–brain communication. *Front. Endocrinol.* 11, 25
42. Frost, G. *et al.* (2014) The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat. Commun.* 5, 3611
43. Caspani, G. and Swann, J. (2019) Small talk: microbial metabolites involved in the signaling from microbiota to brain. *Curr. Opin. Pharmacol.* 48, 99–106
44. Swann, J.R. *et al.* (2020) Developmental signatures of microbiota-derived metabolites in the mouse brain. *Metabolites* 10, E172
45. Arentsen, T. *et al.* (2017) The bacterial peptidoglycan-sensing molecule Pglyrp2 modulates brain development and behavior. *Mol. Psychiatry* 22, 257–266
46. Chu, H. and Mazmanian, S.K. (2013) Innate immune recognition of the microbiota promotes host-microbial symbiosis. *Nat. Immunol.* 14, 668–675
47. Beutler, B. (2004) Inferences, questions and possibilities in Toll-like receptor signalling. *Nature* 430, 257–263
48. Moresco, E.M. *et al.* (2011) Toll-like receptors. *Curr. Biol.* 21, R488–R493
49. Philpott, D.J. *et al.* (2014) NOD proteins: regulators of inflammation in health and disease. *Nat. Rev. Immunol.* 14, 9–23
50. Mukherjee, T. *et al.* (2019) NOD1 and NOD2 in inflammation, immunity and disease. *Arch. Biochem. Biophys.* 670, 69–81
51. Royet, J. and Dziarski, R. (2007) Peptidoglycan recognition proteins: pleiotropic sensors and effectors of antimicrobial defences. *Nat. Rev. Microbiol.* 5, 264–277
52. Royet, J. *et al.* (2011) Peptidoglycan recognition proteins: modulators of the microbiome and inflammation. *Nat. Rev. Immunol.* 11, 837–851
53. McFall-Ngai, M. *et al.* (2013) Animals in a bacterial world, a new imperative for the life sciences. *Proc. Natl. Acad. Sci. U. S. A.* 110, 3229–3236
54. McFall-Ngai, M. *et al.* (2010) The role of the immune system in the initiation and persistence of the *Euprymna scolopes–Vibrio fischeri* symbiosis. *Semin. Immunol.* 22, 48–53
55. Bouskra, D. *et al.* (2008) Lymphoid tissue genesis induced by commensals through NOD1 regulates intestinal homeostasis. *Nature* 456, 507–510
56. Clarke, T.B. *et al.* (2010) Recognition of peptidoglycan from the microbiota by Nod1 enhances systemic innate immunity. *Nat. Med.* 16, 228–231
57. Molinaro, R. *et al.* (2019) Trace levels of peptidoglycan in serum underlie the NOD-dependent cytokine response to endoplasmic reticulum stress. *J. Biol. Chem.* 294, 9007–9015
58. Huang, Z. *et al.* (2019) Antibody neutralization of microbiota-derived circulating peptidoglycan dampens inflammation and ameliorates autoimmunity. *Nat. Microbiol.* 4, 766–773
59. Schrijver, I.A. *et al.* (2001) Bacterial peptidoglycan and immune reactivity in the central nervous system in multiple sclerosis. *Brain* 124, 1544–1554
60. Laman, J.D. *et al.* (2020) Bacterial peptidoglycan as a driver of chronic brain inflammation. *Trends Mol. Med.* Published online February 21, 2020. https://doi.org/10.1016/j.molmed.2019.11.006
61. Krueger, J.M. and Opp, M.R. (2016) Sleep and microbes. *Int. Rev. Neurobiol.* 131, 207–225
62. Kubota, K. (1989) Kuniomi Ishimori and the first discovery of sleep-inducing substances in the brain. *Neurosci. Res.* 6, 497–518
63. Johannsen, L. *et al.* (1994) Somnogenic activity of muramyl peptide-derived immune adjuvants. *Int. J. Immunopharmacol.* 16, 109–116
64. Johannsen, L. *et al.* (1989) Somnogenic activity of O-acetylated and dimeric muramyl peptides. *Infect. Immun.* 57, 2726–2732
65. Krueger, J.M. *et al.* (1982) The composition of sleep-promoting factor isolated from human urine. *J. Biol. Chem.* 257, 1664–1669
66. Martin, S.A. *et al.* (1984) Peptidoglycans as promoters of slow-wave sleep. I. Structure of the sleep-promoting factor isolated from human urine. *J. Biol. Chem.* 259, 12652–12658
67. Bicks, L.K. *et al.* (2015) Prefrontal cortex and social cognition in mouse and man. *Front. Psychol.* 6, 1805
68. Boulanger, L.M. and Shatz, C.J. (2004) Immune signalling in neural development, synaptic plasticity and disease. *Nat. Rev. Neurosci.* 5, 521–531
69. Djurisic, M. *et al.* (2013) PirB regulates a structural substrate for cortical plasticity. *Proc. Natl. Acad. Sci. U. S. A.* 110, 20771–20776
70. Lee, H. *et al.* (2014) Synapse elimination and learning rules co-regulated by MHC class I H2-Db. *Nature* 509, 195–200
71. Lathia, J.D. *et al.* (2008) Toll-like receptor 3 is a negative regulator of embryonic neural progenitor cell proliferation. *J. Neurosci.* 28, 13978–13984
72. Ma, Y. *et al.* (2006) Toll-like receptor 8 functions as a negative regulator of neurite outgrowth and inducer of neuronal apoptosis. *J. Cell Biol.* 175, 209–215
73. Okun, E. *et al.* (2011) Toll-like receptor signaling in neural plasticity and disease. *Trends Neurosci.* 34, 269–281
74. Okun, E. *et al.* (2010) TLR2 activation inhibits embryonic neural progenitor cell proliferation. *J. Neurochem.* 114, 462–474
75. Shechter, R. *et al.* (2008) Toll-like receptor 4 restricts retinal progenitor cell proliferation. *J. Cell Biol.* 183, 393–400
76. Okun, E. *et al.* (2012) Evidence for a developmental role for TLR4 in learning and memory. *PLoS One* 7, e47522
77. Okun, E. *et al.* (2010) Toll-like receptor 3 inhibits memory retention and constrains adult hippocampal neurogenesis. *Proc. Natl. Acad. Sci. U. S. A.* 107, 15625–15630
78. Tosoni, G. *et al.* (2019) Bacterial peptidoglycans as novel signaling molecules from microbiota to brain. *Curr. Opin. Pharmacol.* 48, 107–113
79. Harris, N. *et al.* (2015) The innate immune receptor PGRP-LC controls presynaptic homeostatic Plasticity. *Neuron* 88, 1157–1164
80. Thul, P.J. *et al.* (2017) A subcellular map of the human proteome. *Science* 356, eaal3321
81. Eagleson, K.L. *et al.* (2017) The pleiotropic MET receptor network: circuit development and the neural-medical interface of autism. *Biol. Psychiatry* 81, 424–433
82. Park, H. and Poo, M.M. (2013) Neurotrophin regulation of neural circuit development and function. *Nat. Rev. Neurosci.* 14, 7–23
83. Arentsen, T. *et al.* (2018) Sex-dependent alterations in motor and anxiety-like behavior of aged bacterial peptidoglycan sensing molecule 2 knockout mice. *Brain Behav. Immun.* 67, 345–354
84. Rehman, A. *et al.* (2001) The cloning of a rat peptidoglycan recognition protein (PGRP) and its induction in brain by sleep deprivation. *Cytokine* 13, 8–17
85. Pusceddu, M.M. *et al.* (2019) Nod-like receptors are critical for gut–brain axis signalling in mice. *J. Physiol.* 597, 5777–5797
86. Sterka Jr., D. and Marriott, I. (2006) Characterization of nucleotide-binding oligomerization domain (NOD) protein expression in primary murine microglia. *J. Neuroimmunol.* 179, 65–75
87. Sterka Jr., D. *et al.* (2006) Functional expression of NOD2, a novel pattern recognition receptor for bacterial motifs, in primary murine astrocytes. *Glia* 53, 322–330
88. Al-Haddad, B.J.S. *et al.* (2019) The fetal origins of mental illness. *Am. J. Obstet. Gynecol.* 221, 549–562
89. Lee, B.K. *et al.* (2015) Maternal hospitalization with infection during pregnancy and risk of autism spectrum disorders. *Brain Behav. Immun.* 44, 100–105
90. Atladottir, H.O. *et al.* (2010) Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. *J. Autism Dev. Disord.* 40, 1423–1430
91. Bokobza, C. *et al.* (2019) Neuroinflammation in preterm babies and autism spectrum disorders. *Pediatr. Res.* 85, 155–165
92. Patterson, P.H. (2011) Maternal infection and immune involvement in autism. *Trends Mol. Med.* 17, 389–394
93. Brown, A.S. and Meyer, U. (2018) Maternal immune activation and neuropsychiatric illness: a translational research perspective. *Am. J. Psychiatry* 175, 1073–1083
94. Estes, M.L. and McAllister, A.K. (2016) Maternal immune activation: implications for neuropsychiatric disorders. *Science* 353, 772–777

95. Reed, M.D. *et al.* (2020) IL-17a promotes sociability in mouse models of neurodevelopmental disorders. *Nature* 577, 249–253
96. Kim, S. *et al.* (2017) Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. *Nature* 549, 528–532
97. Codagnone, M.G. *et al.* (2019) Programming bugs: microbiota and the developmental origins of brain health and disease. *Biol. Psychiatry* 85, 150–163
98. Jasarevic, E. and Bale, T.L. (2019) Prenatal and postnatal contributions of the maternal microbiome on offspring programming. *Front. Neuroendocrinol.* 55, 100797
99. Kimura, I. *et al.* (2020) Maternal gut microbiota in pregnancy influences offspring metabolic phenotype in mice. *Science* 367, eaaw8429
100. Humann, J. *et al.* (2016) Bacterial peptidoglycan traverses the placenta to induce fetal neuroproliferation and aberrant postnatal behavior. *Cell Host Microbe* 19, 388–399
101. Courchesne, E. *et al.* (2001) Unusual brain growth patterns in early life in patients with autistic disorder: an MRI study. *Neurology* 57, 245–254
102. Hazlett, H.C. *et al.* (2005) Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. *Arch. Gen. Psychiatry* 62, 1366–1376
103. Hahamy, A. *et al.* (2015) The idiosyncratic brain: distortion of spontaneous connectivity patterns in autism spectrum disorder. *Nat. Neurosci.* 18, 302–309
104. Just, M.A. *et al.* (2012) Autism as a neural systems disorder: a theory of frontal-posterior underconnectivity. *Neurosci. Biobehav. Rev.* 36, 1292–1313
105. Schipul, S.E. *et al.* (2011) Inter-regional brain communication and its disturbance in autism. *Front. Syst. Neurosci.* 5, 10
106. Courchesne, E. *et al.* (2019) The ASD living biology: from cell proliferation to clinical phenotype. *Mol. Psychiatry* 24, 88–107
107. Goldman, S.M. *et al.* (2014) Peptidoglycan recognition protein genes and risk of Parkinson's disease. *Mov. Disord.* 29, 1171–1180
108. Eberl, G. and Lochner, M. (2009) The development of intestinal lymphoid tissues at the interface of self and microbiota. *Mucosal Immunol.* 2, 478–485
109. Moore, R.E. and Townsend, S.D. (2019) Temporal development of the infant gut microbiome. *Open Biol.* 9, 190128