

THE MICROBIOTA-GUT-BRAIN AXIS IN NEURODEVELOPMENT: EMERGING ROLES OF PEPTIDOGLYCAN SIGNALLING

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SUMMARY

The gut microbiota has emerged as an important regulator of brain development, function and behaviour. Studies using germ-free, gnotobiotic and other experimental models have demonstrated that microbial colonization influences multiple processes involved in brain development, including blood-brain barrier formation and integrity, microglial maturation, and myelination. A major challenge is now to identify the molecular mechanisms underlying these effects, translate findings from experimental models to humans, and develop strategies to harness the gut microbiota to promote brain health. Microbiota-brain communication involves multiple neuronal, metabolic, immune and endocrine pathways. While considerable attention has focused on microbial metabolites, accumulating evidence indicates that structural bacterial components may also function as physiological signals. In particular, peptidoglycan (PGN) fragments released by commensal bacteria can cross biological barriers and be recognized by host PGN-sensing pathways. Experimental studies demonstrate that microbiota-derived PGN can reach the brain and that PGN sensing influences brain development and behaviour, with sex-dependent effects. More recent findings extend this concept to prenatal life, showing that maternal exposure to the PGN fragment muramyl dipeptide (MDP) can alter the foetal environment and PGN sensing and transport pathways in the foetal brain, with persistent sex-specific effects on offspring behaviour in the absence of overt foetal neuroinflammation. Translating these mechanisms to humans will require integration of experimental models with human stem cell-based model systems and prospective longitudinal studies. Early-life differences in microbial composition and metabolic function have been detected in infants at elevated likelihood of developing autism spectrum disorder (ASD) before measurable behavioural differences emerge, supporting a potential contribution of the gut microbiota to the neurobiology of ASD. At the same time, experimental and emerging clinical studies with *Limosilactobacillus reuteri* demonstrate the potential, as well as the complexity, of microbiota-based interventions to modulate neural circuits underlying social behaviour. Their effects depend on microbial strain, developmental timing and host characteristics, including biological sex. Together, these findings support a shift from largely descriptive microbiota studies towards understanding the precise molecular mechanisms mediating gut-brain interactions. Such mechanistic knowledge will be essential for translating microbiota-gut-brain research into targeted strategies to promote brain health in humans.

INTRODUCTION

The microbiota-gut-brain axis has emerged as an important framework for understanding how indigenous microorganisms residing in the gastrointestinal tract interact with the brain. The gut microbiota is a complex and dynamic ecosystem comprising bacteria, archaea, fungi and viruses that contributes to host physiology through a broad repertoire of metabolic, immune, endocrine and neuronal signals. Over the past two decades, evidence from germ-free (GF), antibiotic-exposed, gnotobiotic and other animal models has demonstrated that the gut microbiota can influence brain development, function and behaviour, with sex-specific effects. These studies have implicated the microbiota in processes ranging from blood-brain barrier (BBB) formation and integrity and microglial maturation to stress responses, myelination, synaptic development and complex behaviours (*Sudo et al., 2004; Diaz Heijtz et al., 2011; Braniste et al., 2014; Erny et al., 2015; Hoban et al., 2016*). Together with a growing, although still largely associative, body of evidence from human studies, these findings support the possibility that microbiota-host interactions contribute to brain development and function across the lifespan and that disruption of these interactions may be relevant to neurodevelopmental, psychiatric, neurological and neurodegenerative disorders (*Cryan et al., 2020*).

Gut microbiota and brain interactions may be particularly important during early life, although the nature of microbial influence differs fundamentally between the prenatal and postnatal periods. Current evidence does not support the presence of a resident foetal gut microbiota during healthy, uncomplicated pregnancy (*Kennedy et al., 2023*). Before birth, the maternal microbiota is therefore more likely to influence the

developing foetus indirectly through maternal and microbiota-derived signals than through direct microbial colonization. These signals may include microbial metabolites, immune mediators and structural microbial products that enter the maternal circulation and, in some cases, reach the placenta and foetal compartment (*Pessa-Morikawa et al., 2022; Wang et al., 2025*). Maternal infection, inflammation, stress, antibiotic exposure, diet and other environmental or pregnancy-related factors may alter the maternal microbiota and the nature or magnitude of these signals, thereby modifying the foetal environment. Following birth, exposure to maternal and environmental microorganisms initiates establishment of the infant gut microbiota (*Stewart et al., 2018; Sinha et al., 2026*), which subsequently undergoes substantial ecological and functional maturation during the first years of life.

During prenatal and early postnatal life, the brain undergoes extensive cellular and structural changes, including neurogenesis, neuronal migration, axonal and dendritic growth, synaptogenesis, synaptic pruning, myelination and maturation of glial populations (*Semple et al., 2013; Silbereis et al., 2016*). Maternal microbiota-derived signals may therefore influence the developing brain even before establishment of the infant gut microbiota and, after birth, microbial signals increasingly originate from the infant's own colonizing microbial community. Compelling evidence that postnatal microbial colonization contributes to these processes has come from GF and gnotobiotic models. GF mice exhibit alterations in behaviour and stress responsiveness together with changes in synaptic pathways, BBB integrity, myelination and microglial maturation (*Cryan, 2025*).

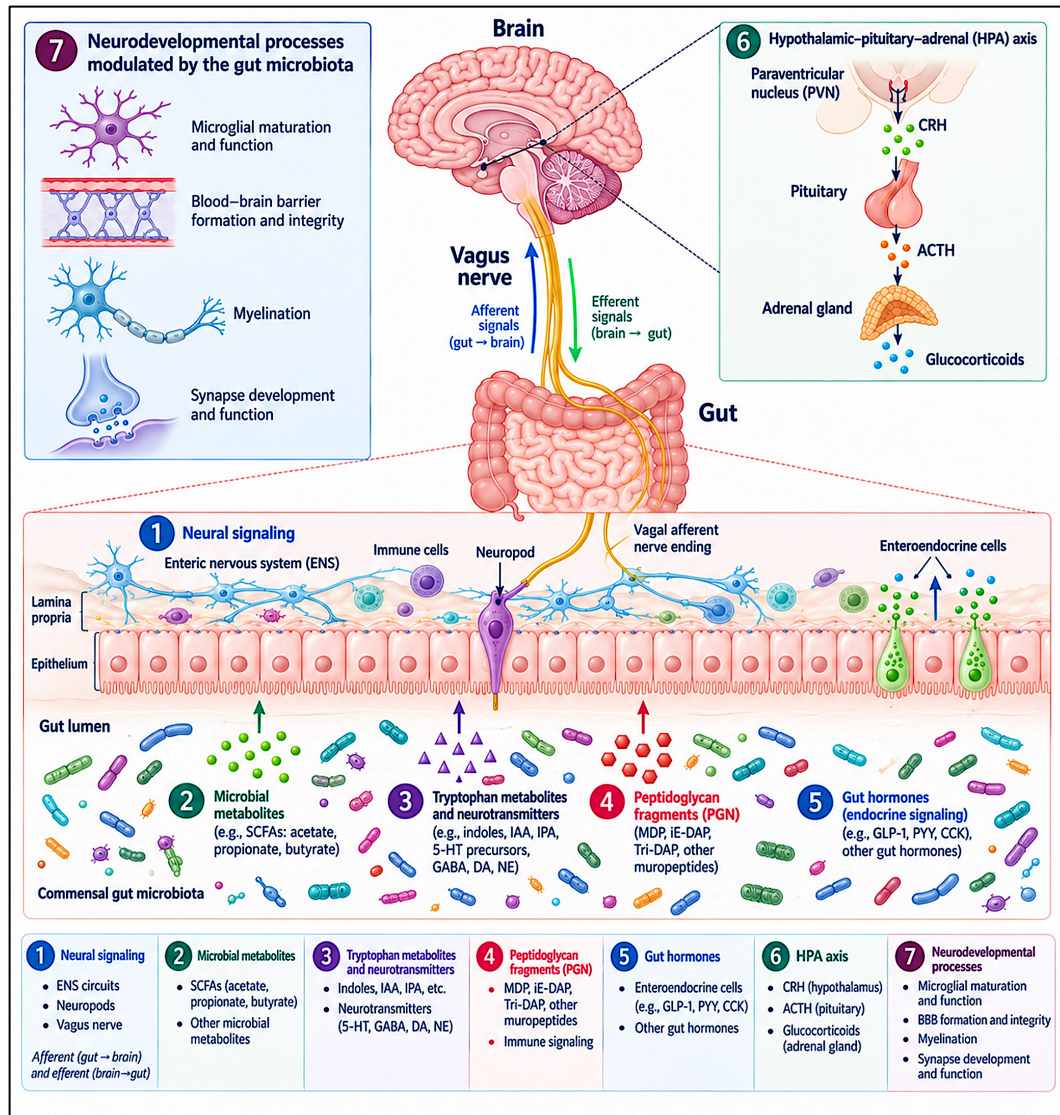


Figure 1: Biological signalling pathways and processes involved in microbiota–gut–brain communication.

The gut microbiota communicates with the developing brain through multiple, interconnected pathways. These include neural signalling through the enteric nervous system (ENS), neuropods, and bidirectional vagal pathways (1); microbial metabolites, including short-chain fatty acids (SCFAs) (2); tryptophan metabolites and neurotransmitter-related signalling (3); peptidoglycan (PGN) fragments and associated immune signalling (4); gut hormones released by enteroendocrine cells (5); and modulation of the hypothalamic–pituitary–adrenal (HPA) axis (6). Together, these signals can influence neurodevelopmental processes, including microglial maturation and function, blood–brain barrier formation and integrity, myelination, and synapse development and function (7). The relative contribution of individual pathways varies across developmental stages and physiological contexts, and substantial bidirectional and functional interactions occur among them. SCFAs, short-chain fatty acids; IAA, indole-3-acetic acid; IPA, indole-3-propionic acid; 5-HT, serotonin; GABA, γ -amino-butyric acid; DA, dopamine; NE, norepinephrine; MDP, muramyl dipeptide; iE-DAP, γ -D-glutamyl-meso-diaminopimelic acid (DAP); Tri-DAP, L-Ala- γ -D-Glu-meso-DAP; GLP-1, glucagon-like peptide-1; PYY, peptide YY; CCK, cholecystikinin; CRH, corticotropin-releasing hormone; ACTH, adrenocorticotrophic hormone; BBB, blood–brain barrier.

Controlled colonization studies have further begun to identify contributions of specific early-life microorganisms, including evidence that human infant-associated *Bifidobacterium* species can influence synaptic development and pruning in the cerebellum (Luck et al., 2020). Importantly, microbiota-dependent effects on the brain can differ between males and females and depend on the developmental period during which microorganisms or their signals are absent, introduced or restored.

Despite substantial progress, three major challenges remain central to the field: defining the mechanisms through which the gut microbiota influences brain development, function and behaviour; translating observations from experimental animal models to human

biology; and determining whether microbiota-based interventions can ultimately be harnessed to promote brain health. These three questions provide the framework for this monograph. Addressing them requires moving beyond associations between microbial community composition and brain-related phenotypes toward identifying the molecular signals responsible for communication with the host. Which microbial molecules are sensed? Can they cross intestinal, placental, blood-brain or other biological barriers? How are they transported through the body? Which receptors, cell populations and brain circuits respond to them? And how do developmental timing, biological sex, host genetics and physiological state shape these responses?

MICROBIAL SIGNALS AND THE BRAIN

Microbiota-brain communication occurs through multiple interacting pathways, including the vagus nerve and enteric nervous system, enteroendocrine signalling, immune mediators, microbial metabolites such as short-chain fatty acids (SCFAs) and tryptophan metabolites, and structural microbial components (Figure 1). One of the most direct routes is the vagus nerve, which conveys sensory information from the gastrointestinal tract to the brain. Experimental studies have shown that the behavioural effects of several probiotic strains depend on intact vagal signalling. For example, the effects of *Lactobacillus rhamnosus* JB1 on stress-related behaviour are abolished by vagotomy (Bravo et al., 2011), while vagal signalling is also required for *Limosilactobacillus reuteri* to restore social deficits in several mouse models relevant to autism spectrum disorder (ASD) (Sgritta et al., 2019). Communication between the intestinal epithelium

and vagal afferents may also involve specialized enteroendocrine cells with axon-like basal processes, commonly referred to as neuropod cells. These cells can form synaptic connections with vagal sensory neurons, providing a rapid route through which luminal signals may be communicated to the brain (Kaelberer et al., 2018). Experimental activation of gut-innervating vagal sensory neurons has also been linked to reward-related behaviour and dopaminergic signalling (Han et al., 2018), illustrating how intestinal signals can influence specific brain circuits.

Other microbiota-derived signals reach distant host tissues through the circulation. SCFAs, particularly acetate, propionate and butyrate, are generated predominantly through microbial fermentation of dietary substrates in the colon and can influence host cells through several mechanisms, including G protein-coupled receptors and the regulation of gene transcription. Studies in

GF animals have provided compelling evidence that SCFAs contribute to brain homeostasis. Erny and colleagues showed that the absence of a microbiota results in immature and functionally altered microglia and that treatment with SCFAs can partially restore these abnormalities (Erny et al., 2015). SCFAs have also been implicated in BBB integrity and in the regulation of microglial function in neurodevelopmental, psychiatric and neurodegenerative animal models. The molecular repertoire generated through microbiota-host interactions extends considerably beyond SCFAs. Commensal microorganisms can produce or modify neurotransmitters and numerous metabolites that enter the circulation, and microbial or microbiota-host co-metabolites have been detected in the brain from early postnatal life (Swann et al., 2020). Although the functional significance of many of these molecules for brain development remains poorly understood, their presence demonstrates that the developing brain is exposed to a chemically diverse repertoire of signals whose abundance depends, at least in part, on the gut microbiota.

Microbial signals may influence brain development even before establishment of the infant gut microbiota. Although current evidence does not support colonization of the foetus by a resident microbiota during healthy pregnancy, functional molecular signals generated by the maternal gut microbiota can enter the maternal circulation and reach the foetal compartment. Experimental studies have demonstrated that maternal microbiota-derived metabolites can influence foetal devel-

opment, including the programming of peripheral tissues (Kimura et al., 2020) and thalamocortical axon development in the foetal brain (Vuong et al., 2020). These findings establish an important conceptual principle: microbial colonization of the developing organism is not required for microbial signals to influence brain development. Before birth, the maternal microbiota can provide molecular information to the developing foetus; after birth, microbial signals increasingly originate from the infant's own colonizing microbial community. The nature and magnitude of these signals are likely to vary with the maternal and postnatal environment. Infection, inflammation, stress, diet, antibiotic exposure and other factors can alter microbial composition, microbial metabolism, intestinal barrier function or combinations of these processes and may consequently modify host exposure to microbiota-derived molecules. Their biological effects are therefore likely to depend on the identity and concentration of the signal, developmental timing, biological sex, host genetics and physiological state.

While much attention has focused on microbial metabolites, bacteria also continuously release structural components during growth, division, remodelling and lysis. These molecules can be recognized by host pattern-recognition systems and provide another potential source of molecular information about the microbial environment. Among them, PGN represents a particularly intriguing and comparatively underappreciated signal in microbiota-brain communication.

PEPTIDOGLYCAN: AN UNDERAPPRECIATED SIGNAL IN MICROBIOTA-BRAIN COMMUNICATION

PGN is an essential structural component of the bacterial cell wall and is

absent from mammalian cells. It consists of glycan chains composed of

alternating N-acetylglucosamine and N-acetylmuramic acid residues cross-linked by short peptide chains (*Wheeler et al., 2014*). Despite its structural role, PGN is highly dynamic. During bacterial growth, division, remodelling and turnover, fragments of the cell wall are continuously released into the surrounding environment. Different bacterial groups generate structurally distinct PGN motifs. Meso-diaminopimelic acid (DAP)-containing PGN is characteristic of Gram-negative bacteria and some Gram-positive species, whereas lysine-type PGN is found in many Gram-positive bacteria. Muramyl dipeptide (MDP) represents a minimal bioactive motif shared by PGN from both Gram-positive and Gram-negative bacteria.

The mammalian host possesses specialized pattern-recognition systems capable of detecting these structures. Two major families are the intracellular nucleotide-binding oligomerization domain receptors NOD1 and NOD2 and the peptidoglycan recognition proteins PGLYRP1-4 (*Royet et al., 2011; Mukherjee et al., 2019*). NOD1 preferentially detects DAP-containing PGN motifs, whereas NOD2 recognizes MDP. Historically, these pathways have been studied primarily in the context of bacterial infection and innate immune defence. However, the enormous and continuous source of PGN provided by the commensal microbiota raises a different possibility: PGN sensing may also participate in physiological host-microbe communication. An important demonstration of this principle came from Clarke and colleagues, who showed that PGN originating from the indigenous intestinal microbiota can translocate across the intestinal barrier and reach systemic tissues in healthy mice. Microbiota-derived PGN was sufficient to prime neutrophil function through NOD1 signalling (*Clarke et al., 2010*). More recent demonstrations of

systemic dissemination of microbiota-derived PGN have reinforced the concept of a circulating repertoire of PGN fragments generated by commensal bacteria (*Wheeler et al., 2023*).

Our work extended this concept to the developing brain. Arentsen and colleagues demonstrated that PGN fragments derived from the indigenous gut microbiota reach the developing mouse brain (*Arentsen et al., 2017*). Brain PGN levels increased in parallel with postnatal microbial colonization, suggesting that exposure of the brain to bacterial cell-wall components is part of normal postnatal development rather than being restricted to infection or pathological disruption of the BBB. Importantly, molecules required for PGN sensing are expressed in the developing brain. Members of the PGLYRP and NOD receptor families show developmentally regulated expression, as does the peptide transporter PepT1. Their expression varies according to brain region, developmental stage and sex, suggesting that the biological effects of PGN signalling are likely to be strongly context dependent. The functional importance of this pathway was demonstrated using mice lacking *Pglyrp2*. Juvenile *Pglyrp2*-deficient mice displayed alterations in the expression of genes involved in brain development and synaptic function, including *c-Met* and *Bdnf*, together with changes in social behaviour. Male, but not female, *Pglyrp2*-deficient mice exhibited a pronounced hypersocial phenotype, and additional sex-dependent behavioural alterations emerged later in life (*Arentsen et al., 2017, 2018*). Other studies have subsequently strengthened the connection between PGN sensing and brain function. NOD1-dependent signalling in intestinal epithelial cells has been implicated in stress-related behavioural responses, while NOD2 has been identified in hypothalamic neurons, where bacterial

PGN sensing contributes to the regulation of appetite and body temperature (Pusceddu et al., 2019; Gabanyi et al., 2022).

The biological activity of microbiota-derived PGN may depend not only on bacterial abundance or total PGN production but also on how the bacterial cell wall is remodelled. As a proof of principle, bacterial PGN hydrolases, including DL-endopeptidases, can alter the repertoire of soluble muropeptides available for host sensing and thereby modify NOD-dependent signalling (Gao et al., 2022). This emerging concept suggests that the functional PGN

output of a microbial community cannot necessarily be inferred from its taxonomic composition alone. Together, these findings suggest that PGN-sensing pathways should not be regarded exclusively as components of antimicrobial defence. Instead, they may represent an evolutionarily conserved molecular interface through which the host, including the developing brain, obtains information about its microbial environment. The finding that PGN reaches the brain during postnatal development raises a further question: could PGN signalling begin even earlier, before birth?

PEPTIDOGLYCAN SIGNALLING AT THE MATERNAL-FOETAL INTERFACE

During pregnancy, the maternal intestinal microbiota provides a continuous source of microbial molecules, including bacterial structural components, while the placenta and other maternal-foetal barriers regulate their access to the developing foetus. Changes in maternal intestinal permeability, microbial composition, bacterial growth or cell-wall turnover could therefore alter the amount and composition of PGN fragments entering the maternal circulation and reaching the foetal compartment. To investigate whether maternal PGN exposure could influence the foetal environment, we used MDP as a defined bioactive PGN motif. Pregnant mice were exposed to MDP through drinking water during late gestation, from gestational day 16 to 19. Using a cellular reporter assay based on HEK-Blue cells expressing mouse NOD2, we detected increased NOD2-activating activity in amniotic fluid 24 hours after maternal MDP exposure, with a significant increase observed in samples from male foetuses (Martinez Sanchez et al., 2025). This observation provides evi-

dence that changes in maternal exposure to a defined PGN fragment can influence the foetal environment and alter the molecular milieu surrounding the developing foetus.

We next examined whether the foetal brain showed molecular responses to this altered PGN environment. We focused on candidate transport systems for small bacterial peptides. Members of the SLC15 family, including SLC15A1/PepT1, SLC15A2/PepT2, SLC15A3/PHT2 and SLC15A4/PHT1, together with members of the SLC46 family, have been implicated in the transport of small PGN fragments in peripheral tissues (Bastos et al., 2021). In the foetal brain, maternal MDP exposure produced sex-dependent changes in the mRNA expression of genes associated with PGN transport and sensing. Male foetuses showed increased expression of *Slc15a1*, *Slc15a2* and *Slc46a2*, whereas *Nod2* expression was increased in females (Martinez Sanchez et al., 2025). One particularly important observation was the absence of significant changes in the mRNA expression

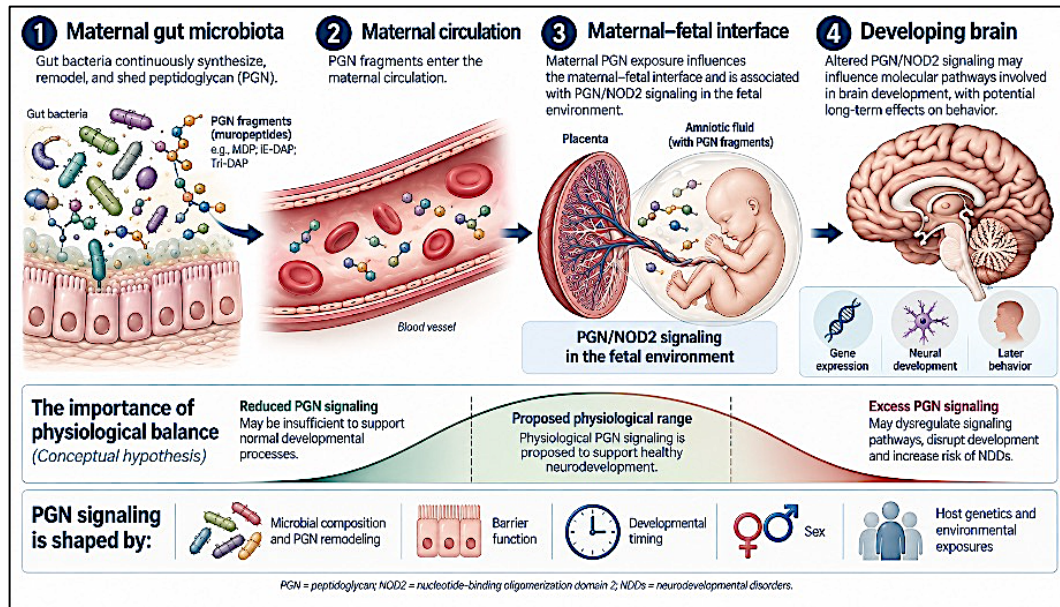


Figure 2. Maternal microbiota-derived peptidoglycan signalling during neurodevelopment.

The maternal gut microbiota continuously generates peptidoglycan (PGN) fragments, or muropeptides, through bacterial growth and cell-wall remodelling. These fragments can enter the maternal circulation and potentially influence the maternal-foetal interface and foetal environment, where PGN-sensing pathways, including NOD2 signalling, may contribute to developmental processes. Experimental studies indicate that perturbation of PGN/NOD2 signalling during gestation can alter molecular pathways involved in brain development and produce long-term effects on behaviour. The lower panel illustrates a conceptual model in which PGN signalling operates within a physiological range that supports normal neurodevelopment, whereas reduced or excessive signalling may disrupt developmental processes and increase susceptibility to neurodevelopmental disorders. The magnitude and consequences of PGN signalling are likely shaped by microbial composition and PGN remodelling, barrier function, developmental timing, sex, host genetics, and environmental exposures. MDP, muramyl dipeptide; iE-DAP, γ -D-glutamyl-meso-diaminopimelic acid; Tri-DAP, L-Ala- γ -D-Glu-meso-DAP; NOD2, nucleotide-binding oligomerization domain-containing protein 2; NDDs, neurodevelopmental disorders.

of inflammatory and microglia-related genes in the foetal brain. This distinction is conceptually important. Maternal immune activation models commonly rely on strong inflammatory stimuli and are generally interpreted through cytokine-mediated effects on foetal brain development. The MDP findings suggest an additional possibility: bacterial molecules may modify developmental programs through changes in PGN-associated sensing and transport pathways without necessarily provoking classical neuroinflammation. However, given that these observations were based on gene expression, further stud-

ies will be required to establish the underlying cellular mechanisms. The consequences of prenatal PGN exposure were not restricted to foetal molecular responses. Juvenile offspring displayed distinct sex-specific behavioural phenotypes. Prenatally exposed males showed reduced direct social interaction, whereas females displayed reduced novelty-induced locomotion and impaired social recognition. These behavioural alterations were accompanied by changes in the expression of synaptic and microglia-related genes in the prefrontal cortex (Martinez Sanchez et al., 2025).

Taken together with our earlier work showing that microbiota-derived PGN reaches the postnatal brain, these observations suggest a broader developmental framework (Figure 2). PGN signalling may operate across multiple developmental windows, beginning with maternal microbial signals during foetal life and continuing as the infant acquires its own intestinal microbiota after birth. The identity and concentration of PGN fragments, developmental stage, expression of sensing and transport pathways, brain region, biological sex, host genetics and physiological context may

collectively determine the biological response.

This raises an important question for future research. Rather than asking whether PGN is simply “beneficial” or “harmful,” should we instead consider the existence of an optimal developmental range of PGN signalling? Both insufficient and excessive exposure could potentially disturb developmental processes. Such a model would also help explain why environmental factors that alter microbial composition, bacterial turnover or barrier permeability may have different consequences depending on when they occur.

TRANSLATING MICROBIOTA-BRAIN FINDINGS TO HUMANS

Experimental animal models have been essential for establishing causal relationships between the gut microbiota and brain development. GF animals, antibiotic exposure, controlled microbial colonization and administration of defined microbial molecules make it possible to manipulate individual components of the microbiota-brain axis and examine their effects on brain development and behaviour. Translation to humans, however, remains challenging. Human microbial communities are highly heterogeneous and are shaped by host genetics, diet, medications, lifestyle, geography and numerous environmental exposures. Moreover, many experimental manipulations routinely used in animal models cannot be applied during human pregnancy or infancy. A translational approach therefore requires integration of complementary experimental systems. Human neural stem cell-based models and brain organoids may help determine whether microbial metabolites or structural bacterial components act directly on human brain cells and identify candidate receptors, transporters and molecular pathways

involved. Prospective human cohort studies can then determine whether related microbial features or molecular signals are detectable during relevant developmental windows and whether they precede differences in developmental outcomes. Metagenomic and metabolomic approaches provide an additional opportunity to move beyond taxonomic descriptions of the microbiota toward characterizing its functional capacity and molecular output.

Our longitudinal study of infants at elevated likelihood of developing ASD provides one example of the importance of studying the microbiota before behavioural differences become apparent. Infants with and without a family history of ASD were followed across the first 36 months of life (Zuffa et al., 2023). At 5 months, infants at elevated likelihood harboured lower abundances of *Bifidobacterium* and higher abundances of *Clostridium* and *Klebsiella* species compared with low-likelihood infants. Faecal γ -aminobutyric acid (GABA) was also lower in the elevated-likelihood group. Integrated microbiome-metabolome analysis revealed pos-

itive associations between GABA and *Bifidobacterium* species and negative associations with *Clostridium* species. *In vitro* experiments further demonstrated that bifidobacteria and clostridia differentially influence GABA availability, with *Clostridium* species consuming GABA. Importantly, these microbial and metabolic differences were already present at 5 months, when behavioural measures did not differ significantly between the groups. Differences in developmental and ASD-related measures became evident later, at 36 months. Although these observational findings cannot establish causality, they demonstrate the value of longitudinal studies beginning before measurable behavioural differences emerge and raise the possibility that early microbial functional differences may contribute to later atypical neurodevelopmental trajectories (Zuffa et al., 2023).

The same emphasis on microbial function rather than taxonomy may ultimately be important for PGN. Recent work provides proof of principle that bacterial PGN-remodelling capacity can determine the production of host-active

muropeptides (Gao et al., 2022). A recent study in preterm infants further linked early antibiotic exposure with altered microbial PGN-processing capacity and host responses (Shen et al., 2026). These findings concern intestinal and immune physiology rather than brain development and should therefore not be extrapolated directly to neurodevelopment. Nevertheless, they demonstrate that environmental perturbations in early life can modify the functional PGN output of the human microbiota.

The strongest translational evidence will ultimately emerge from convergence across experimental systems: mechanistic studies in animals, validation of candidate pathways in human cell-based models and longitudinal human studies demonstrating that related microbial functions or molecular signals are relevant during human development. For PGN, important questions include which muropeptides circulate during pregnancy and infancy, how their abundance and molecular composition change with development, and whether variation in these signals is associated with different trajectories of brain development.

TARGETING THE MICROBIOTA TO PROMOTE BRAIN HEALTH

Understanding the molecular mechanisms linking microbes to the brain is also essential for developing rational microbiota-based interventions. Rather than seeking to establish a universally “healthy microbiome,” a more realistic translational goal may be to identify microbial functions, molecules, and host signalling pathways that can be targeted to modulate specific brain circuits and functions in a predictable manner. Studies with *Limosilactobacillus reuteri* illustrate both the potential and the complexity of microbiota-based interventions to modulate neural circuits under-

lying social behaviour. Buffington and colleagues showed that maternal high-fat diet altered the offspring gut microbiota and was associated with impaired social behaviour (Buffington et al., 2016). These behavioural changes were accompanied by reduced numbers of oxytocin-immunoreactive neurons in the hypothalamus and impaired synaptic potentiation in the ventral tegmental area (VTA) in response to social interaction. Microbial transfer experiments supported a causal role for the altered gut microbiota in the social deficits. Remarkably, reconstitution

with *L. reuteri* restored hypothalamic oxytocin levels, social interaction-induced synaptic plasticity in the VTA and social behaviour (Buffington et al., 2016). Subsequent work demonstrated that the effects of *L. reuteri* are not restricted to a single environmental model. Sgritta and colleagues showed that *L. reuteri* treatment selectively rescued social deficits across genetic, environmental and idiopathic mouse models relevant to ASD (Sgritta et al., 2019). Importantly, the behavioural effects were not mediated by restoration of the overall gut microbiota composition. Instead, *L. reuteri* acted in a vagus nerve-dependent manner and restored social interaction-induced synaptic plasticity in the VTA, while its effects were absent in oxytocin receptor-deficient mice. Together, the Buffington and Sgritta studies established a mechanistic connection between a defined commensal bacterium, vagal signalling, oxytocin-dependent modulation of dopaminergic reward circuitry and social behaviour.

More recent work has reinforced this pathway in another environmental context. In offspring exposed to prenatal stress, *L. reuteri* supplementation during critical developmental windows restored oxytocin levels in the paraventricular nucleus, improved dopamine reward pathway function and ameliorated social deficits. These effects were abolished by either oxytocin receptor antagonism or subdiaphragmatic vagotomy, further supporting the importance of vagal afferent signalling and oxytocin-dopamine circuitry in *L. reuteri*-mediated modulation of social behaviour (Zhang et al., 2026). Importantly, early clinical studies suggest that some of these preclinical observations may translate to humans. In a pilot double-blind, randomized, placebo-controlled trial in children with ASD, treatment with a combination of

L. reuteri strains ATCC-PTA-6475 and DSM-17938 improved social functioning across several measures, although it did not alter overall autism severity, restricted and repetitive behaviours, microbiome composition or immune profiles (Mazzone et al., 2024). Preclinical comparison of the strains further indicated strain specificity: ATCC-PTA-6475, but not the parental strain of DSM-17938, rescued social deficits in an ASD mouse model. These findings illustrate an important principle for microbiota-based therapeutics: closely related bacterial strains cannot be assumed to exert equivalent effects on brain function and behaviour.

Developmental timing and biological sex add further layers of complexity. We recently investigated the long-term consequences of *L. reuteri* supplementation during a defined perinatal window, from gestational day 6 to postnatal day 7, in BALB/c mice (Sieglar Lathrop et al., 2025). Perinatal supplementation produced persistent improvements in sociability and social recognition in adult males, together with changes in gut microbiota composition and increased *Il10* gene expression in the striatum and colon. Expression of the oxytocin receptor (*Oxtr*) was increased in both the prefrontal cortex and striatum in males and females. However, females showed a distinct behavioural profile, including impaired social recognition, demonstrating that the same early-life microbial intervention can produce divergent long-term outcomes according to sex. Interestingly, expression of the PGN-associated transporters *Slc46a2* and *Slc46a3* was also altered in a sex-dependent manner, raising the possibility that microbial interventions may influence not only vagal and neuroendocrine pathways but also host responsiveness to structural bacterial signals. Collectively, these observations highlight several variables likely to

determine the success of early-life microbial interventions. Timing matters because prenatal and postnatal microbial signals encounter fundamentally different developmental environments. Host factors, including sex, genetics and immune status, can modify responsiveness. Microbial characteristics, particularly strain identity, dose and mode of administration, determine the functions delivered to the host. Finally, environmental context, including maternal diet, stress, inflammation and antibiotic exposure, may enhance, modify or inter-

fere with an intervention.

These considerations argue against a one-size-fits-all approach to microbiota-based therapeutics. Instead, future strategies will require increasing precision in defining when, how and for whom microbial interventions are likely to be effective. Ultimately, identifying the molecular functions and signals generated by individual microbial strains may allow interventions to be selected on the basis of biological mechanism rather than microbial taxonomy alone.

CONCLUSIONS AND PERSPECTIVES

Research on the microbiota-gut-brain axis has progressed from demonstrating that microorganisms influence brain development, function and behaviour toward identifying the molecular signals responsible for these effects. This transition from association toward mechanism is essential if microbiome research is to explain how microbial environments shape brain development and ultimately determine whether these pathways can be modified to promote brain health. PGN illustrates this conceptual shift particularly well. Once viewed primarily as a pathogen-associated molecule that activates innate immunity during infection, PGN is increasingly recognized as a component of normal host-microbiota communication. The intestinal microbiota continuously generates a diverse repertoire of PGN fragments, collectively forming a peptidoglycome shaped by bacterial growth, turnover and cell-wall remodeling. A proportion of these fragments can cross biological barriers and reach distant host tissues. PGN-sensing and candidate transport pathways are present in the developing brain, and experimental manipulation of PGN exposure or sensing can alter molecular programs

involved in brain development and behaviour.

Our recent findings extend this framework into prenatal life. Maternal exposure to the NOD2 ligand MDP alters NOD2-activating activity within the amniotic fluid and alters the expression of genes associated with PGN sensing and candidate transport pathways in the foetal brain in a sex-dependent manner. These molecular changes occur without detectable changes in the expression of microglial or inflammatory markers in the foetal brain, while transient prenatal PGN overexposure is followed by persistent, sex-specific behavioural and molecular alterations in juvenile offspring. These findings raise the possibility that PGN can function not simply as an inflammatory stimulus but as a developmental microbial signal whose biological consequences depend on molecular structure, concentration, developmental timing, tissue context and host characteristics.

An important next step will be to understand how the microbial PGN signal itself is generated. PGN is continuously remodelled by bacterial enzymes that determine the molecular repertoire of soluble fragments released into the

intestinal environment. Recent evidence that bacterial DL-endopeptidase activity can regulate the generation of host-active mucopeptides provides proof of principle that microbial PGN remodelling can influence host signalling. The functional PGN output of the microbiota may therefore depend not simply on which bacteria are present, but on the enzymatic activities that determine which PGN fragments become available for host sensing. This concept may be particularly important during early development. The infant microbiota undergoes profound ecological and functional maturation during the same period in which brain circuits, glial populations, the immune system and physiological barriers undergo extensive development and maturation. It is therefore conceivable that maturation of the microbiota is accompanied by maturation of its PGN-remodelling capacity, generating developmentally distinct peptidoglycomes. Antibiotics, infection, diet and other environmental perturbations could modify this process by changing not only microbial community structure but also the quantity and molecular composition of PGN fragments presented to the host.

Many fundamental questions remain. Which PGN fragments circulate under physiological conditions during pregnancy and infancy? How does their molecular composition change during development? How do individual mucopeptides cross intestinal, placental and brain barriers? Which transport systems regulate their tissue and cellular distribution? Which brain and peripheral cell populations respond to particular PGN structures? And how do developmental timing, biological sex, host genetics and environmental exposures modify these responses? Extending this framework to humans represents a major challenge. Interindividual variation in microbial PGN biosynthesis and remodelling,

intestinal permeability, systemic clearance, transport and innate immune sensing could generate substantially different PGN exposures even among individuals with apparently similar microbiota compositions. Longitudinal studies integrating metagenomic analysis of PGN-related microbial functions with characterization of circulating mucopeptides will be required to determine whether distinct PGN signatures accompany typical developmental trajectories and whether altered PGN signalling is associated with atypical brain development.

The same mechanistic precision will be required for microbial interventions. The *L. reuteri* studies illustrate both the promise and complexity of this approach. Developmental timing, biological sex, host genetics, microbial strain and environmental context can all influence the response. Identifying the molecular signals generated by specific microorganisms may therefore allow the field to move beyond interventions defined principally by microbial taxonomy toward approaches based on defined microbial functions.

The developing brain exists in continuous molecular dialogue with the microbial world. Deciphering this dialogue will require understanding not only which microorganisms are present, but also which molecules they generate, how those molecules are modified, where they travel and how they are sensed by the developing host. PGN provides a compelling model for this emerging view of microbiota-brain communication. Defining the developmental peptidoglycome, and the microbial and host mechanisms that shape it, may ultimately reveal new principles governing brain development and identify molecular pathways through which early-life microbial environments influence vulnerability or resilience to neurodevelopmental and neurological disorders.

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