BMC Medicine

REVIEW

Open Access

Gut-immune-brain interactions during neurodevelopment: from a brain-centric to a multisystem perspective

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Abstract

Background Neurodevelopmental disorders (NDDs) and epileptic syndromes are complex neurological conditions linked by shared abnormal neurobiological processes. Existing therapies mostly target symptoms, rather than addressing the underlying causes of the disease, leaving a burden of unmet clinical needs.

Main body Emerging evidence suggests a significant role for the gut microbiota and associated immune responses in influencing brain development and function, changing the paradigm of a brain-centric origin of NDDs. This review discusses the pivotal interactions within the gut-immune-brain axis, highlighting how microbial dysbiosis and immune signaling contribute to neurological pathologies. We also explore the potential of microbial management and immunomodulation as novel therapeutic avenues, emphasizing the need for a shift towards addressing the root causes of these disorders rather than just their symptoms.

Conclusions This integrated perspective offers new insights into the biological underpinnings of NDDs and epilepsy, proposing innovative biomarkers and therapeutic strategies.

Keywords Autism, ADHD, Epilepsy, Gut-microbiota, Neurodevelopment

Background

The human gut microbiome: our second genome

The collection of bacteria, archaea, eukaryotes, and to a lesser extent yeasts, parasites, and viruses, colonizing the gastrointestinal (GI) tract is called the "*gut microbiota*". This complex ecosystem has co-evolved with the host to establish an intricate and mutually beneficial relationship [1]. *Eubiosis* is a condition of interspecies balance, which is beneficial for the host as it allows the microbiota community to perform metabolic functions (such as the synthesis of neuroactive metabolites, neurotransmitters, and their precursors), enzymatic functions, and stimulation of the immune system. An alteration of the gut bacterial composition due to overgrowth of potentially pathogenic bacteria, or loss of overall bacterial diversity, is called *dysbiosis* [2, 3]. Dysbiosis has been associated with the pathogenesis of many disorders such as immunological, cutaneous, cardiovascular, and even neurological diseases [4, 5].

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Gut colonization: from pregnancy to infancy

Starting from intrauterine life, there are different environmental factors that can influence microbial development, including the mother's lifestyle, oral broad spectrum antibiotics exposure, and infections [6]. It is well-known that unhealthy dietary habits can shape the maternal gut microbiota and influence the baby's risk for disease later in life [7–9]. As pregnancy progresses, the maternal gut microbiota composition becomes enriched in bacterial populations (i.e., *Lactobacillus, Streptococcus,* and *Enterococcus*) found later in the infant gut [8].

Depending on the type of delivery, the newborn comes into contact with different microbial populations [10]. During a vaginal delivery, the newborn skin and mucosal surfaces are seeded with maternal vaginal and fecal microorganisms [6, 11]. At birth, the neonate gut provides an aerobic environment colonized primarily by tolerant aerobes, such as *Enterobacteriacee* and *Clostridiacee*. Within a few days, however, these organisms deplete the oxygen levels, transforming the intestinal lumen into an anaerobic environment, thus allowing the subsequent colonization by strict anaerobes such as *Bifidobacterium* (phylum: *Actinobacteria*), *Clostridium* (phylum: *Firmicutes*), and *Bacteroides* (phylum: *Bacteroidetes*) [12]. Differently, babies born by cesarean section (CS) show a significantly lower abundance of *Bacteroides* and *Bifidobacterium* spp. and an overrepresentation of species associated with the hospital environment and the skin surface, such as *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp. [13, 14] Moreover, compared to vaginally delivered babies, the gut microbiome of children born from CS shows enhanced strain turnover in early life, leading to functional differences in the immunostimulatory potential of the gut microbial community [15, 16]. The disruption of microbial transmission from the mother to the neonate is linked to conditions more frequently observed in CS-born individuals, including allergies, chronic immune syndromes, and metabolic disorders [17–19] (Fig. 1).

Another important factor influencing the newborn gut microbiota is the type of feeding. While infant formula is produced to carefully mimic the nutritional composition of breast milk, it is well known that breastfeeding provides better protection against a wide variety of early life medical conditions, such as respiratory infections and neonatal fever, sudden infant death syndrome (SIDS), and childhood obesity and cancer, together with an array of other adult disorders including cardiovascular disease, hypertension, diabetes, depression, and Alzheimer's disease [20–24]. As solid food is introduced in the child's diet during weaning, the gut microbiota composition becomes increasingly more similar to that of the

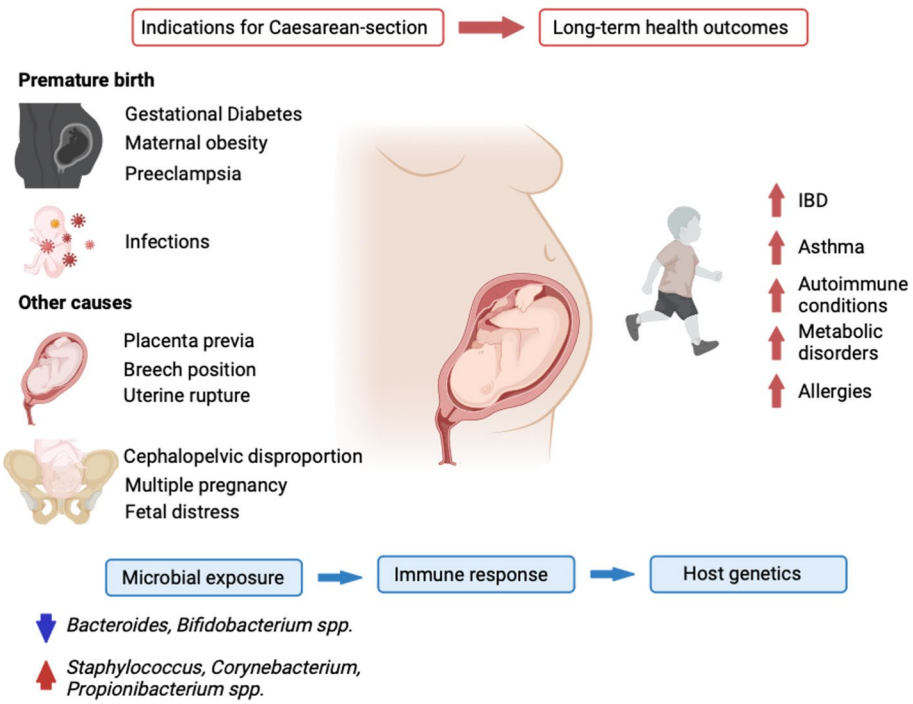

Fig. 1 Conditions linked to Caesarean section and related long-term health outcomes. Several indicators of Caesarean section, including premature birth, placental disorders, and cephalopelvic disproportion, may cause microbial dysbiosis. This microbial substrate shapes the immune system and exposes the infant to a wide range of autoimmune, allergic, and metabolic conditions throughout the lifespan. Created with Biorender.com

adult, with the main bacterial groups being *Streptococcus fecalis* (30%), *Staphylococcus epidermidis* (20%), *Streptococcus faecium* (10%), *non-hemolytic streptococci* (10%), *Staphylococcus aureus* (4%), and *Enterobacteriaceae* (i.e., *E. coli* [20%], *Klebsiella aerogenes* [20%], *Proteus mirabilis* [2%], *Enterobacter cloacae* [1%], *Serratia* sp. [1%],and *Pseudomonas aeuroginosa* [0.5%]) [13]. The first 1000 days of life constitute a critical time window susceptible to insults that can have long-lasting effects on the correct development and future health of the child [25, 26].

Gut-organ axes

During the developmental period, the gut microbiome matures along with the host's organs and interacts with them through multi-directional communication systems. Exploring these gut-organ axes is fundamental to understand the role of the gut microbiome in human health and disease [27]. In particular, emerging evidence suggests a significant role for the gut microbiota and associated immune responses in influencing brain development and function. This review will focus on the gut-immune-brain interactions and the pathological roles that dysbiosis plays in the development of neurological disorders, changing the paradigm of a brain-centric origin for these conditions.

The microbiota-gut-brain axis: from a brain-centric to a multisystem perspective

Developmental stage

The connection between the gut and the brain finds its origin in their embryological development, as both the GI tract and the central nervous system (CNS) originate from the neural crest, strongly influencing each other's maturation [28]. Various stimuli contribute to shape the CNS increasingly complex neuronal circuitry, including hormones (i.e., oxytocin), neurotransmitters (i.e., serotonin), the immune system, and the gut microbiota. Particularly, the relevance of the gut microbiome in early brain development has been established thanks to germ-free (GF) murine models [29]. Genome-wide transcriptomic analyses have demonstrated several brain-specific molecular modifications in GF mice, including decreased expression of 5-hydroxytryptamin 1 A (5-HT1 A) receptor genes in the hippocampal dentate gyrus; upregulation of myelination-related genes in the prefrontal cortex; alteration of synaptic plasticity-related genes such as the brain-derived neurotrophic factor (BDNF) in brain areas such as the striatum; increased transcription of early response genes such as *Fos*, *Fosb*, *Egr2*, and *Nr4a1*, as well as the transcription factor cAMP response element-binding protein (CREB) in the amygdala. Other important cellular changes included increased neurogenesis in the hippocampus; increased amygdala volume and structural changes of pyramidal neurons in the basolateral amygdala; alteration in microglial phenotypes [30, 31]. These results support that gut microbiota crucially contribute to a correct development of the CNS, starting from intrauterine life. Impaired neurodevelopment of the macro areas of the brain can lead to behavioral and neurological disorders. Neurogenesis in the hippocampus, for instance, is involved in spatial learning and memory; the amygdala is linked to autism spectrum disorders (ASD) and anxiety disorders; while the prefrontal cortex is the main area of neuropsychiatric disorders, such as attention deficit/hyperactivity disorder (ADHD), ASD, depression, and schizophrenia [30, 31] (Fig. 2).

Microbiota-gut-brain axis routes

Beyond the developmental stage into adulthood, the gut and the brain continue their bidirectional communication through the spinal cord, the autonomic nervous system (ANS), the hypothalamic–pituitary–adrenal axis (HPA), and the enteric nervous system (ENS) [32, 33]. The brain can stimulate the ENS and gut function to such an extent that the ENS can be considered as an extension of the limbic system in the gut [34]. Furthermore, the vagus nerve relays both orthodromic information, to regulate the contraction of smooth muscles and glandular secretion in the intestine, and antidromic information to several regions of the CNS, such as the locus coeruleus (LC), the rostral ventrolateral medulla, the thalamus, and the amygdala [35]. Several studies showed that probiotic treatment with *Lactobacillus rhamnose* improved anxiety and depressive-like behavior in naïve mice, and this effect was precluded by vagotomisation, indicating that the beneficial properties of this bacterial strain are dependent upon gut-brain signaling via the vagus nerve [24]. Similarly, probiotic treatment with *Bifidobacterium longum* failed to produce an anxiolytic effect in a vagotomised colitis mouse model [24]. Finally, the gut microbiota release molecules that can cross the intestinal barrier and disseminate into the systemic circulation. For instance, short-chain fatty acids (SCFAs) are produced through fermentation of undigested polysaccharide and oligosaccharides by gut bacteria and cross the blood brain barrier (BBB) to modulate neurons and glia at the cellular level. In the CNS, butyrate inhibits histone deacetylase (HDAC), reduces inflammation, increases fatty acid oxidation, and promotes BBB integrity [36].

When dysfunction of the microbiota-gut-brain axis occurs, multiple pathologies can arise, including irritable bowel syndrome (IBS), diabetes, obesity, and also neurological disorders such as depression, post-traumatic stress disorder (PTSD), and ASD [24]. Interestingly, many of these conditions have a systemic or local inflammatory component, suggesting that the immune system

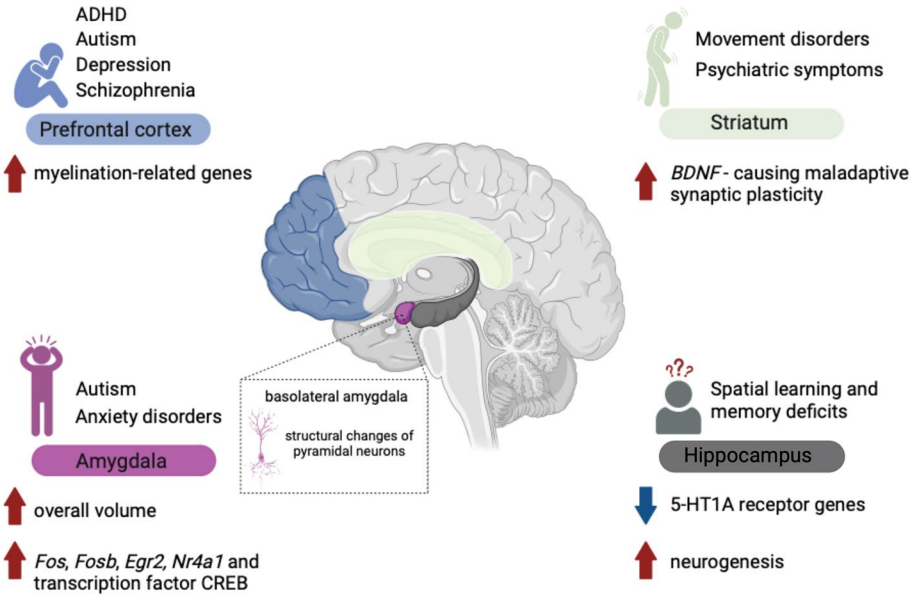

Fig. 2 Gut microbiota-mediated transcriptomic and structural changes in brain macro areas. Brain macro areas known to be involved in the behavioral and neurological disorders caused by an altered gut microbiota, as demonstrated by transcriptomics and brain histology studies in GF mice. Created with Biorender.com

might also be involved in the cross-talk between the gut and the brain (Fig. 3). For instance, chronic stress can enhance gut epithelial permeability, leading to increased translocation of endotoxins and induction of low-grade inflammation, a phenomenon associated with many neurological disorders (i.e., major depressive disorder) [37].

The gut-immune-brain axis: adding a layer of complexity

Local immunity in the brain

Resident immune cells in the brain, such as microglia and astrocytes, perform homeostatic functions and protect the CNS from insults. Empirical evidence suggests that gut microbial communities and their metabolites can influence CNS-resident immune cell structure and function during neurodevelopment, with long lasting effects. This section will highlight the most recent pre-clinical and clinical studies showing a link between gut microbiota and CNS immunity (Table 1).

Gut microbiota modulates CNS-resident immune cells

Along with inducing broad changes in the macro areas of the developing brain, the gut microbiota can affect CNS fine tuning at the cellular level. In particular, different gut microbiota populations modulate intestinal, systemic, and CNS-resident immune cell function, highlighting that gut-brain interactions may involve the host immune system as well. Noteworthy is the role of microglia during development. Microglia are the tissue-resident macrophages of the CNS, responsible for phagocytizing dying cells, protein aggregates, and other soluble antigens that might endanger the brain. The homeostatic functions of microglia influence brain development by affecting synaptic patterning and neurotransmission [38, 39], neuronal cell migration, and survival, myelinogenesis, and axon dynamics [31, 40]. Gut microbiota has been implicated in reducing oxidative stress and improving mitochondrial dysfunction in microglia, especially in the aged brain [41]. Erny and colleagues also demonstrated that GF mice display global defects in microglia with altered cell proportions, phenotype, and effector functions, leading to an impaired immune response [40]. Furthermore, they analyzed microglial function in mice with limited microbiota complexity, exclusively colonized by a standardized microbiota cocktail (the so called "altered Schaedler flora" [ASF]), which includes *Bacteroides distasonis* (strain ASF 519), *Lacotbacillus salivarius* (strain ASF 361), and *Clostridium cluster XIV* (strain ASF 356). In this model, microglia showed a structural and expression alteration pattern comparable to that observed in GF mice, suggesting that a gut microbiota with limited diversity is not sufficient to induce comprehensive microglial maturation [40]. Interestingly, when the tri-colonized mice were co-housed with specific-pathogen free (SPF) mice, they displayed a normal microglia density and partially restored microglia processes (i.e., normal length, number of segments, and branching points), highlighting that reconstitution of a rich and complex gut microbiota

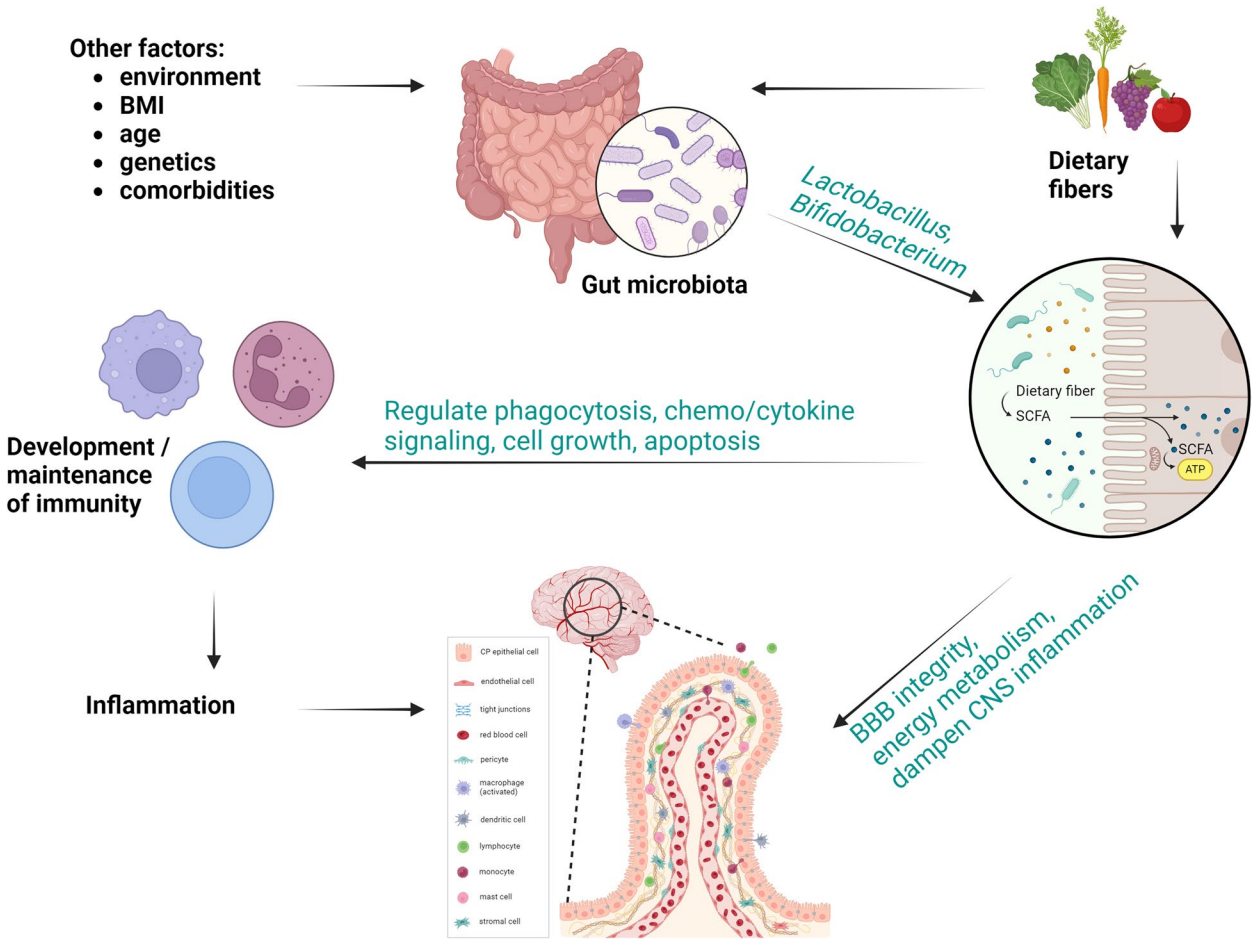

Fig. 3 Gut-immune-brain interactions. An overview of the cross talk between the gut microbiota, the immune system, and the brain, with a focus on the role of short-chain fatty acids (SCFAs) as signaling mediators in this network. Created with Biorender.com

could rescue the microglia phenotype. Similarly, administering SCFAs to GF mice also reversed changes in the microglial structural and maturation patterns [40].

Microglia are involved in many neurodegenerative and neuroinflammatory disorders. Classically activated microglia assume an inflammatory profile characterized by the production of pro-inflammatory cytokines and chemokines, as well as reactive oxygen and nitrogen species. Alternatively activated microglia secrete anti-inflammatory mediators and neurotropic factors, which are involved in restoring homeostasis [42]. Overactivation of microglial functions can lead to brain pathology and is associated with Parkinson's (initiation and progression phase) [43] and Huntington disease (all grades) [44, 45]. Furthermore, a pro-inflammatory microglia phenotype has been described in the acute or early phase of disease development in multiple sclerosis patients and experimental autoimmune encephalomyelitis in mice. Microglia then progress towards an anti-inflammatory polarization during remyelination, and finally present a pro-inflammatory phenotype during relapse [46, 47]. Interestingly, in Alzheimer's disease (AD) [48, 49] and amyotrophic lateral sclerosis [50, 51] the opposite occurs, where microglia start out with an anti-inflammatory phenotype, followed by a pro-inflammatory phenotype during the advanced stage. In the context of epilepsy, microglia can have both pro and anti-epileptic properties, as their transcription, morphology, and effector functions vary dynamically in a context-dependent manner during disease progression. Interestingly, while neuroinflammation can promote the development and recurrence of seizures by lowering seizure threshold and increasing neuronal excitability, it can also be the other way around [52]. Seizures can cause brain injury and cell death, therefore activating microglia to produce inflammatory mediators. Murine studies showed that these molecules perpetrate neuronal excitation, neurotoxicity, and BBB dysfunction, giving rise to a vicious cycle [53].

Table 1 The gut microbiota-immuno-brain axis

Gut microbiota	Immune cells	Brain
Structural and maturation pattern [30, 31, 40]; reduce oxidative stress and mitochondrial dysfunction [41]	Microglia	Implicated in Parkinson's [43], Huntington disease [44, 45], MS [46, 47]
Regulate levels [68]; promote recruitment and migration [71]; inhibit nitric oxide generation [72]; enhance effector responses [73–75]	Neutrophils	Implicated in stroke, Alzheimer's, Parkinson's, Huntington disease, MS, autism [76], epilepsy [77]
Regulate intestinal levels [69]; dampen degranulation and development food allergies [176]	Mast cells	Involved in neurogenesis, neuroinflammation, neurodegeneration, disruption of the BBB. Implicated in Alzheimer's, Parkinson's, Huntington disease, ALS [78]
Promote anti-inflammatory phenotype [29, 65]	Dendritic cells	Implicated in stroke, brain tumors, MS, Alzheimer's, Parkinson's, and epilepsy [82]
Promote anti-inflammatory phenotype, chemotaxis, and phagocytosis [29, 65]; affect polarization and reduce glycolysis [79, 80]	Macrophages	Implicated in neurodegenerative (i.e., Parkinson's and Alzheimer's) and neuroinflammatory disorders (MS and ALS) [81]
Promote differentiation of NKp46 + cells expressing RORγt [65], which regulate mucosal immunity [85]	NK	Reside in the meninges at steady state and migrate to the brain parenchymal during stroke and MS [85]
Modulate CD4 + Th differentiation (Th1, Th2, Th17 or Treg) [29, 65, 88, 91, 92] Regulate CD8 + cell function [65, 95] and maintenance [96]. SCFAs enhance CD8 + T cells activation and differentiation into long-lived memory cells [98, 99]	T cells	CD4 + accumulation is associate with hemorrhagic brain injury pathology [93] CD8 + are implicated in neurodegenerative conditions (i.e., Parkinson's, Alzheimer's, and MS) [100]; as well as in neuroinflammation, endotheliopathy [101], limbic encephalitis [102], and Rasmussen encephalitis [103]
Impact B cell anatomical clustering and function [104]. SCFAs can play contrasting roles on B cell epigenetics, class switching, antibody responses, and differentiation [107, 108]	B cells	Implicated in MS, neuromyelitis optica spectrum disorder, MOG antibody-associated disorder, Anti-N-methyl-D-aspartate receptor encephalitis, autoimmune epilepsy, Parkinson's and Alzheimer's disease [109]

Table 2 The interplay between SCFAs and the immune system

Cell/tissue type	Butyrate	Propionate	Acetate
Neutrophils	Increases recruitment and migration [71]		Enhances responses via FFAR2 to promote IL-1β and inflammosome [73–75]
	Specifically inhibit generation of nitric oxide [72]		
Macrophages	Reduces glycolysis in vitro resulting in increased AMP-activated protein kinase (AMPK) and decreased mTOR activity [79, 80]	Affect polarization [79, 80]	
T cells	Support the development of CD4 + Th1, Th17, and IL-10-secreting Tregs, inhibition of HDACs, and the increase of mTOR-S6 K activity [88]. Also control CD8 + immune response [95]		
	Promotes memory potential of antigen-activated CD8 + [98, 99]		
B cells	Have contrasting functions by regulating B cell epigenetics, class switching, antibody responses, and differentiation [107, 108]		
Brain	Inhibits HDAC, reduces inflammation, increases fatty acid oxidation, and promotes BBB integrity [36]		

Other players of CNS local immunity

Gut bacteria-derived SCFAs (Table 2) can modulate the activation of astrocytes, essential for maintaining CNS homeostasis, via mitochondrial modulation [54]. Astrocyte dysfunction is involved in neuroinflammation [54] and plays a role in a range of neurological conditions [55]. Astrocytes recognize and are activated by inflammatory cytokines produced by gut resident macrophages in response to dysbiosis [54]. Furthermore, Caldwell et al. demonstrated that increased secretion of insulin like growth factor binding protein 2 (Igfbp2), an inhibitor of insulin-like growth factor (IGF), contributes to altered neuronal development in mouse models of Rett, Fragile X, and Down syndrome. Administration of IGF or inhibition of Igfbp2 can partially rescue neuronal deficits associated with these NDDs [56]. Moreover, astrocytes can mediate changes in brain metabolism and neuronal excitability, and dysregulation of these mechanisms can play a role in the onset and progression of epilepsy [57].

Taken together, these results show that both microglia and astrocytes are involved in the pathogenesis of several CNS disorders; therefore, alterations in their activation state and their homeostatic role resulting from their interaction with gut microbiota may have relevance for the genesis and progression of such diseases.

Systemic immunity

The gut microbiota influences immune induction, training, and effector functions during development and throughout life. Recent evidence suggests that systemic immune cells can cross the BBB in certain conditions and modulate the local environment in the brain. This section will discuss the mutual relationship between gut microbiota and the innate and adaptive immune systems, with a focus on the role of the gut-immune-brain axis in neurological disorders.

The brain: immune-privileged or not?

For a long time, the CNS was classified as immune-privileged due to the BBB, the lack of conventional draining lymphatics, and other factors. Aside from microglia, the CNS was thought unable to mount a functional immune response to local insults. However, more recently this dogma has been revised as new evidence came to light suggesting a role for peripheral innate and adaptive immune cells in higher CNS functions, response to insults, homeostasis, and tissue repair [58, 59]. In particular, alongside microglia and astrocytes, the brain immunological functions are mediated by myeloid cells (both CNS-residing and peripheral), lymphocytes entering through the BBB, and neural cells (i.e., neurons and oligodendrocytes) [59]. During injury or infection, activated glial cells release chemokines to attract peripheral innate and adaptive immune cells into the CNS through the parenchymal and leptomeningeal blood vessels, as well as via the choroid plexus [60, 61]. The gut microbiota can influence BBB permeability directly (i.e., via the secretion of SCFAs) or indirectly, for example by influencing the pool size and composition of bile acids [62]. Secondary bile acids, for instance, are only produced by microbial biotransformations in the large intestine, and each bile acid can have a different impact on epileptigenesis depending on its properties [62, 63]. Furthermore, bile acids can promote the colonization of bacterial species promoting BBB leakage and decrease the levels of species promoting anti-inflammatory effects, potentially contributing to the establishment of a hyperexcitable milieu [64]. Gut microbiota-derived changes in BBB permeability affect immune cell migration into the CNS. The bidirectional cross-talk between immune and neural cells is mediated by the release of cytokines, neurotransmitters, and neuropeptides, both during homeostatic conditions and in response to local insults of different nature [59]. While peripheral immune cells are important

for detecting and responding to harmful agents which would disrupt homeostasis, a dysregulated inflammatory response can become damaging if not properly controlled [61].

Gut microbiota modulates systemic innate immunity

The innate immune system represents the first line of defense against infections and injurious events by detecting exogenous and endogenous antigens, microbe-associated molecular patterns (MAMPs), and damage-associated molecular patterns (DAMPs) respectively, through pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs) [29, 65]. PRRs that recognize bacterial surface molecules such as peptidoglycans (PGNs), have been identified as potential regulators of gut microbiota-brain interactions [66]. In fact, under physiological conditions, PGN from gut-microbiota can be transported into the developing brain and sensed by specific PRRs on CNS-resident immune cells. These PRRs, as well as the PNG transporter PepT1, are abundantly expressed in the developing brain [66], highlighting a role for the PGN signaling pathway in regulating neurodevelopment.

Seventy to eighty percent of all immune cells reside in the gut, where the intestinal microbiota, the intestinal epithelial layer, and the local mucosal immune system interact in a complex interplay [67]. The gut microbiota plays a crucial role in immune induction, training, and effector functions. On the other hand, the immune system has evolved to tolerate the gut flora and maintain a mutually beneficial symbiotic relationship. In fact, commensal bacteria-derived MAMPs continuously stimulate the gut-associated lymphoid tissue (GALT) to induce local and systemic immune unresponsiveness to innocuous antigens, which suppresses inflammation and maintains immune tolerance [67].

Dysregulation of the innate immune system in experimental models

GF mice show reduced levels of neutrophils [68] and intestinal mast cells [69], supporting the role of the gut microbiota in the development of these cells. Furthermore, SCFAs can modulate innate immune cell function by rewiring their metabolism and interfering with cell signaling [36]. To support this, recent studies have explored the temporal pattern of immune cell activation demonstrating that metabolic changes occur prior to, and may cause, immunological changes [70]. In neutrophils, SCFAs promote recruitment and migration (butyrate) [71], inhibit nitric oxide generation (butyrate, propionate, and acetate) [72], and enhance effector responses via free fatty acid receptor 2 (FFAR2) to induce interleukin (IL)−1β and the inflammasome (acetate) [73–75]. Neutrophils are the most abundant leukocytes in the circulations and are considered the first line of defense to infection or injury. However, neutrophils have also been involved in the pathology of several neurological diseases, such as ischemic stroke, AD, Parkinson's disease (PD), Huntington disease (HD), multiple sclerosis (MS), and ASD [76]. The ratio between neutrophils and lymphocytes has also been implicated in the acute and subacute phases of epilepsy [77]. Furthermore, mast cells are involved in neuroinflammation, neurogenesis, neurodegeneration, and in the disruption of the BBB. These functions can give rise to pathogenic mechanisms involved in PD, HD, AD, and amyotrophic lateral sclerosis [78].

The gut microbiota can also affect the activation and effector functions of antigen presenting cells (APCs) in the gut, crucial to bridge the innate and adaptive immune responses. While the presence of eubiotic gut microbiota promotes a tolerant and anti-inflammatory phenotype in Peyer's patches dendritic cells (DCs) and intestinal macrophages, the absence of these microbes can impair chemotaxis and phagocytosis of peritoneal macrophages [29, 65]. These observations show that the gut microbiota mediate a fine tuning between tolerance and activation of innate immunity. Similarly to their effect on neutrophils, SCFAs affect macrophage polarization and reduce glycolysis, resulting in increased AMP and decreased mTOR activity (butyrate) [79, 80]. Macrophage dysregulation has been heavily implicated in neurodegenerative (i.e., PD and AD) and neuroinflammatory (i.e., MS and amyotrophic lateral sclerosis [ALS]) disorders. Interestingly, neurodegenerative diseases can in turn impact the phenotype of brain macrophage populations, ranging from microglia to monocyte-derived macrophages [81]. DCs have also been implicated in CNS conditions such as stroke, MS, brain tumors, AD, PD, and epilepsy [82].

The phenotype of natural killer (NK) cells is also influenced by the gut microbiota and in particular the differentiation of gut NKp46 + cells expressing the nuclear hormone receptor retinoic acid receptor-related orphan receptor gamma t (RORγt) [65]. These cells, called innate lymphoid cells 3 (ILC3 s), show a general NK phenotype, yet differ from classical NK cells as they produce IL-22 [65, 83]. Interestingly, IL-22 secretion is repressed by intestinal epithelial cells (IECs) upon adequate gut colonization by commensal bacteria [84]. ILC3 s play a role in the formation of lymph nodes and Peyer's patches in the embryo and regulate mucosal immunity [85]. Because of their properties, ILCs constitute part of type 3 immunity, a RORγt-mediated response fundamental for the containment of symbiotic microbiota at mucosal surfaces and for the defense against bacterial and fungal pathogens [86]. Of note, RORγt can be regulated by its natural ligands "oxysterols," endogenous 27-carbon derivatives of

cholesterol, and by the circadian rhythm through Nfil3 [86], a transcriptional regulator expressed by the small intestine epithelium under light–dark circadian rhythm [87]. These observations highlight the importance of the neuro-ILC3 crosstalk. To support this, it is known that ILC3 s reside in the meninges at steady state and migrate to the brain parenchymal during stroke and MS (Anandamide [AEA] model) [85].

Gut microbiota modulates peripheral adaptive immunity

Unlike innate immunity, adaptive immunity is highly specific and composed by two lymphocyte populations, B and T cells, named after the site where they mature: the bone marrow and thymus, respectively.

Cell-mediated immunity and the complex influence of the GM

In the *lamina propria* of the intestine, the gut microbiota plays a fundamental role in mediating the differentiation of CD4 + T helper (Th) cells into different sub-types (i.e., Th1, Th2, Th17, or Treg). CD4 + T cells are major histocompatibility complex (MHC)-II-restricted and are involved in the activation and suppression of inflammation, depending on the subtype. The physiological balance between these Th populations has profound effects on homeostasis and on the outcome of different disorders. Recent studies have demonstrated that specific bacterial populations may modulate Th polarization: *Bacteroides fragilis* and *Lactobacillus* strains induce a systemic Th1 response, *Segmented Filamentous Bacteria* (SBF), and *Prevotella* are inducers of Th17 cells, while in the absence of gut microbes the ratio between Th1/Th2 is dysregulated towards a Th2 response [29, 65]. SCFAs (butyrate, proprionate, acetate) are also involved in supporting the development of Th1, Th17, and IL-10-secreting T regulatory cells (Tregs), as well as the inhibition of HDACs, and the modulation of mTOR-S6 K activity (*88*). Interestingly, microbiota- and antigen-induced Tregs express RORγt, whereas microbiota-independent Tregs express GATA binding protein 3 (GATA3) [86], supporting a role of the gut microbiota in shaping CD4 + T cell profiles. Furthermore, colonic regulatory Tregs have a unique TCR repertoire [89] and are unresponsive to commensal-derived antigens [90]. The gut bacterial populations responsible for this include Clostridia, particularly cluster IV and XIVa [91], and Bacteroida [92], which can induce colonic Treg via the release of lipid metabolites (i.e., linoleic acid derivatives). These observations highlight the crucial role of a balanced gut flora in the induction of Tregs and in mediating immune tolerance [91]. As CD4 + T cells are key players of adaptive immunity, they are also involved in many neurological conditions. For instance they accumulate in perihematomal regions during hemorrhagic brain injury and aggravate the condition [93]. On the other hand, neurological conditions can shape the adaptive immune response, as CD4 + T cells derived from PD patients show altered migration, as well as impaired mitochondrial function and positioning withing the cell [94].

MHC-I-restricted CD8 + T cells are also influenced by the gut microbiota, which regulate their maintenance and cytotoxic functions [65, 95]. GF mice transferred with antigen-activated transgenic gBT-I cells carrying TCRs specific to a herpes simplex virus (HSV) glycoprotein-B-derived epitope (gB498–505), show lower levels of the transferred cells compared to SPF mice [96], highlighting the role of the gut microbiota in mediating the maintenance of these CD8 + populations. Furthermore, SCFAs including acetic, propionic, and butyric acid, but also the less known pentanoate, are involved in CD8 + T cell regulation [95]. SCFAs can act as substrates for fatty acid oxidation and the generation of acetyl-CoA, which in turn enters the tricarboxylic acid (TCA) cycle and oxidative phosphorylation [97]. Due to these properties, SCFAs enhance CD8 + T cell activation and differentiation towards long-lived memory cells with improved recall capacity [98, 99].

Due to their cytotoxic properties, CD8 + T cells have been heavily implicated in neurological diseases. CD8 + T cell infiltration, with a subsequent increase in clonal expansion and enhanced cytotoxic properties have been reported in disease-associated brain areas of patients with neurodegenerative conditions, including AD, PD, and MS [100]. Another study reveals that CD8 + T cells adhere to microvessels in the CNS and release granzyme B, a serine protease involved in cellular apoptosis, resulting in endothelial cell injury and microhemorrhage. Blocking T cell adhesion ameliorates neuroinflammation and endotheliopathy [101]. Lastly, CD8 + T cells play a pathogenic role in limbic encephalitis [102] and Rasmussen encephalitis [103], correlating with disease severity and seizures.

Mounting a humoral response in a bacteria-rich environment

While cell-mediated immunity is driven by T cells, the humoral response is mediated by B cells and antibodies. Gut-associated B cells are mostly located in Peyer's patches and secrete Immunoglobulin (Ig) A, associated with mucosal immunity in physiologically colonized guts. Conversely, the absence of gut microbiota in GF mice deeply impacts B cell anatomical clustering and function [104]. In Peyer's patches, as well as in other secondary lymphoid organs such as the spleen, B cells are activated, proliferate, differentiate, and undergo somatic hypermutation [105]. Interestingly, gut dysbiosis can alter the immune maturity of the spleen, leading to fewer and smaller germinative centers. On the

other hand, splenectomy results in the disappearance of IgA-secreting cells in the intestine and an increase in IgE, also reflected systemically in GF mice [65]. Taken together, these observations highlighting the role of the spleen in the gut-immune system crosstalk [65, 106]. As for many other immune cell types, SCFAs also affect B cells. A murine study shows that butyrate and propionate modulate B cell epigenetics in a dose-dependent manner, inhibiting class switching and reducing T cell-dependent and independent antibody responses both systemically and locally in the gut [107]. On the other hand, Kim et al. show that SCFAs induce plasma cell differentiation and class switching, with a subsequent release of IgA [108]. These inconsistencies highlight the need for further studies to tease out the gut microbiota-derived metabolic drivers of adaptive immunity.

B cells have been shown to play a dual role in promoting and dampening inflammation through the production of different cytokines and co-stimulatory molecules that can influence Th polarization. That said, B cells have been implicated in numerous CNS autoimmune disorders, including MS, neuromyelitis optica spectrum disorder, myelin oligodendrocyte glycoprotein (MOG) antibody-associated disorder, Anti-N-methyl-D-aspartate receptor encephalitis, autoimmune epilepsy, and neurodegenerative disorders such as PD and AD [109]. Interestingly, B cell-depleting therapies have gained an impressive clinical success for the treatment of MS and other CNS autoimmune disorders, highlighting the importance of B cells in their pathogenesis.

Gut-immune-brain (GIB) axis and childhood neurological disorders

GIB axis and NDDs

The Diagnostic and Statistical Manual of Mental Disorders, Fifth edition (DSM-5) defines neurodevelopmental disorders (NDDs) as "a group of conditions with onset in the developmental period." NDDs share a typical onset in the pre-scholar or early scholar age, and as a heterogeneous group, they range from limitations of learning or control of executive functions to severe impairment of social skills or intelligence [110, 111]. Table 3 provides an overview of the disorders falling into this category alongside with their specifiers; their order reflects the impact on adaptive behavior.

Both genetic and epigenetic factors (e.g., preterm birth, early sensory stimuli, gut dysbiosis, and socioeconomic context) have been implicated in the disruption of synaptic pruning, branching, and the overall neuronal plasticity of various CNS areas leading to NDDs [112–115]. Albeit the link between some of these disorders and the gut microbiome have been studied both preclinically and clinically [116, 117], for other NDDs the available evidence is limited to the animal models.

Several studies have also linked maternal gut dysbiosis to immune dysregulation and enhanced systemic inflammation, resulting in atypical brain development and the occurrence of NDDs such as ASD and ADHD [118]. Prolonged inflammation during pregnancy due to maternal gut dysbiosis or infection is a determinant for disease risks in the offspring, including abnormal brain development both pre and neonatally, subsequently leading to NDDs [119]. Microbiota-derived products and maternal inflammatory mediators can cross the placenta and the immature fetal BBB to induce neuroinflammation and affect fetal brain development, increasing the risk of ASD [120]. Furthermore, neuropoietic inflammatory cytokines (i.e., IL-6, IL-1β, and TNF-α) can directly affect dendrite development, neural activity, long-term potentiation, neurite outgrowth, and regulation of synaptic plasticity in the hippocampus, with consequences on neurodevelopment and behavior [121]. A recent study also shows an association between dysbiosis-mediated neuroinflammation and hippocampal neurogenesis, which led to impaired learning, anxiety, and depressive-like behaviors [122]. Lastly, the maternal gut microbiota can directly influence the effector functions of certain immune cells, for example by inducing long-term transcriptomic and chromatin accessibility alterations in microglia, leading to an underdeveloped phenotype [123]. Taken together, these studies highlight the importance of the GIB axis in shaping the correct development of the CNS, starting from intrauterine life (Fig. 4). The increasing incidence of NDDs has created a clinical need to further characterize pathophysiological mechanisms such as transplacental immune signaling, epigenetic priming of offspring microglia, and postnatal crosstalk between the CNS and the immune system, with the aim of developing preventive strategies both in pregnancy and in the postnatal phase.

GIB axis and specific learning disorders (SLDs)

Children with SLDs show adequate intelligence quotient (IQ) and below average academic performance and an impairment in reading (dyslexia), writing (dysorthography and dysgraphia), or mathematics (dyscalculia) [124]. The link between GIB and this set of disorders is poorly characterized in humans; however, a 2009 study demonstrated an association between diet-induced alteration of gut microbiota and learning and memory in mice [125]. Furthermore, recent clinical study demonstrated higher levels of neutrophil/lymphocyte ratio, platelet/lymphocyte ratio, and systemic inflammation index in 90 SLD-diagnosed patients compared to matched healthy controls, highlighting a role for inflammation in SLD etiopathogenesis [126]. Additional studies are needed in

Table 3 Neurodevelopmental disorders (NDDs) with a clear link to the gut microbiota. This table lists the categories and subcategories of the NDDs included in the diagnostic category of the Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM-5) and highlights the main pre-clinical and clinical studies showing empirical evidence of a link between the gut microbiota and these disorders

DSM-5 NDDs category	Subcategories	Link to the gut microbiome
Specific learning disorders -below average academic performance after 6 months of adequate instruction. No other sensory, motor, or developmental disorders identified [124]	1. Impairment in reading—dyslexia 2. Impairment in mathematics—dyscalculia 3. Impairment in writing—dysorthography and dysgraphia	Poorly characterized in humans with pure learning disorders Ascertained in mouse models using proper tests (Hole-board apparatus) [125]
Communication disorders -deficits in verbal and nonverbal communication skills [127]	1. Language disorders 2. Speech sound disorder 3. Childhood-onset fluency disorder 4. Social (pragmatic) communication disorder (SPCD)* 5. Unspecified communication disorder	Limited evidence available. *L. reuteri* can reverse social deficits (comparable to SPCD) but not repetitive behaviors in ASD children [145] SPCD ascertained in mouse models using the isolation-induced ultrasonic vocalizations test, the 3-chamber social approach and the free social interaction tests [128] *SPCD differs from full autism for the absence of repetitive behaviors
Motor disorder -involuntary or uncontrolled movements or actions of the body [129]	1. Developmental coordination disorder 2. Stereotypic movement disorder 3. Tic disorders	Studies on tic disorders, especially Tourette Syndrome (TS), have shown a reduction of *Prevotella* spp. and *Bifidobacteria* at the genus level (*130–132*) Different microbial signatures were identified between healthy mice and the TS mouse model established with 3,3′-iminodipropionitrile (i.e., increase in *Turicibacteraceae* and *Ruminococcaceae*). Fecal matter transplantation (FMT) of TS mice with the microbiota of healthy mice or probiotics alleviated the TS symptoms at the mice stereotyped behavior score by modulating the microbiota and the levels of 5-HT (*133*)
Attention-deficit/hyperactivity disorder (ADHD) -impairing levels of inattention, disorganization, and/or hyperactivity-impulsivity [134]	1. ADHD, primarily inattentive type (ADHD-I) 2. ADHD, primarily hyperactive-impulsive type (ADHD-H) 3. ADHD, combined type (ADHD-C)	In a randomized clinical trial, 10-week dietary micronutrient supplementation leads to an increase in *Bifidobacterium* and *Collinsella* and a relative reduction of *Actinobacteria* (*135*). Results in cohort studies seem contradictory in identifying changes in specific *genera* which may derive from different statistical analyses (e.g., unsupervised vs supervised methods) [136, 137]; although, in [136] three differential abundance methods (i.e., LEfSe, DESeq, LADEx2) identified increased *Turicibacter* in controls compared to adult ADHD individuals. *Odoribacter* and *Butyricimonas* were mildly elevated in the ADHD group. [138] demonstrated increased *Dialister* and *Megamonas*, and reduced *Anaerotaenia* and *Gracilibacter* in over 100 untreated adult ADHD patients. No correlation of these *genera* with the ADHD rating scale. In children with ADHD, the SNAP-IV parent form and the Child Behavior Checklist (CBCL) were used to identify correlations within the ADHD group. No correlation with the SNAP-IV, but significant association between withdrawal and depression symptoms and *Agathobacter* and between rule-breaking behavior and *Ruminococcus gnavus* [139]. Decreased structural integrity of the internal capsule and hippocampus, as well as decreased connectivity between the right motor and right visual cortices and increased anxiety at the open-field test demonstrated in germ-free C57BL/6 JOlaHsd male mice colonized with the gut microbiota from ADHD individuals, compared to those colonized by control microbiota. At the family level mice^{ADHD} showed increased *Clostridiales* [unknown] [140]

Table 3 (continued)

DSM-5 NDDs category	Subcategories	Link to the gut microbiome
Autism spectrum disorder (ASD) -persistent deficits of social communication and interaction; repetitive patterns of behaviors, activities or interests, including sensory issues [129, 177]	No subcategories The current DSM-V category groups the formerly called "pervasive development disorders", including autistic disorder; Asperger syndrome; childhood disintegrative disorder; pervasive developmental disorder-not otherwise specified (PDD-NOS) [129]	A recent systematic review (*143*) identified some common trends between available studies: a decrease of the *Bacteroidetes/Firmicutes* ratio in ASD children compared to healthy ones; the long-lasting effects of FMT over ASD symptoms severity. Different mixture of both pre- and pro-biotics have also been tested, with reduction of ASD symptoms, anxiety, and systemic inflammatory status [144] Differential expression of genes (i.e., *Daglb*) was found in germ-free mice transplanted with gut microbiota (GM) from human donors with ASD, as compared to control mice transplanted with the GM from healthy human donors [117]. Oral supplementation of 5-aminovaleric acid (5 AV) or taurine to BTBR T^{+} tf/J (BTBR) ASD-mouse models significantly reduced repetitive behaviors with the marble burying test and increased social duration with the direct social interaction test [117]. Other works tested whether different pre- or probiotics could reverse the behavioral abnormalities in ASD-mouse models (*146*, *147*). Recently, [148] used oral supplementation of fibers (galacto- and fructo-oligosaccharides, GOS/FOS) in male offspring of BALB/cByJ dams injected with valproate (VPA) during gestation. Fibers restored changes induced by VPA administration, including reduced neuroinflammation in the cerebellum and impairment in behavior and cognition
Intellectual disorders -childhood onset of intellectual difficulties, defined as an intelligence quotient (IQ) two or more standard deviations below the population mean, associated with difficulties in conceptual, social, and practical areas of living (adaptive functioning) [129]	1. Intellectual developmental disorder 2. Global developmental delay 3. Unspecified intellectual disability	Poorly characterized in humans with pure and isolated intellectual disorders. Mostly associated with other NDDs. [149] demonstrated a positive correlation with *Barnesiella* and *Lachnospiraceae,* as well as a negative correlation with *Sutterella* on different clinic-administered cognitive tests (e.g., the Montreal Cognitive Assessment (MoCA) and Digit Symbol Substitution Test (DSST)) in a cohort of 597 adult patients. Moreover, [150] showed different microbial communities in infants with high or low composite cognition (CC) scores at the Bayley Scale of Infant Development, third edition (BSID-III). Fecal transplant from high or low CC infants to germ-free mice depicted different memory profiles in the two groups, with better results at the open-field test and novel object recognition test for mice transplanted with high CC infant feces. Moreover, the latter group had enrichment with *Bacteroides* and *Bifidobacterium*, as well as *Phocaeicola* (including butyrate-producing species) in the gut microbiota [150]

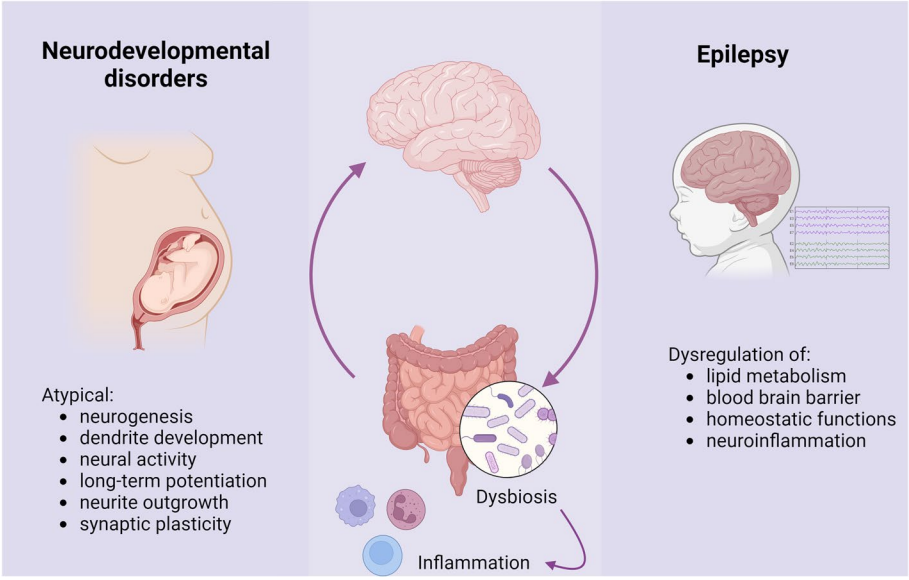

Fig. 4 The role of dysbiosis in neurodevelopmental disorders and epilepsy. An overview of the effects of dysbiosis on proper brain development and on the modulation of molecular and cellular mechanisms in epilepsy. Created with Biorender.com

order to further elucidate the relationship between gut dysbiosis and inflammation in SLD patients.

GIB axis and communication disorders

Communication disorders lead to deficits in verbal and nonverbal communication skills. The diagnostic categories include language disorder, speech sound disorder, childhood-onset fluency disorder (stuttering), social (pragmatic) communication disorder (SPCD), and unspecified communication disorder [127]. As for SLD, there is limited evidence suggesting a role for the gut microbiota in communication disorders. A recent preclinical study demonstrated that perturbations in maternal gut microbiota influenced the offspring's gut microbiota composition, which in turn resulted in alterations in neonatal communications and juvenile socio-emotional behavior. This behavioral phenotype, characteristic of SPCD, was associated with a mild inflammation in the colon and a reduced gene expression of the oxytocin receptor, as well as several tight-junction proteins in the prefrontal cortex [128], pointing to a link between the GIB axis and the symptoms of communication disorders.

GIB axis and motor disorders

Motor disorders are described as involuntary or uncontrolled movements or actions of the body. The DSM-5 motor disorders include developmental coordination disorder, stereotypic movement disorder, and the tic disorders of Tourette Syndrome (TS), persistent (chronic) motor or vocal tic disorder, and provisional tic disorder [129]. Different gut microbial signatures are associated with TS. TS patients show a reduction of *Prevotella* spp. and *Bifidobacteria* at the genus level [130–132], while a TS mouse model show an increase in *Turicibacteraceae* and *Ruminococcaceae*, compared to healthy controls [133]. Interestingly, modulating the gut microbiota by probiotic administration or by transplantation of fecal matter from healthy mice leads to increased serotonin secretion and to an amelioration of tic severity in this murine model [133]. Taken together, these studies highlight a causal relationship between gut microbiota composition and motor disorder phenotypes, mediated by a beneficial metabolic shift.

GIB axis and attention-deficit/hyperactivity disorder (ADHD)

Individuals suffering from ADHD show impairing levels of inattention, disorganization, and/or hyperactivity-impulsivity (*134*). In the recent years, more research has emerged on the relationship between the gut microbiota and ADHD; however, there is still limited consensus on which specific populations are involved, possibly due to different statistical analyses (e.g., unsupervised vs supervised methods) [135–137]. In a randomized clinical trial in children with ADHD, 10-week dietary micronutrient supplementation (a mixture of vitamins, minerals, amino acids, and antioxidants) leads to an increase in Bifidobacterium and Collinsella and to a relative reduction of Actinobacteria [135]. A different study using three differential abundance methods (LEfSe, DESeq, LADEx2) identified decreased *Turicibacter* and a mild increase in *Odoribacter* and *Butyricimonas* in adult

ADHD individuals [136]. Furthermore, [138] demonstrated increased *Dialister* and *Megamonas*, and reduced *Anaerotaenia* and *Gracilibacter* in over 100 untreated adult ADHD patients. In an attempt to correlate these *genera* with ADHD symptoms, Lee et al. showed a significant association between withdrawal and depression symptoms and *Agathobacter*, and between rule-breaking behavior and *Ruminococcus gnavus* [139]. A murine model of GF C57BL/6 JOlaHsd male mice colonized with the gut microbiota from ADHD individuals also demonstrated decreased structural integrity of the internal capsule and hippocampus, as well as decreased connectivity between the right motor and right visual cortices and increased anxiety at the open-field test. The mice^{ADHD} also showed increased *Clostridiales* [unknown] [140]. Taken together, these results highlight an important association between gut microbiota, brain structure, and ADHD symptoms. While empirical evidence points to a state of low-grade inflammation in ADHD children and adults, it is not fully clear how this correlates with the composition and diversity of the gut microbiome and the other biological changes discussed in this Sect. [141, 142]. Additional studies are needed to identify the direct or indirect pathways that might be involved.

GIB axis and ASD

As the literature on the link between the gut microbiota and ASD is vast and rapidly expanding, this section will only highlight the main studies on this topic. For further information, we direct the readers to more comprehensive review papers dedicated exclusively to this disorder [143, 144]. These studies identify common trends in ASD individuals compared to healthy controls, such as the decrease of the Bacteroidetes/Firmicutes ratio, and highlight the long-lasting effects of fecal microbiota transplantation (FMT) over ASD symptoms severity [143]. Along with FMT, different mixtures of pre- and pro-biotics have been tested, resulting in the reduction of ASD symptoms, anxiety, and systemic inflammatory [144]. Furthermore, a differential expression of genes (i.e., Daglb) involved in neuronal growth and alternative splicing in the prefrontal cortex and the striatum was found in germ-free mice transplanted with gut microbiota (GM) from human donors with ASD, compared to control mice transplanted with GM from healthy human donors [117]. Oral supplementation of 5-aminovaleric acid (5 AV) or taurine to BTBR T^+ tf/J (BTBR) ASD-mouse models significantly reduced repetitive behaviors, as measured by the marble burying test, and increased social duration, as measured by the direct social interaction test [117]. On the other hand, L. reuteri administration reversed the social deficits of ASD children comparable to those of children with SPCD, but it did not ameliorate repetitive behavior [145]. The mechanism of action of L. reuteri treatment acts directly via the vagus nerve and is independent from other gut microbial populations [145]. Other studies also tested whether different pre- or probiotics could reverse the behavioral abnormalities in ASD-mouse models [146, 147]. Recently, Prince et al. [148] used oral supplementation of fibers (galacto- and fructo-oligosaccharides, GOS/FOS) in male offspring of BALB/cByJ dams injected with valproate (VPA) during gestation. Fibers restored changes induced by VPA administration, including reduced neuroinflammation in the cerebellum and impairment in behavior and cognition.

GIB axis and intellectual disorders

Intellectual disorders are characterized by an IQ two or more standard deviations below the population mean, associated with difficulties in conceptual, social, and practical areas of living [129]. While evidence from preclinical and small clinical studies supports a role for the gut microbiota in cognitive functioning, there is only a limited number of studies investigating this relationship in large cohorts. Meyer et al. demonstrated a positive correlation with Barnesiella and Lachnospiraceae, as well as a negative correlation with Sutterella on different clinic-administered cognitive tests (e.g., the Montreal Cognitive Assessment (MoCA) and Digit Symbol Substitution Test (DSST)) in a cohort of 597 adult patients [149]. Moreover, Cerdó et al. [150] showed different microbial communities in infants with high or low composite cognition (CC) scores at the Bayley Scale of Infant Development, third edition (BSID-III). Interestingly, fecal transplant from high or low CC infants to germ-free mice depicted different memory profiles in the two groups, with better results at the open-field test and novel object recognition test for mice transplanted with high CC infant feces [150]. The latter group also showed an enrichment of Bacteroides and Bifidobacterium, as well as Phocaeicola (including butyrate-producing species). Lastly, the results showed lower fecal levels of histidine and lower ratios of urocanate:glutamate in the perirhinal cortex in mice transplanted with high CC infant feces, pointing at the gut microbiota-mediated modulation of histidine metabolism as a potential underlying mechanism [150].

GIB axis and epilepsy

Epilepsy affects about 65 million people worldwide and the World Health Organization (WHO) ranks it as the 4th most common and burdensome neurological condition (0.56% of the total global disability-adjusted life-years). First-line treatments include >20 symptomatic anti-seizure medications (ASMs) often endowed of serious side effects. Furthermore, up to 40% of the patients have drug-resistant seizures, together with other

neurological comorbidities. Thus, epilepsy has a strong negative impact on the quality of life (QoL) of patients, associated with a substantial economic burden for the health care system and society [151].

Epilepsy is not classified within the DSM-5, but its most recent classification is based onto the guidelines provided by the International League Against Epilepsy (ILAE) guidelines [152]. Given the shared neurobiological basis, epilepsy comorbidity is frequent in NDDs [153]. The resulting pathophysiological mechanism disrupts the excitation/inhibition (E/I) balance of the brain and leads to some phenotypic overlap [154].

The idea of a relationship between the gut microbiota and epilepsy was first introduced at the beginning of the twentieth century with the hypothesis of a *Bacillus Epilepticus* related to the onset and maintenance of epilepsy and constipation [155]. Nowadays, researchers are studying the potential role of the microbiota to affect seizures and epileptogenesis (Fig. 4) [156]. Interestingly, a rat model of acquired epilepsy revealed distinct metagenomic and metabolomic signatures associated with epilepsy, suggesting a dysregulation in SCFA and lipid metabolism. These changes were accompanied by molecular, cellular, and structural alterations characteristic of a dysfunctional gut, as well as local inflammation in the small intestine [157]. Dysbiosis may also induce a peripheral inflammatory state which can contribute to neuro-inflammation in the CNS [158, 159]. Sustained CNS inflammation can lower seizure threshold and contribute to epileptogenesis, neuronal death, the loss of homeostatic functions of glial cells, BBB dysfunction, and activation of microglia, with the production of pro-inflammatory cytokines. This cycle reinforces the already ongoing inflammatory state and contributes to the development of epilepsy [159–161] and, through neuronal and humoral pathways, to structural alterations in the gut [156, 157]. A higher level of complexity has been more recently uncovered, as microglia transcription, morphology, and effector functions can vary dynamically in a context-dependent manner during epilepsy progression [52], highlighting the need for multi-system studies to unravel the pro and anti-epileptic properties of these CNS resident immune cells. Furthermore, neutrophils, DCs, and lymphocytes have been implicated in epilepsy [77, 82]; however, the role of the gut microbiome in modulating the levels and migration of these cells is still not fully understood.

Intervening on the GIB axis for therapeutic purposes

Gut supplements (i.e., *prebiotics*, *probiotics*, and *postbiotics*), diet (e.g., the ketogenic diet), antibiotics, and FMT could be adjuvants in treating epilepsy given their effect on the gut microbiota, and may prevent the use of more invasive treatments (i.e., vagal nerve stimulation or surgery) [162]. A recent study has shown that administration of the antibiotic rifaximin during epileptogenesis ameliorates GI structural alterations and reduces seizure duration in a mouse model of temporal lobe epilepsy [163], highlighting the therapeutic effects of antibiotics as gut-based therapies in epilepsy. Furthermore, the ketogenic diet (KD) was shown to prevent electrically induced seizures and spontaneous tonic–clonic seizures by altering the gut microbiota and their metabolic output [164]. Another proposed mechanism of action is by indirectly reducing inflammation. A low calory KD dampens the adaptive and innate immunity inflammatory response, which occurs in refractory status epilepticus and in drug-resistant epilepsy. These immunomodulatory effects are not fully understood, but they might involve gut microbiota modifications, along with caloric restriction and the modulation of ketones bodies and polyunsaturated fatty acids [165]. Furthermore, the use of supplements such as SCFAs could be beneficial as it is well established that these metabolites exert anti-seizure effects by modulating the ratio between inhibitory and excitatory neurotransmitters in the brain [166]. Furthermore, gut dysbiosis and the concomitant alteration of SCFA levels have been implicated as potential risk factors also in NDDs, as SCFA-mediated effects on the CNS start during neural development, with the differentiation of neural cells from embryonic stem cells and the growth of neurospheres from neural stem cells. Recent studies have also shown that SCFAs promote human neural progenitor cell proliferation in vitro [167] and significantly remodel the brain lipidome to regulate apoptosis in vivo and in vitro [168]. However, the specific mechanisms at play are not yet fully characterized due to the low reproducibility of in vivo conditions. A deeper understanding of the impact of SCFA exposure during brain development will shed light on the molecular mechanisms at play and aid in the discovery of specific biomarkers and novel therapeutic strategies for different neurological conditions. However, no consensus or shared guidelines are yet available for preclinical and clinical studies, which are heterogeneous in terms of experimental design and type of supplement [169].

An alternative, strategy could derive from administering immune-regulatory drugs to modulate the gut microbiota. Immunotherapies include cell trafficking inhibitors, immune checkpoint inhibitors, immunomodulators, anti-proliferative drugs, and inflammatory cytokine inhibitors. Although most of these treatments were associated with shifts in specific bacterial taxa, there were no association between immunotherapy class and the microbiota profile [170], highlighting the need

for further studies to fully uncover the clinical significance of these findings.

Aside from their effect on gut, immunomodulators can be used to regulate the effector function of CNS resident cells. In particular, the pathogenic role of microglia makes these cells a candidate target for novel therapeutic strategies to treat different neurological conditions. Minocycline blocks proliferation and activation of microglia and administration post status epilepticus mediates a decrease in pro inflammatory cytokine production and a reduction in the duration, severity, and number of seizures in preclinical models [171]. A more recent study also identified the role of the gap junction pathway mediated by astrocyte connexin 43 in modulating the epilepsy-induced neuroinflammatory cascade, making this protein a potential therapeutic target for epileptic inflammatory reactions [172]. Other proposed targets studied in experimental models include cyclooxygenase-2, prostaglandin EP2, monoacylglycerol lipase, IL-1β, HMGB1/Toll-like receptor signaling, P2X7 receptor, immunoproteasome, mTOR, TGF-β, metalloproteinases, cytokines, and chemokines [173], all molecules which have an effect on, or are affected by, the gut microbiota.

Conclusions

Microbiota colonization of the gut is crucial for the proper development and maturation of the immune, endocrine, and CNS systems, influencing their functions (*115*). Throughout life, host-microbe interactions play a role in maintaining homeostasis between these systems through complex communication networks. Notably, changes in the gut microbiota composition can lead to altered behavior and cognition, highlighting the importance of the microbiota-gut-brain axis [115]. The gut flora also influences the induction, training, and effector function of the immune system at a local and systemic level [67]. The cross talk between the gut microbiota, the immune system, and the CNS can have profound effects on neurodevelopment, and dysfunction of this network can lead to increased risk of neurological conditions. NDDs and epilepsy are serious health condition impacting on the quality of life of patients and their caregivers, and existing therapies target symptoms, rather than addressing the underlying abnormal biological processes. There is a need to advance knowledge of the GIB mechanisms at play to guide the development of novel therapies and early interventions for these multi-faceted conditions.

This review article discussed the most recent studies exploring the complex interactions between the gut microbiota and their associated immune responses with the developing brain. Despite the advances made with murine preclinical models, the specific mechanisms at play in humans are not yet fully characterized. In particular, the current research mostly focuses on finding associations, rather than establishing causality, and there is a lack of functional and interventional clinical studies due to limitations including resource intensiveness, restricted sample sizes, lack of longitudinal time points and proper control groups. A new avenue to study the gut-immune-brain axis in a controlled system could be provided by organ-on-a-chip devices, which allow for in vitro modeling of the multi-organ connection pathways in physiological and pathological conditions [174, 175]. Understanding how each branch of this complex network functions synergistically during neural development and early life is imperative to learn how to modulate them and help guide future interventions with immunomodulators, diet, pro/pre-biotics, etc. to promote a shift towards a health-like state. These interventions would be more economical and less invasive compared to the current available options, improving the management and quality of life of patients with epilepsy and NDDs, and striving towards a non-invasive personalized medical approach.

Abbreviations

AD	Alzheimer's disease
ADHD	Attention deficit/hyperactivity disorder
AEA	Anandamide
ALS	Amyotrophic lateral sclerosis
AMPK	AMP-activated protein kinase
ANS	Autonomic nervous system
APCs	Antigen presenting cells
ASD	Autism spectrum disorders
ASF	Altered Schaedler flora
ASMs	Anti-seizure medications
5 AV	5-Aminovaleric acid
BBB	Blood brain barrier
BDNF	Brain-derived neurotrophic factor
BSID-III	Bayley Scale of Infant Development, third edition
CC	Composite cognition
CNS	Central nervous system
CREB	CAMP response element-binding protein
CS	Cesarean section
DAMPs	Damage-associated molecular patterns
DCs	Dendritic cells
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, Fifth edition
DSST	Digit Symbol Substitution Test
E/I	Excitation/inhibition
ENS	Enteric nervous system
FFAR2	Free fatty acid receptor 2
FMT	Fecal microbiota transplantation
GALT	Gut-associated lymphoid tissue
GATA3	GATA binding protein 3
gB498–505	Glycoprotein-B-derived epitope
GF	Germ-free
GI	Gastrointestinal
GIB	Gut-immune-brain
GM	Gut microbiota
GOS/FOS	Galacto- and fructo-oligosaccharides
HD	Huntington disease
HDCA	Inhibits histone deacetylase
HPA	Hypothalamic-pituitary-adrenal axis
HSV	Herpes simplex virus
5-HT1 A	5-Hydroxytryptamin 1A
IBS	Irritable bowel syndrome

IECs	Intestinal epithelial cells
Ig	Immunoglobulin
Igfbp2	Insulin like growth factor binding protein 2
IGF	Insulin-like growth factor
IL	Interleukin
ILAE	International League Against Epilepsy
ILC3 s	Innate lymphoid cells 3
IQ	Intelligence quotient
LC	Locus coeruleus
MAMPs	Microbe-associated molecular patterns
MHC	Major histocompatibility complex
MoCA	Montreal Cognitive Assessment
MOG	Myelin oligodendrocyte glycoprotein
MS	Multiple sclerosis
NDDs	Neurodevelopmental disorders
NK	Natural killer
PD	Parkinson's disease
PGNs	Peptidoglycans
PRRs	Pattern recognition receptors
PTSD	Post-traumatic stress disorder
QoL	Quality of life
RORγt	Receptor-related orphan receptor gamma t
SBF	*Segmented Filamentous Bacteria*
SCFAs	Short-chain fatty acids
SIDS	Sudden infant death syndrome
SLDs	Specific learning disorders
SPCD	Social (pragmatic) communication disorder
SPF	Specific-pathogen free
TCA	Tricarboxylic acid
Th	T helper
TLRs	Toll-like receptors
TS	Tourette syndrome
VPA	Valproate
WHO	World Health Organization

Acknowledgements
Supported by PNRR-MUR-M4 C2 PE0000006 Research Program "MNESYS"—A multiscale integrated approach to the study of the nervous system in health and disease. IRCCS 'G. Gaslini' is a member of ERN-Epicare.

Authors'contributions
G.V, A.R, collection of data, drafting; L.N, F.Z, T.R, P.S, revision of the data. A.R, P. S, conception of the study, revision and final approval of the manuscript. All authors read and approved the final manuscript.

Funding
No targeted fundings to be reported.

Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. Ethics approval was not needed as no human subjects were involved in the study.

Consent for publication
Not applicable as no human subjects were involved in the study.

Competing interests
The authors declare no competing interests.

Received: 24 November 2024 Accepted: 24 April 2025
Published online: 06 May 2025

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