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Review article

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Gastrointestinal and brain barriers: unlocking gates of communication across the microbiota–gut–brain axis

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Abstract

Crosstalk between gut and brain has long been appreciated in health and disease, and the gut microbiota is a key player in communication between these two distant organs. Yet, the mechanisms through which the microbiota influences development and function of the gut–brain axis remain largely unknown. Barriers present in the gut and brain are specialized cellular interfaces that maintain strict homeostasis of different compartments across this axis. These barriers include the gut epithelial barrier, the blood–brain barrier and the blood–cerebrospinal fluid barrier. Barriers are ideally positioned to receive and communicate gut microbial signals constituting a gateway for gut–microbiota–brain communication. In this Review, we focus on how modulation of these barriers by the gut microbiota can constitute an important channel of communication across the gut–brain axis. Moreover, barrier malfunction upon alterations in gut microbial composition could form the basis of various conditions, including often comorbid neurological and gastrointestinal disorders. Thus, we should focus on unravelling the molecular and cellular basis of this communication and move from simplistic framing as 'leaky gut'. A mechanistic understanding of gut microbiota modulation of barriers, especially during critical windows of development, could be key to understanding the aetiology of gastrointestinal and neurological disorders.

Sections

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Key points

- Barriers across the microbiota–gut–brain axis are key elements in the communication across this axis.
- By enabling a physical segregation between the host and microbiome, barriers have played key parts in the evolution of the holobiont.
- Gut and brain barriers are epithelial or endothelial in nature and they have different levels of permissiveness under physiological conditions, which is important for their function.
- Barriers are dynamic in nature and their function varies across the lifespan.
- Preclinical studies provide direct evidence of microbial metabolites influencing barrier functioning, linking gut microbial alterations to barrier dysfunction and the subsequent abnormal passage of substances (microbial and non-microbial) along the gut–brain axis.
- Barrier dysregulation has been shown to be a hallmark in disorders of the gut and of the brain, and could underlie some of their comorbidities.

Introduction

Emerging data suggest that the gut microbiota, the trillions of microorganisms that live in the gastrointestinal tract, has the potential to strongly influence brain physiology[1]. Despite strong associative evidence underpinning microbiota–brain interactions with abnormalities in brain function and behaviour, the conduits of communication between the gut microbiota and the brain are not fully understood. Much like Tolkien's *Lord of The Rings*' new roads or secret gates that run West of the Moon, East of the Sun, referring to the routes connecting Valinor and Middle Earth[2], we are beginning to witness lesser-known conduits of communication between the distant microbial ecosystem and the brain. In this context, the various cellular barriers beyond the gut epithelial barrier existing across the gut–brain axis are arising as new roads, or secret gates, that communicate between the gut microbiome and the brain. Our understanding of barriers has evolved from considering them strict impermeable cellular barriers to dynamic and exquisitely regulated communication interfaces with different levels of permissiveness.

This Review provides an overview of barrier function across the gut–brain axis, the interactions of this axis with the gut microbiome, and the implications for communication across this microbiota–gut–brain axis. We propose that microbiota-mediated multi-barrier modulation can be at the basis of comorbidity in neurological and gastrointestinal disorders.

Barriers across evolution

The influence of microorganisms on the host's physiology results from a long-standing evolutionary relationship between microorganisms and multicellular organisms. Evolution of multicellular organisms occurred in microbially-dominated ecosystems. Hence, every multicellular organism forms a partnership in which the larger host and various microbial prokaryotic and eukaryotic species, encompassing bacteria, archaea, fungi and viruses commonly known as microbiota, rely on each other in a synergistic manner. These associations are denoted by the terms of 'metaorganisms' or 'holobionts'[3]. Most of the holobiont's symbiotic microorganisms reside in the host's gastrointestinal tract, and we have come a long way in our understanding on how this complex and diverse ecosystem, the gut microbiota, modulates a bewilderingly wide range of host physiological processes. Among the processes known to be modulated by gut microorganisms, the connection between gut and brain through the gut–brain axis has long been appreciated, but it is only during the past two decades that research has cemented the role of gut-resident microorganisms as strong modulators of brain development, physiology and host behaviour[1,4].

A key feature for symbiotic coexistence of microbial communities and the host is the ability to maintain a physical segregation between the two. Throughout evolution, with the emergence of the first multicellular organisms, a need for establishing compartments isolated from the external environment arose. This feat was achieved through the establishment of cellular barriers in the form of simple epithelia that would still allow a controlled interaction with that external environment. These barriers are based on cell–cell junctions that restrict diffusion of microorganisms and solutes through the paracellular route. These cell–cell junctions, designated generally as occluding junctions, are found across the animal kingdom, indicating their ancient phylogenetic origin[5] (Box 1). The evolution of these physical structures, termed compartmentalization, has been a common feature used by hosts to establish a controlled relationship with symbiotic microorganisms[6].

The gut mucosa, which lines the gut lumen, forms the first cellular barrier between the host and gut microorganisms, but remarkably, along the pathway of communication between the distant gut and brain, we find a series of additional cellular barriers that create compartments where maintaining different compositions is essential for homeostasis. Despite our advances in understanding how the gut microbiota modulates a wide array of host physiological processes, there are still many unknowns, especially in relation to the molecular and mechanistic nature of this communication. Understanding the cooperation between host and symbiotic partners should encompass the cellular barriers that have allowed a controlled communication between both parties across evolution.

The gut barriers

The gastrointestinal tract can be viewed as a passageway crossing the body that opens to the external environment through the mouth and the anus. With that in mind, the main function of the gut barriers is to separate the body from the external environment, that is the luminal part of the gastrointestinal tract, whilst facilitating nutrient absorption. The gut epithelial barrier comprises a mucosal surface composed of a layer of simple columnar epithelial cells or enterocytes (the gut epithelial barrier)[7]. As mentioned above, the gastrointestinal tract contains most of the symbiotic microorganisms in the host, the gut microbiota. In a similar manner to ancestral mucosal barriers, such as the body surface in cnidarians, the gut mucosa prevents gut microorganisms entering the host, whilst allowing a symbiotic interaction with some of these microorganisms[8]. This process is achieved through various components of the gut mucosa that support barrier function and enhance the gut barrier's role in establishing host–microbiota symbiosis, including: occluding junctions at the gut epithelial barrier[9] (Box 1); antimicrobial peptides[10]; secretory IgA[11]; and a gel-like layer consisting of glycosylated proteins (mucins) forming the mucus layer, that prevent direct contact of big particles and gut microbes with the epithelial layer[12] (Fig. 1). Apart from establishing a physical barrier,

Box 1

Occluding junctions: master molecular complexes in barrier biology

The molecular structures that mediate cell–cell junctions sealing the paracellular transport across cellular barriers are evolutionarily ancient and collectively termed occluding junctions. Their appearance was key to the adaptation of metazoans to microbially-dominated ecosystems. Although their function is conserved, there is a high degree of structural variation across the animal kingdom, indicating evolutionary reconfiguration of these structures for adaptation of different species to different niches. The main role is a 'fence and gate' function: they restrict the passage of unwanted elements whilst tightly modulating the access of some molecules. Thus, alterations in their structure can be harmful to the organism[28].

In vertebrates, occluding junctions are known as tight junctions, in contrast to invertebrates, in which they are called septate junctions[5] (Fig. 2). Most of the molecular features of septate junctions in invertebrates have been characterized in fruit fly (*Drosophila melanogaster*). Among the molecular components, an array of >20 proteins, such as Megatrachea (Mega) and Kune-kune (Kune), have structural similarities to the vertebrate tight junction claudin proteins[5]. Moreover, claudin-like proteins CLC1 to CLC4 have also been identified in the nematode *Caenorhabditis elegans*[263].

The molecular components of tight junctions have been extensively studied. Claudins are four-pass transmembrane proteins that form the main structural intercellular component of tight junctions, interacting with neighbouring claudins at the intercellular side and with scaffolding proteins at their cytoplasmic side[264]. Other structural components of tight junctions are MAL and related proteins for vesicle trafficking and the membrane link (MARVEL) domain-containing proteins tricellulin and occludin, junctional adhesion molecules (JAMs)[25,27] (Fig. 1). The assembly of these proteins, and interactions among them and with membrane lipids, are essential features for tight junction properties. In relation to their organization, tight and septate junctions differ in their relative positions within the junctional complexes: tight junctions are apically located in relation to adherens junctions and septate junctions lie basolaterally (Fig. 2). Interestingly, tight junctions are not exclusive to vertebrates. Some invertebrates, such as selected arthropods and tunicates, have tight and septate junctions[5,63]. The presence of tight junctions in both tunicates (the closest invertebrate relatives to vertebrates) and all vertebrates implies that these junctions were favoured as structural elements for paracellular barriers in a shared ancestor. Nevertheless, the precise evolutionary relationship between tight junctions and septate junctions, and whether they emerged independently, remains unclear[264].

Beyond barrier function, tight junctions are key signalling molecules, serving as conduits for transmitting information to the cell, influencing cytoskeleton dynamics, cell proliferation, differentiation and gene expression[25]. Notably, proteins within tight junctions undergo various post-translational modifications, including palmitoylation, glycosylation, phosphorylation, methylation and acetylation. These modifications alter interactions within the junctional complex, which can result in the redistribution or degradation of these proteins[265]. Many of the functional aspects of transmembrane tight junction proteins depend on their interaction with cytosolic proteins. Some of the multiple cytosolic adaptor proteins are zonula occludens (ZO) and cingulin (Fig. 1). This array of proteins can interact with each other and form dynamic complexes, allowing barrier cells to constantly adapt to environmental factors by opening or tightening paracellular transport under various physiological conditions. Both cytosolic and transmembrane tight junction proteins recruit and bind signalling proteins such as kinases, phosphatases and G protein-coupled receptors, which modulates different signalling pathways[25].

Research is moving towards a view of tight junctions as spatiotemporally dynamic macromolecular complexes. Further research will advance our understanding of how tight junctions are modulated to regulate barrier properties and other functions at the cellular barriers. Despite evidence about microbial metabolites having roles in modulating tight junction molecular components (Table 2), mechanistic detail is lacking. Microbial modulation of barriers has been shown at the gene expression and protein levels (Table 2), suggesting a multilevel regulatory role of microbial signals in barrier function.

mucosal surfaces also constitute an immune barrier interfacing with the external world, which together control 'external' microbial access into underlying tissues and dissemination into the circulation. Excellent reviews on mucosal immunity have been published[8,13,14]. After the mucosal layer, a second layer of defence is established by the gut vascular barrier, consisting of intestinal endothelial cells that create a physical barrier that further prevents bacterial dissemination into the systemic circulation of the host[15] (Fig. 1).

The importance of the gut microbiota in maintaining homeostatic gut barrier function has been widely described (Table 1). For example, acute depletion of the bacterial fraction of the holobiont induces an increase in gut permeability in both male and female mice[16], whereas in another study, germ-free mice showed a lower paracellular uptake of an inert probe in the proximal colon[17]. Importantly, the gastrointestinal tract presents marked physiological and organizational heterogeneity along its length. In terms of cellular composition, the small intestine is rich in enterocytes and contains crypts and villi, whereas the large intestine is richer in goblet cells and contains crypts, but no villi[18] (discussed in section 'The gut epithelial barrier'). This heterogeneity is also reflected in distinct gradients of oxygen, nutrients, pH and antimicrobial agents, which altogether strongly determine the local composition of the gut microbiota along the gastrointestinal tract[18]. The most abundant bacterial phyla in the gut are Bacillota, Bacteroidota, Pseudomonadota, Actinomycetota and Verrucomicrobia[19].

However, there are considerable differences in the dominant families along the gut. The local environment in the small intestine is characterized by a higher pH, oxygen levels and presence antimicrobial agents, which limits bacterial growth, whereas the colon, with a lower pH and oxygen, harbours the highest density of microorganisms[18]. Similarly, there is also a spatial stratification of microbiota composition across the transverse biogeography of the gut, with some microorganisms more prominent at the mucosal surface of the gut lumen and others more prominent in the mucosal folds[18]. In terms of gut barrier heterogeneity, several studies have found barrier changes in specific gut regions (Table 1), which suggests differential modulation along gut biogeography, in which local gut microorganisms could have an important role. All in all, biogeographical considerations of gut epithelium organization, microenvironment and microbial communities are essential in our understanding of microbial modulation of gut barrier function in health and disease.

Notably, the first layer of defence in the gut is formed by the above-mentioned mucus layer. This mucus barrier is formed by mucins secreted by goblet cells and is critically important for limiting the exposure of gut epithelial cells to potentially harmful substances, and the gut microbiota and its thickness increases along the length of the gut, mirroring the higher abundance of resident microrganisms[12]. Additionally, the mucus barrier serves as both a nutrient source and a colonization niche for the microbiota. Thus, any disruptions in this delicate balance can lead to infections and contribute to the initiation of inflammatory responses, which are often associated with the development of intestinal inflammatory disorders such as Crohn's disease and ulcerative colitis[12].

The gut epithelial barrier

The gut epithelial barrier resides below the mucus layer and provides a semipermeable physical and biochemical barrier that allows an optimally orchestrated balance of communication and physical segregation between gut microbes and the host. The epithelial monolayer is organized into a series of protrusions and invaginations, called villi and crypts (or crypts of Lieberkühn[20]), respectively. The complex functionality of the gut epithelium is reflected in the diversity of epithelial cell types that compose it. Absorptive enterocytes comprise the majority of gut epithelial cells, but other types of specialized intestinal epithelial cells are enteroendocrine cells, goblet cells, Paneth cells, tuft cells, M cells and stem cells[7,21–23] (Fig. 1). As part of the organizational heterogeneity mentioned above, the relative composition of the different cell types varies along the gut length[24].

Adjacent enterocytes are connected by junctional complexes composed of tight junctions and adherens junctions that limit paracellular transport and therefore intestinal permeability (Box 1). Tight junctions in the gut epithelial barrier are composed of several transmembrane proteins including those of the claudin family, the MARVEL-domain proteins occludin, tricellulin (also known as MARVELD2) and MARVELD3 (ref. 25) (Box 1 and Fig. 1). The fast proliferation and renewal of intestinal epithelial cells makes it imperative that tight junctions are also exquisitely regulated to avoid any dysregulated barrier integrity.

Transport across the gut epithelium includes: transcellular pathways, including passive diffusion; receptor-mediated transport; vesicular transport or endocytosis; and paracellular transport, which includes the 'pore' and the 'leak' pathways[9,26–28]. These two pathways are complementary and strictly modulated, involving transport across tight junctions. By contrast, an additional pathway named the 'unrestricted' pathway, applies when the epithelial barrier is discontinuous due to epithelial cell damage or death[9]. The gut barrier also possesses ATP-binding cassette (ABC) efflux transporters, which protect the gut from accumulation of toxins and other molecules and prevent excessive inflammation[29]. Importantly, gut microorganisms have been shown to modulate their expression in the gut[30,31].

Unsurprisingly, disruption of the fine balance between gut barrier function and selective permeability has been associated with a wide range of intestinal and other disorders[26,32–35] (discussed in section 'Barrier dysfunction in disease'). Finally, gut epithelial barrier function has been shown to be both positively and negatively modulated by enteric glia[36,37], in a similar way to astrocytes in the neurovascular unit (NVU) and their importance for modulating blood–brain barrier (BBB) function[38].

The gut vascular barrier

The presence of a gut vascular barrier enables the active exclusion of gut microorganisms from the systemic and portal circulation. This vascular barrier controls the access of gut microorganisms, microbially-derived substances and dietary compounds into the circulation[15], acting like an ultimate checkpoint (after the gut mucosa and gut epithelial barrier) for gut microorganisms to access the systemic circulation of the host. The gut endothelium is fenestrated, but paracellular transport is restricted by tight junctions formed by claudins, occludin, ZO-1, cingulin and JAM-A[15] (Box 1). Importantly, the gut vascular barrier allows the passage of molecules of up to 4 kDa[15]. Gut endothelial cell fenestrae are closed by plasmalemma vesicle-associated protein 1, a key protein for the formation of diaphragms associated with vascular fenestrae and other structures. Moreover, other cell types are closely associated with this endothelium forming a gut–vascular unit, such as glial cells and pericytes[15] (Fig. 1), although the specific functions of these cell types in maintaining gut vascular barrier function has not been investigated.

The gut vascular barrier is also essential for communication across the gut–brain axis. A landmark publication showed how gut vascular barrier disruption due to an inflammatory insult can induce closure of the choroid plexus vascular barrier in mice[39], which suggests that barriers along the gut–brain axis are functionally linked and that this link can underly the often comorbid neurological symptoms and gastrointestinal symptoms[40].

The brain barriers

In Tolkien's *The Fellowship of the Ring*, Gandalf the Grey famously pronounces "You cannot pass" to the Balrog of Moria[2]. Similarly, our brains have their own wizardry in not allowing foes to pass, as brain homeostasis relies on a highly controlled and stable microenvironment. To establish this milieu, the interstitial fluids within the central nervous system (CNS) are partitioned from the ever-changing blood environment at two key interfaces: at the brain vasculature by the BBB[38] and at the epithelial layer of the choroid plexus by the blood–cerebrospinal fluid barrier (BCSFB), which separates cerebrospinal fluid (CSF) from the choroid plexus interstitial fluid[41] (Fig. 1). The existence of a barrier between the brain and the circulation was first described by Ehrlich who observed that cerulean-S sulfate injected intravenously into the brain does not extravasate[42]. Perhaps more famous are the experiments of Goldmann, Bouffard and Franke who showed that trypan blue, methylene blue and trypan red do not reach the brain when injected intravenously[43]. A further brain barrier is constituted by the meningeal barrier, located within the meninges, which collectively provide the most exterior protection of the brain. Briefly, the meninges are composed of pia, arachnoid and dura mater. Barrier-forming cells

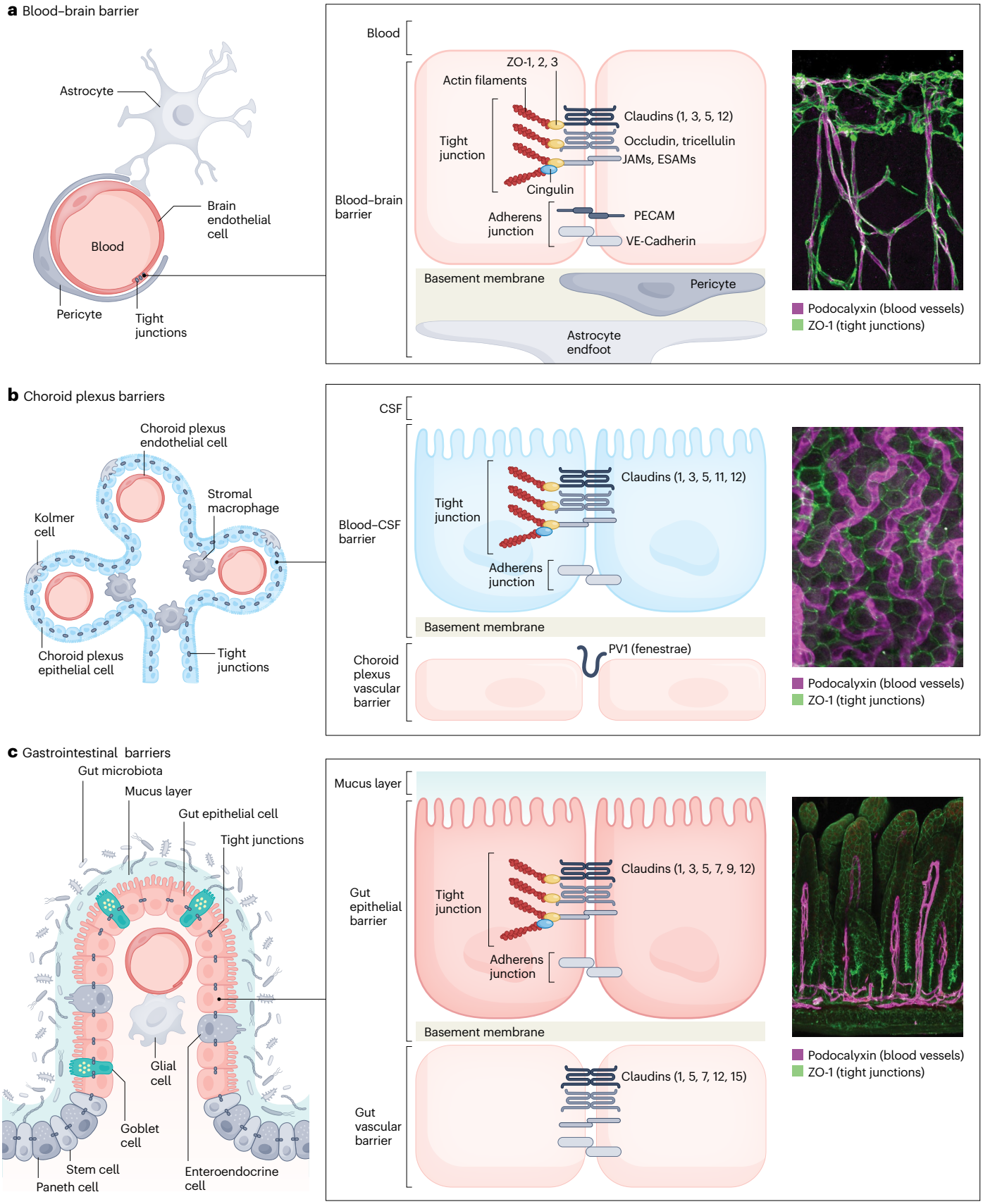

a Blood–brain barrier
Astrocyte
Brain endothelial cell
Blood
Pericyte
Tight junctions
Blood
Blood–brain barrier
ZO-1, 2, 3
Actin filaments
Tight junction
Claudins (1, 3, 5, 12)
Occludin, tricellulin
JAMs, ESAMs
Cingulin
Adherens junction
PECAM
VE-Cadherin
Basement membrane
Pericyte
Astrocyte endfoot
Podocalyxin (blood vessels)
ZO-1 (tight junctions)
b Choroid plexus barriers
Choroid plexus endothelial cell
Stromal macrophage
Kolmer cell
Choroid plexus epithelial cell
Tight junctions
CSF
Blood–CSF barrier
Tight junction
Claudins (1, 3, 5, 11, 12)
Adherens junction
Basement membrane
Choroid plexus vascular barrier
PV1 (fenestrae)
Podocalyxin (blood vessels)
ZO-1 (tight junctions)
c Gastrointestinal barriers
Gut microbiota
Mucus layer
Gut epithelial cell
Tight junctions
Glial cell
Goblet cell
Stem cell
Paneth cell
Enteroendocrine cell
Mucus layer
Gut epithelial barrier
Tight junction
Claudins (1, 3, 5, 7, 9, 12)
Adherens junction
Basement membrane
Gut vascular barrier
Claudins (1, 5, 7, 12, 15)
Podocalyxin (blood vessels)
ZO-1 (tight junctions)

Fig. 1 | Schematic cross section of gastrointestinal and brain barriers in vertebrates. a, Structure of the blood–brain barrier (BBB). The BBB consists of endothelial cells sealed by junctional complexes of tight junctions and adherens junctions. As part of the neurovascular unit, BBB endothelial cells are in close contact with pericytes, which are embedded in the basement membrane. Astrocytic end-feet line the endothelium and connect vascular and neuronal cells. The confocal microscopy micrograph shows cortical brain blood vessels (magenta) and vascular tight junctions (green). **b**, Structure of choroid plexus barriers. The blood–cerebrospinal fluid barrier consists of choroid plexus epithelial cells sealed by junctional complexes of tight and adherens junctions. The choroid plexus vascular barrier is composed of a fenestrated endothelium. The choroid plexus is also a reservoir of immune cells, such as Kolmer and epiplexus cells and macrophages. The confocal microscopy micrograph shows cortical choroid plexus blood vessels (magenta) and choroid plexus epithelial tight junctions (green). **c**, Structure of gastrointestinal barriers. The gut endothelial barrier consists of gut epithelial cells or enterocytes sealed by junctional complexes of tight and adherens junctions. A layer of mucus covers the gut epithelium at the luminal side. The gut epithelium contains a series of specialized epithelial cells: enteroendocrine cells secrete gut hormones and represent an important link in the communication between the central and enteric nervous systems; goblet cells secrete the mucins that compose the mucous layer; Paneth cells secrete antimicrobial peptides that further contribute to the modulation of gut microbes; tuft cells are secretory and chemosensory epithelial cells important in modulating the immune host response and for sensing diverse chemical information from the gut lumen; M cells mediate antigen sampling and presentation to dendritic cells in the lamina propria; and stem cells or pluripotent intestinal epithelial cells are at the base of crypts, where they proliferate and differentiate and then migrate up the crypts along the epithelium to renew the different types of intestinal epithelial cells. The gut vascular barrier consists of a semipermissive fenestrated epithelium that is also sealed with junctional complexes of tight and adherens junctions. The confocal microscopy micrograph shows tight junctions at the gut epithelial barrier in the ileum of the small intestine (green) and gut vasculature (magenta). CSF, cerebrospinal fluid; ESAMs, endothelial cell-selective adhesion molecules; JAMs, junctional adhesion molecules; PECAMs, platelet endothelial cell adhesion molecules; PV1, plasmalemmal vesicle associated protein 1; ZO, zonula occludens.

are tight junction-expressing epithelial-like cells at the outer layer of the arachnoid membrane and endothelial cells in subarachnoid blood vessels. Meningeal barrier function and structure have been extensively reviewed elsewhere[40,44,45] and are not discussed in detail in this Review.

Brain barriers restrict paracellular diffusion into the brain but are also ideally positioned as communication interfaces to receive peripheral circulating signals, including circulating inputs from the microbiota. Research on microbial modulation of brain barriers is an area of increasing interest, and a potential way for how gut microorganisms can influence the distant brain function. In this context, it is worth noting the various similarities between brain and gut barriers, at both the cellular level and the molecular level, which makes them amenable to modulation by common signals, including those derived from the gut microorganisms.

The blood–brain barrier

The BBB controls the exchange of cells and molecules between the circulating blood and the CNS, and prevents the passage of pathogens, toxins and cells into the brain, which is fundamental to protecting and maintaining homeostasis and function. The main elements contributing to BBB function are endothelial cells bound by tight junctions and adherens junctions, which prevent paracellular transport across the vascular wall (Fig. 1). Tight junctions in the BBB endothelial cells are composed of a complex of transmembrane proteins such as claudins, occludins and JAMs, which are associated with cytosolic adapter proteins such as ZO-1 and ZO-2 that provide a structural link between tight junctions and the cytoskeleton[46,47] (Fig. 1 and Box 1). However, other cell types such as astrocytes and pericytes contribute to BBB function and serve as a link between the vasculature and neurons. Microglia and neurons have also been shown to influence BBB function[48,49]. This complex cellular assembly constitutes the NVU[50] (Fig. 1).

BBB dysfunction is well known to play a part in different pathological conditions[38,51–53], but the existence of dynamic and tightly regulated physiological changes in BBB function to maintain brain homeostasis in response to different environmental factors has now been highlighted[54]. Endothelial cells restrict movement of substances from the circulation into the CNS by the presence of tight junctions, specific transporters and a limited rate of transcytosis[55–57]. Thus, endothelial function is central to BBB function, but the modulation by the other cell types at the NVU provide additional capacity for fine-tuning BBB function[50]. To overcome this restrictive nature of the BBB endothelium, there are a wide range of specific transporters in the brain endothelium needed to ensure a supply of molecules for CNS function such as nutrients, hormones and proteins. Several mechanisms of selective transport across the BBB endothelium exist: transcellular passive diffusion of small lipophilic molecules; paracellular diffusion of small hydrophilic molecules[58]; and receptor-mediated transport, such as ion transporters, carrier-mediated transport via solute carrier transporters (SLC) (for example, the glucose transporter SLC2A1 or GLUT1); and transcytosis of macromolecules, which can be receptor mediated (for example, insulin) or adsorptive (for example, plasma proteins). However, overall transcytosis rates are very low in the BBB endothelium, which is largely attributed to the presence of MFSD2A, a lipid transporter that is enriched in brain endothelial cells in both humans and animal models[59,60]. ABC efflux transporters at the BBB prevent the accumulation of endogenous molecules (for example, aldosterone and nucleosides) and exogenous molecules (for example, drugs and xenobiotics) in the brain by actively transporting these back into circulation[52]. These efflux transporters are partially responsible for the poor penetration of some therapeutic agents into the brain, and therefore there is large therapeutic value in getting a better insight into their modulation.

Like the gastrointestinal barrier, the NVU also shows marked heterogeneity in different brain regions as well as in different types of blood vessels. This heterogeneity includes different cell types (astrocytes, pericytes and endothelial cells) at the level of gene expression of specific receptors and transporters, which is suggested to allow local fine regulation of solute transport and blood flow[52]. One of the main features of the heterogeneous nature of the NVU is the presence of the circumventricular organs. These specialized areas feature capillaries that do not possess typical barrier functions and are therefore well-suited to detecting signals circulating in the bloodstream, including hormones, and possibly even microbial metabolites and structural components[61]. Notably, substances entering circumventricular organs cannot freely diffuse to other brain regions because of the presence of a tanycytic barrier. However, these circumventricular organs relay peripheral information to other areas of the brain through neuronal connections[62].

Throughout evolution, BBB-like cellular complexes have appeared independently multiple times, which is in contrast to the simplified

Table 1 | Selected examples of microbial metabolites as modulators of barriers across the gut–brain axis in preclinical models

Metabolite or condition	Microbial signal	Species, model or strain	Effect in host
Gut epithelial barrier			
Germ-free	None	Mice (ICR and IQI outbred strains)	Decreased levels of tight and adherens junctions mRNA (claudin 7, occludin, ZO-1, E-cadherin 1) in colonic tissue[215]
		Mice (germ-free and specific pathogen-free C57BL/6 inbred strain)	Increased claudin 1 and occludin protein intensity in colonic tissue and increased paracellular permeability in specific pathogen-free vs germ-free, recovered upon colonization[17]
Short-chain fatty acids	Butyrate	Human E12 colonic cell line	Improved barrier function via MUC2 increase at 1–10 mM (increased TEER and decreased 4 kDa FITC–dextran permeability); a higher dose had the opposite effect[216]
	Butyrate	Human Caco-2 colonic cell line	Improved barrier function at 2 mM (increased TEER and decreased inulin permeability), 8 mM had opposite effect and induced apoptosis[217]; increased AMP-activated protein kinase, which promotes tight junction assembly reorganization[218]
	Acetate, propionate, butyrate	Human Caco-2 colonic cell line	Protection from LPS-mediated morphological disruption of ZO-1 and occludin and from LPS-mediated barrier disruption (measured by TEER and FITC–dextran permeability) through: NLRP3 inflammasome inhibition (acting as HDAC inhibitors) and autophagy inhibition (acting as energy substrates)[219]
	Butyrate	Rat IEC6 intestinal epithelial cell line	Improved barrier function (increased TEER and decreased 4 kDa FITC–dextran permeability); increased claudin 1 protein expression by facilitating association between transcription factor SP1 and claudin 1 promoter region; induced ZO-1 and occludin redistribution[220]
	Butyrate (30 mg/kg body weight)	Mice (C3H/HeJ strain)	Reduction in gut permeability (measured by 4 kDa FITC–dextran permeability); increase in *Ocln*, *TJP1* (also known as *Zo1*) and *Muc2* gene expression[221]
	Acetate, propionate, butyrate	Piglets (weaned)	Increased mRNA levels of occludin and claudin 1 in the duodenum and ileum[222]
Tryptophan metabolites or indole derivatives	Indole-3-propionic acid	Caco-2 and HT29 co-culture model	Improved barrier function (increased TEER and decreased paracellular permeability to sodium fluorescein); increased protein and mRNA levels of ZO-1, occludin and claudin 1 (ref. 223)
		Mice (Swiss Webster outbred strain)	Improved barrier function (measured by 4 kDa FITC–dextran permeability) through pregnane X receptor[224]
	Indole-3-ethanol, indole-3-pyruvate and indole-3-aldehyde	Mice (DSS-induced colitis model)	Protection against increased gut permeability by maintaining the integrity through AhR and modulation of tight and adherens junctions via actin regulatory proteins[113]
	Indole	Mice (germ-free)	Increased claudin 7, occludin and ZO-1 mRNA levels[215]
		Human Caco-2 colonic cell line	Increased claudin 7, occludin and ZO-1 mRNA levels[215]
	3-Phenylpropionic acid	Mice (Kunming outbred strain)	Improved barrier function (measured by the lactulose:mannitol test) and adherens and tight junction gene expression (ZO-1 and E-cadherin)[225]
	Indoxyl sulfate	Mice (Balb/c strain)	Reduced mRNA levels of tight junction proteins (ZO-1, occludin, claudin 1 and claudin 2)[141]
		Human Caco-2 colonic cell line	Disrupted barrier function (reduced TEER); reduced mRNA levels of tight junction (ZO-1, occludin, claudin 1 and claudin 2)[141]
	Kynurenine	Mice (DSS-induced colitis model; C57B6-129 strain)	Reduction in intestinal permeability (measured by FITC–dextran)[116]
Polyphenolic derivatives	Urolithin A	Mice C57BL/6 (wild-type, *Nrf2*$^{-/-}$, *Ahr*$^{-/-}$) and TNBS-induced colitis model	Increased colonic claudin 4, occludin, ZO-1 and ZO-3 mRNA in wild-type, but not in *Nrf2*$^{-/-}$ or *Ahr*$^{-/-}$ mice; attenuated TNBS-induced increased FITC–dextran permeability[226]
		Caco-2 cells and HT29 cells	Increased claudin 4 immunoreactivity; increased barrier function (measured by 4 kDa FITC–dextran permeability and TEER) through AhR activation and NRF2 pathway[226]
Bile acid metabolites	Lithocholic acid	T84 colonic adenocarcinoma cell line	Protected barrier integrity after exposure to primary bile acid, chenodeoxycholic acid and pro-inflammatory cytokines[121]
	Deoxycholic acid	Jejunum/colon explants (ex vivo) and mice (in vivo)	Increased gut permeability (4 kDa FITC permeability ex vivo and in vivo)[119]
		Human Caco-2 colonic cell line	Decreased TEER and increased permeability (measured by 10 kDa Cascade Blue–dextran permeability) via EGFR activation[120]

Table 1 (continued) | Selected examples of microbial metabolites as modulators of barriers across the gut–brain axis in preclinical models

Metabolite or condition	Microbial signal	Species, model or strain	Effect in host
Gut epithelial barrier (continued)			
Polyamines	Polyamines	Rat intestinal epithelial IEC-6 cell line	Enhanced E-cadherin transcription through MYC activation and barrier function (measured by mannitol and inulin paracellular transport); promoted occludin synthesis and stability[227,228]
Other metabolites	TMAO	Mice (C57BL/6 inbred strain)	Decreased claudin 1 immunoreactivity in ileum, jejunum and colon[229]
Gut vascular barrier			
Germ-free	None	Mice (germ-free, C57BL/6 inbred strain)	Increased vascular permeability (measured by PV1 immunoreactivity) and decreased claudin 5 in gut vasculature[230]
BBB			
Germ-free	None	Mice (germ-free inbred strain; embryonic and adult)	Increased permeability (IgG or Evans blue extravasation); decreased mRNA and protein levels of occludin and claudin 5 in cortex, striatum and hippocampus[93]
Short-chain fatty acids	Butyrate, propionate	Murine bEnd.3 brain endothelial cell line	Protection against LPS-induced barrier disruption via remodelling of tight junction–cytoskeleton interactions (claudin 5 and ZO-1)[94]
	Propionate	Human hCMEC/D3 brain endothelial cell line	Protection against LPS-induced barrier disruption[88]
	Butyrate	Mice (C57BL/6 germ-free)	Ameliorated barrier function and normalized tight junction protein levels[93]
Bile acid metabolites	Chenodeoxycholic acid and deoxycholic acid	Rats (Sprague Dawley strain)	Increased permeability via elevated RAC1 signalling[122]
Tryptophan metabolites or indole derivatives	Indoxyl sulfate	Rats (models of chronic kidney disease)	Indoxyl sulfate induced BBB disruption and associated cognitive impairment through binding to AhR[110]
Other metabolites	Methylamines trimethylamine and TMAO	Mice (C57Bl/6J strain)	TMAO enhanced BBB integrity and protection from LPS inflammatory insult (measured by Evans blue extravasation)[105]
		Human hCMEC/D3 brain endothelial cell line	TMAO enhanced BBB integrity; TMAO impaired BBB function (measured by TEER and 70 kDa FITC–dextran permeability); TMAO acted through annexin A1 signalling[105]
	p-Cresol glucuronide	Mice (C57Bl/6J strain)	Enhanced BBB integrity (measured by Evans blue extravasation)[107]
		Human hCMEC/D3 brain endothelial cell line	Enhanced barrier function (increased TEER)[107]
	p-Cresol	Human hCMEC/D3 brain endothelial cell line	Decreased barrier function (decreased TEER)[107]
Choroid plexus epithelial barrier			
Germ-free	None	Mice (C57BL/6 strain, germ-free)	Decreased network of tight junction protein ZO-1 (ref. 138)
		Mice (C57BL/6 strain, germ-free and antibiotic-treated)	Increased permeability (higher IgG levels in CSF); decreased integrity of ZO-1, claudin 1, occludin and cadherin 1 proteins[96]
Short-chain fatty acids	Butyrate, propionate	Mice (C57BL/6 strain, antibiotic-treated)	Recovery of BCSFB function and tight junction gene expression (claudin 1, occludin and ZO-1) upon antibiotic treatment through vagal and humoral pathways[96]
		Primary choroid plexus epithelial cells	Protection against LPS-induced barrier disruption[96]

AhR, aryl hydrocarbon receptor; BBB, blood–brain barrier; BCSFB, blood–cerebrospinal fluid barrier; CSF, cerebrospinal fluid; DSS, dextran sulfate sodium; FITC, fluorescein isothiocyanate; HDAC, histone deacetylase; LPS, lipopolysaccharide; NLRP3, NLR family pyrin domain-containing 3; TEER, transendothelial–transepithelial resistance; TMAO, trimethylamine *N*-oxide; TNBS, 2,4,6-trinitrobenzene sulfonic acid; ZO-1, zonula occludens 1.

concept of a linear evolution of the BBB in a single ancestor[63]. Moreover, BBB function is conserved across many taxa[63]. Given that the BBB is at the interface of the CNS and periphery, it probably evolved to adapt and respond to the environmental conditions or stressors that are particular for different species. Invertebrate barriers are mostly based on glial cells rather than endothelial cells; moreover, invertebrates often lack a vascular endothelium. In vertebrates, both glial cell barriers (for example, in elasmobranchs or cartilaginous fish, which include sharks, rays and skates) and endothelial cell barriers (for example, in teleost fish and mammals) are found[63,64] (Fig. 2).

The blood–cerebrospinal fluid barrier

The choroid plexus (in each of the brain ventricles) consists of specialized ependymal-derived structures that are mostly known for producing

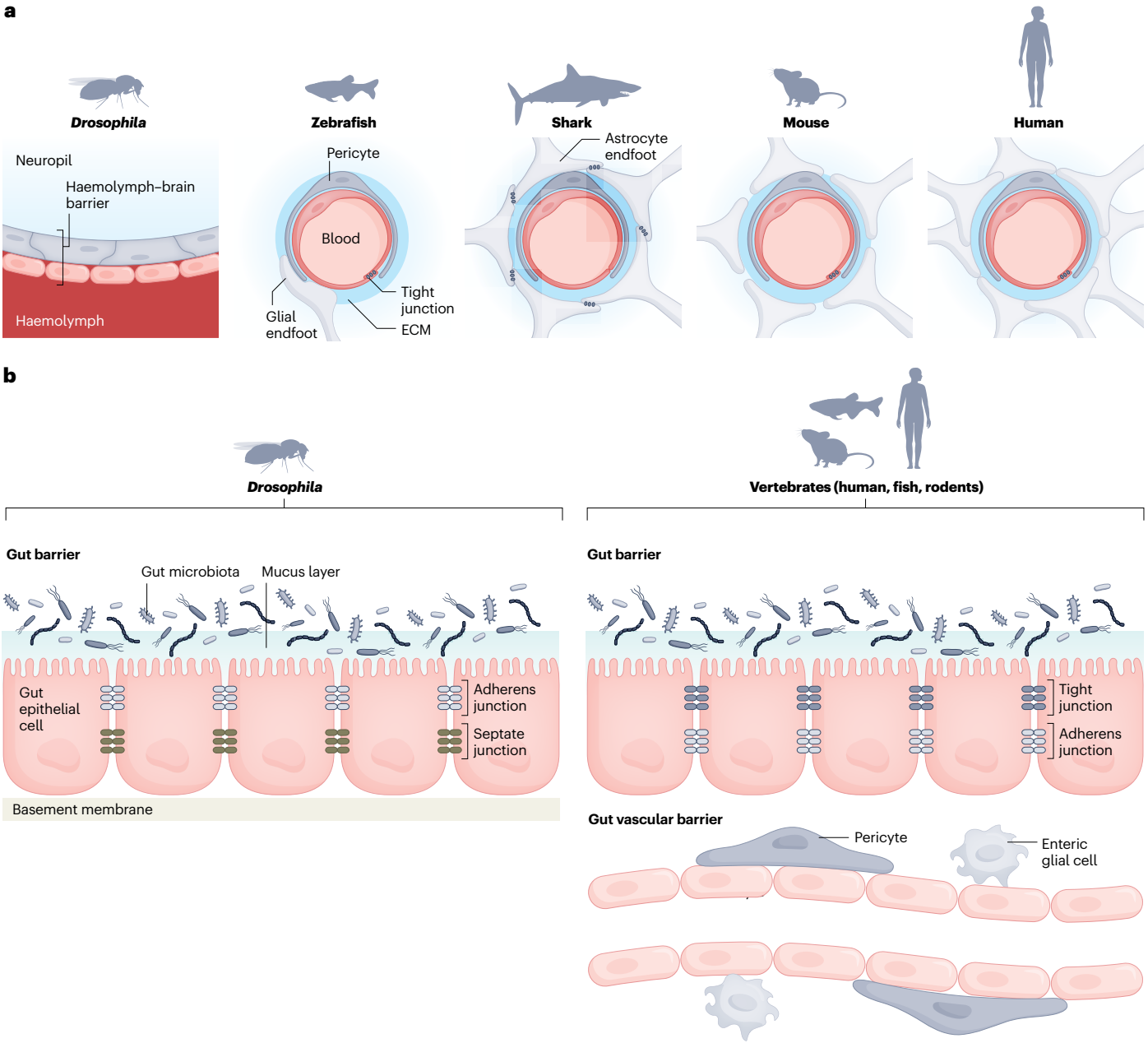


Fig. 2 | Cross-species comparison of gastrointestinal and brain barriers. **a**, The blood–brain barrier (BBB) is the barrier that has been best-studied in terms of its cross-species characteristics. *Drosophila melanogaster* (fruit fly), like most invertebrates, has an open circulatory system, but its brain is still separated from the haemolymph by a BBB homologue structure called the haemolymph–brain barrier that consists of so-called subperineurial glial cells, which have septate junctions and specialized transporters (not depicted). Invertebrates have mostly glial-based barriers, and they generally lack an endothelium. Zebrafish (*Danio rerio*) and other teleost fish have a BBB very similar to that of mammals[256] with endothelial tight junctions, pericytes and glial processes, which in zebrafish are mostly radial glial progenitors, but also bona fide astrocytes[257]. Interestingly, both glial and endothelial BBB exist within vertebrates. Elasmobranch fish, such as sharks and skates, have tight junctions in glial cells instead of endothelial cells[64]. A choroid plexus epithelium and associated vasculature (not shown) have also been described in zebrafish[258,259], sharks[260] and mammals, but a cross-species comparison is not as exhaustive as for the BBB. **b**, The gut barrier in *Drosophila* is composed of septate junctions and adherens junctions sealing the paracellular pathway in epithelial cells, and most likely lacks a gut vascular barrier[261]. Note the difference between tight junctions and septate junctions in their relative position to adherens junctions within the junctional complex. The gut barrier in zebrafish is highly similar to that in mammals[262]. The gut barrier in elasmobranchs has not been described as such, but it is likely to be present due to the general conservation across the animal kingdom. Although the gut vascular barrier has not been described in non-mammalian vertebrates, it is likely to be present due to the high homology to the mammalian vascular system and gut structure. ECM, extracellular matrix. Part **a** reprinted with permission from ref. 64, CSH Press.

and modulating the composition of the CSF that fills the brain ventricles and bathes the cellular lining of the brain ventricles and surface. CSF composition is complex and dynamic across the lifespan, as CSF-borne molecules have key roles in brain development and neurogenesis[65–68].

The choroid plexus also constitutes an interface of exchange between the circulating blood and the CSF, which in turn contributes to the homeostasis of the extracellular fluid in the brain. As such, it is imperative for the choroid plexus to provide a barrier function that prevents paracellular transport of blood-borne substances into the CSF and hence into the brain parenchyma, in a similar way to the BBB in the brain vasculature. The BCSFB also contributes to controlling access of nutrients and hormones to the CSF, and to mediating the efflux of xenobiotics from the CSF into the circulation[41,65]. For these functions, the choroid plexus epithelium, like other barriers, presents a series of specialized ion transporters, channels and efflux transporters[41]. Unlike the BBB, the BCSFB is an epithelial barrier constituted by choroid plexus epithelial cells bound together by tight junctions mainly constituted by claudins, occludin and ZO-1 (refs. 69,70) (Fig. 1). Despite the differences in the cellular nature of the BBB and BCSFB, they both share molecular components and work in conjunction to maintain brain homeostasis. From an evolutionary perspective, the choroid plexus is conserved across most vertebrates[71] but there are no reports of a choroid plexus-like structure in invertebrates.

The choroid plexus vascular barrier

Apart from the epithelial cells present, the choroid plexus is composed of a variety of other cell types, including mesenchymal, glial, neuronal and various immune cells[72]. Moreover, and in contrast to the BBB, the choroid plexus vasculature is fenestrated and permissive to 70 kDa molecules, water and solutes, which is important for the production of CSF[39]. The choroid plexus endothelial cells also include associated mesenchymal cells (fibroblasts and pericytes)[72]. Choroid plexus vessels can respond to different stimuli from the CSF by modifying their diameter, which is mediated by vascular associated pericytes, and innervating nerves[73]. A landmark discovery in animal models demonstrated that the choroid plexus vasculature actually constitutes a vascular barrier that is permissive under normal physiological conditions, but can close in response to certain insults, such as intestinal inflammation and systemic inflammation[39]. The choroid plexus vasculature expresses the vascular diaphragm-associated protein plasmalemma vesicle-associated protein 1, as is also the case for the gut vascular barrier (Fig. 1). Upon closure of the choroid plexus vascular barrier, plasmalemma vesicle-associated protein 1 immunodetection decreases, probably due to a conformational change associated with the closure of the vascular fenestrae[39]. Interestingly, despite the fenestrated nature of the choroid plexus vascular barrier, it also expresses tight junction proteins such as claudin 5, but their possible function in modulating this dynamic barrier remains to be determined[69].

Gut microbiota and barrier function

Pathways of communication

The gut microbiota communicates with the host in many ways, and host factors also influence gut microbiota composition and function[74], reflecting the evolutionarily ancient nature of this interdependent relationship. This bidirectional communication relies on different channels, such as the vagus nerve, the enteric nervous system, the immune system, products of microbial metabolism such as short-chain fatty acids (SCFAs), microbial structural components such as lipopolysaccharide (LPS) and peptidoglycans[1,75,76], and microbial membrane vesicles[77]. The presence of barriers across the microbiota–gut–brain axis can be considered another pathway of communication across the axis: barriers maintain the composition in the different compartments along the axis; barriers are vital for balancing the containment of microorganisms within the gut (including both commensal and pathogenic microorganisms) and the passage of microbial products across gut barriers and subsequently brain barriers; and microbial metabolites have been shown to directly modulate the various barrier functions along the axis (Table 1 and Fig. 3).

Microbial metabolites as signalling molecules

Gut microorganisms vastly increase the enzymatic functional capacity of the host, allowing biochemical reactions that simply would not be possible in the absence of the microbial component of the holobiont[3]. The gut microbiome produces metabolites that can be absorbed through the gastrointestinal tract to reach circulation, where they can interact with virtually every organ and cell in the host. The amount, diversity and nature of circulating microbial metabolites depends on gut microbiota composition and on any factor that can modify this entity, such as diet, medication, stress, age, metabolic state and circadian rhythms, among other factors, making the interactions between microbial metabolites and host cells highly complex[78] (Fig. 3). Notably, previous research has indicated that when accounting for individual differences in the plasma human metabolomic composition, diet and the gut microbiome have a greater influence than genetics[79]. Currently, one of the remaining challenges in the field is to understand these host–microbial interactions at a mechanistic level.

As a reflection of gut microbial complexity, microbial metabolites are diverse, and can arise from microbial metabolism of host-derived compounds such as mucins and secreted proteins, or of diet-derived compounds such as dietary fibre and proteins. The role of microbial metabolites as mediators in host–microbial communication has been extensively reviewed elsewhere[78,80]. Here, we provide an overview of different classes of microbial metabolites and their role in barrier modulation across the microbiota–gut–brain axis.

Microbial fermentation: SCFAs and beyond. The main examples of products of microbial fermentation are SCFAs, which are derived mainly from fermentation of dietary fibre, with butyrate, acetate and propionate the most abundant. The role of SCFAs in microbiota–gut–brain axis signalling has been expertly reviewed previously[81,82]. SCFAs are known to modulate a wide range of host physiological processes, including signalling across the microbiota–gut–brain axis and modulation of gut and brain barriers (Table 1 and Fig. 3). We also have a good mechanistic knowledge of how SCFAs mediate their effects in the host. SCFAs activate the free fatty acid receptors (FFARs), a class of G protein-coupled receptors, and can also be transported into cells via monocarboxylate transporters, where they can have different roles. Upon microbial production, which occurs mostly in the colonic lumen of the gastrointestinal tract[81,82], SCFAs are transported to colonocytes where they constitute a main source of energy. Remaining SCFAs are then transported through portal circulation to reach hepatocytes, which also use SCFAs as an energy source[83–85]. Thus, only a small fraction of SCFAs will reach the systemic circulation and even a smaller fraction will be taken up by the brain[81], though these small amounts seem to be sufficient to exert their actions in brain function. SCFAs reach the brain most likely through monocarboxylate transporters present in endothelial cells[86,87] and might also exert functions at the brain endothelium without crossing into the brain through the presence of FFARs, such as FFAR3, which has been shown to be present in human

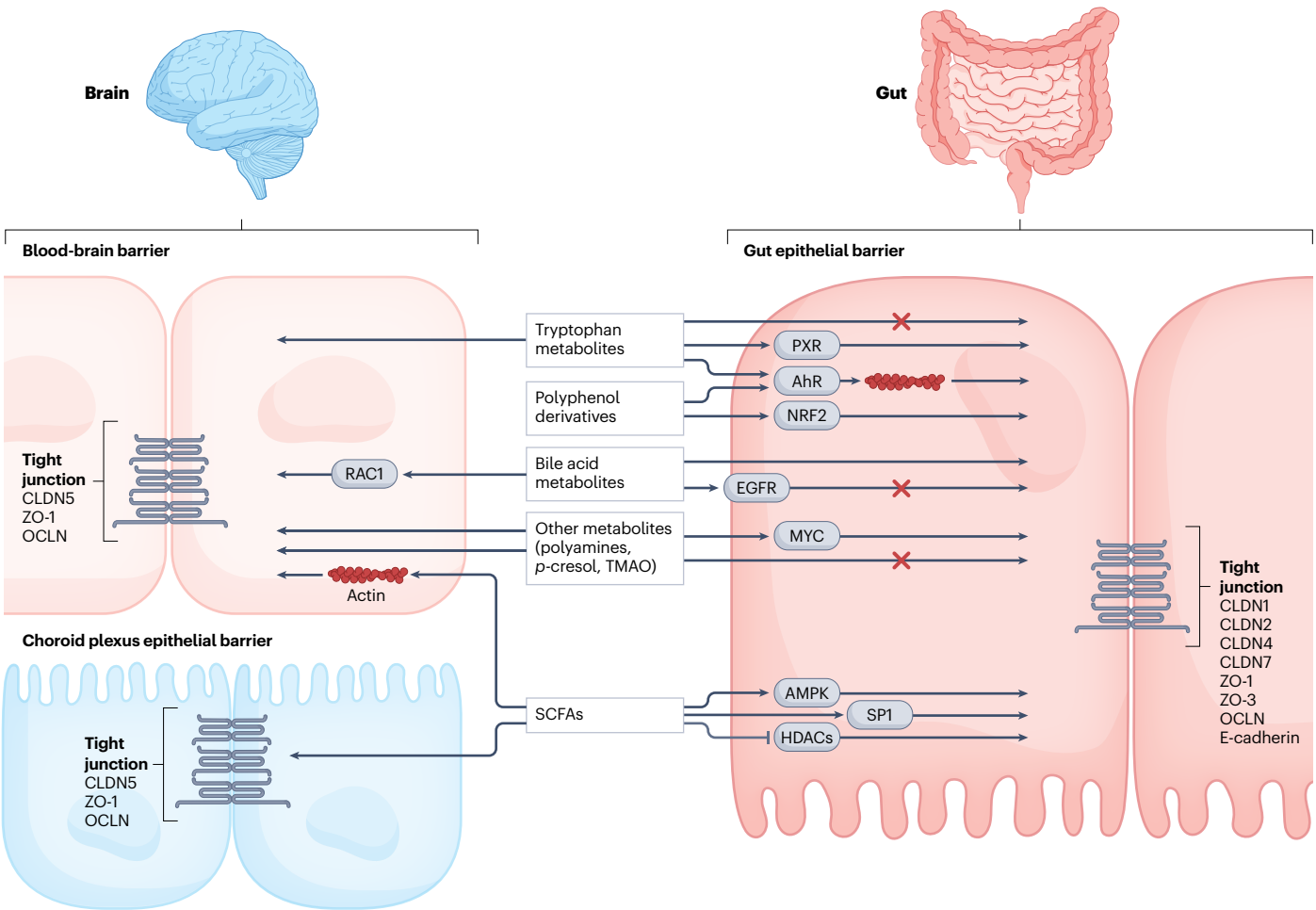


Fig. 3 | Mechanistic overview of microbial modulation of barriers across the microbiota–gut–brain axis. Microbial metabolites are known to modulate barrier function across the microbiota–gut–brain axis. Different types of microbial metabolites have been shown to modulate barrier function by enhancing it (arrows) or disrupting it (crossed arrows). For some of these metabolites, the mechanistic basis of this modulation is known. Most of this mechanistic knowledge comes from effects on the gut epithelial barrier: short-chain fatty acids (SCFAs) are known to protect gut function by inhibiting histone deacetylase (HDAC) activity and promoting AMP-activated protein kinase (AMPK) pathway and SP1 transcription factor. In the brain, they are known to modulate actin cytoskeleton dynamics and interaction of the actin cytoskeleton with tight junction proteins. Some microbially-derived tryptophan metabolites and secondary bile acids are protective (for example, lithocholic acid and indole derivatives), whereas others are disruptive (for example, deoxycholic acid and indoxyl sulfate) of gut and/or brain barriers. Microbially-derived polyphenol derivative urolithin A is protective of barrier function. The effects of other microbial metabolites such as trimethyl *N*-oxide (TMAO) seem to have opposing effects on gut and brain barriers. Moreover, some microbial metabolites have different effects after they are further processed by the host, as is the case with *p*-cresol (microbial) versus *p*-cresol glucuronide (glucuronidated by host enzymes) (see also, Table 1). AhR, aryl hydrocarbon receptor; CLDN, claudin; EGFR, epidermal growth factor receptor; NRF2, nuclear factor erythroid 2-related factor 2; OCLN, occludin; PXR, pregnane X receptor; SP1, specificity protein 1; ZO, zonula occludens.

brain endothelium[88]. Intracellular SCFAs act as inhibitors of histone deacetylases, and in this way, SCFAs promote histone acetylation, leading to an increase in transcriptional activity of chromatin[81]. Interestingly, previous work has shown that systemic administration of SCFAs can modulate HDAC activity in the brain and behaviours in rodents[89–92]. Germ-free mice, which naturally lack SCFA production, present a disrupted BBB across their lifespan, showing an increased extravasation of the classic dye Evans blue into the brain parenchyma and a decrease in tight junction proteins in the endothelium[93]. Remarkably, administration of butyrate or monocolonization with butyrate-producing bacteria such as *Clostridium tyrobutyricum* or *Bacteroides thetaiotaomicron* to germ-free mice ameliorates BBB dysfunction and tight junction protein levels. BBB disruption in germ-free mice is also evident at embryonic stages, suggesting that the maternal gestational microbiome modulates BBB formation[93]. The exact mechanism of how SCFAs modulate barrier function is not fully understood. Our group showed that SCFAs butyrate and propionate promote remodelling of actin cytoskeleton and tight junction proteins (ZO-1 and claudin 5), as well the interaction between these elements, in an in vitro BBB model, without affecting mRNA levels of any of these tight junction proteins[94].

The choroid plexus could also have a role in transporting SCFAs into the brain, as butyrate, propionate and acetate have been shown

to be present in the CSF in healthy adult humans[95]. In fact, adult germ-free mice have been shown to have a disrupted tight junction network (occludin and ZO-1, but not claudin 1) at the choroid plexus[53,96]. Moreover, adult mice treated with antibiotics also show disruption in their tight junction network, suggesting that a constant supply of microbial signals is necessary to maintain barrier integrity at the choroid plexus. Importantly, SCFAs enhanced barrier function and tight junction protein expression at the choroid plexus of antibiotic-treated mice, as well as in cultured primary choroid plexus epithelial cells. Moreover, the authors also showed that modulation of BCSFB integrity depends on both vagal and humoral pathways of communication. Vagotomy in mice is enough to induce disruption of the BCSFB tight junction network, but this vagal pathway could be bypassed by SCFAs through the humoral pathway (systemic circulation)[96].

All in all, in the context of barriers along the microbiota–gut–brain axis, SCFAs have been extensively shown to modulate the gut epithelial barrier as well as the BBB and BCSFB in both in vivo and in vitro models (Table 1 and Fig. 3). However, their role in modulating gut or choroid plexus vascular barriers is yet to be explored. This positive action of SCFAs on pan-barrier homeostasis could be at the core of previously reported associations between SCFAs (especially butyrate) and brain disorders such as depression in humans and animal models[90,92,97,98]. SCFAs are also known to be positive regulators of mitochondrial function[99,100]. Given that mitochondrial dysfunction has been widely shown to be present in several brain disorders[100–102], enhancement of mitochondrial function could also be a mechanism of barrier modulation by SCFAs. In this context, we showed that butyrate and propionate can protect mitochondrial network disruption upon treatment with pathogen-derived LPS in the bEnd.3 brain endothelial cell line[94]. The generalized role of SCFAs in modulating different barriers supports the possible role of microbial signals in orchestrating inter-barrier function to enable communication along the microbiota–gut–brain axis (Fig. 4).

Other metabolites derived from microbial fermentation. Microbial fermentation can also produce other less abundant compounds such as methylamines, indoleacetate, phenylacetate and phenolic compounds[78], and microbial fermentation of branched-chain amino acids (BCAAs) produces the BCAAs 2-methylbutyrate, isovalerate and isobutyrate[78]. However, the role of these relatively minor fermentation products in barrier modulation is yet to be explored. Interestingly, impaired BCAA transport across the BBB has been causally associated with autism spectrum disorder (ASD)-like behaviours in mice[103]. This finding raises the interesting possibility of a role for microbial metabolism of BCAAs in ASD pathophysiology and barrier modulation, among other effects in the host.

Dietary methylamines such as betaine, choline and phosphatidylcholine can be broken down by gut microbes into trimethylamine (TMA), which is subsequently rapidly converted into TMA *N*-oxide (TMAO) in the liver and enters the systemic circulation[78]. TMAO has been shown to have important roles in embryonic axonogenesis[104] and in enhancing BBB function through annexin A1 signalling[105]. However, a dysregulated TMA to TMAO ratio has also been linked to the pathogenesis of cardiovascular disease[106], but causal mechanistic insights need to be further clarified. *p*-Cresol (or 4-methylphenol) is produced by bacterial fermentation of dietary tyrosine and phenylalanine[78], and reaches the liver through the portal circulation. *p*-Cresol undergoes extensive conjugation by the host into *p*-cresol sulfate and *p*-cresol glucuronide[107]. Remarkably, the latter, *p*-cresol glucuronide, has shown protective effects upon LPS challenge in the human brain endothelial cell line hCMEC/D3 (ref. 107) (Table 1 and Fig. 3).

Tryptophan-derived metabolites. Dietary tryptophan can follow various pathways: it can enter the kynurenine pathway, leading to the

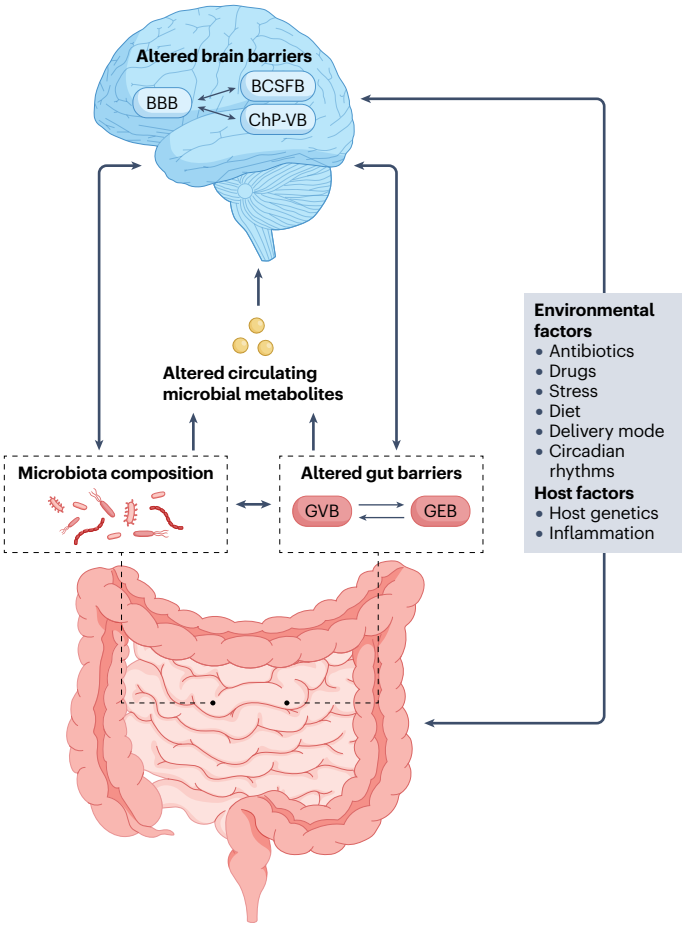


Fig. 4 | The interaction between barriers and the gut microbiota in microbiota–gut–brain axis communication. The presence and relative levels of microbial metabolites and other microbially-derived molecules such as structural components or bacterial membrane vesicles (not shown) depend on microbiota composition, which can be influenced by a diverse array of environmental (diet, stress, mode of birth, among others) and host genetic factors. The complex array of microbial metabolites influence barrier function, and these two factors combined determine the microbial metabolites that reach systemic circulation. These metabolites in turn reach the brain barriers and potentially modulate their function. Other microbiota-independent factors or factors affected indirectly by the gut microbiota affecting gut barrier function, such as inflammatory status, can also alter the brain barriers. Moreover, factors such as psychological stress, that is known to affect the enteric nervous system and gut barrier permeability, modulate the bidirectional microbiota–gut–brain axis in a top-down manner. Disruption of gut and brain barriers have been described in both gut and brain disorders. Thus, given the inter-barrier communication and the gut microbiota as a conduit mediating this communication, an integrative perspective of barrier disruption along the microbiota–gut–brain axis might partially underlie gastrointestinal and neurological comorbidities. BBB, blood–brain barrier; BCSFB, blood–cerebrospinal fluid barrier; ChP-VB, choroid plexus vascular barrier; GEB, gut epithelial barrier; GVB, gut vascular barrier.

production of several intermediates and ultimately NAD^+; it can be converted into serotonin (5-HT) in gut enterochromaffin cells; it can be utilized for protein synthesis; and it can be directly transformed by gut microorganisms into various derivative compounds, including indoles. Notably, some of these indoles serve as ligands for the aryl hydrocarbon receptor (AhR)[78], a ligand-activated transcription factor that integrates environmental and metabolic cues to control complex transcriptional programmes[108]. Microbially-derived AhR ligands have been extensively shown to modulate the gut epithelial barrier (Table 1 and Fig. 3). Moreover, foundational work has demonstrated a key role for AhR signalling in gut endothelial cells in maintaining gut homeostasis in vivo[109]. However, our knowledge of AhR-mediated modulation in brain barriers is more limited, though AhR is present in brain endothelial cells in rodents[110,111] and humans[112]. Several indole metabolites show protective actions in gut barrier function. For example, indole metabolites indole-3-ethanol, indole-3-pyruvate and indole-3-aldehyde, protect against dextran sulfate sodium (DSS)-induced gut barrier disruption in mice by maintaining the integrity of the junctional complex in gut epithelial cells and associated actin regulatory proteins, including by signalling through AhR[113]. Interestingly, SCFAs have been shown to also activate the AhR pathway through their HDAC inhibiting function which, by promoting chromatin decondensation, enhances the availability of AhR–ligand complexes for binding to their designated sites within the promoters of AhR target genes[114].

Tryptophan metabolism through the kynurenine pathway is facilitated by the rate-limiting enzyme indoleamine 2,3-dioxygenase (IDO1), resulting in the production of kynurenine and downstream products, such as kynurenic acid and quinolinic acid[115]. Gut microorganisms have been implicated in inducing IDO1 activity. Moreover, some bacteria harbour enzymes homologous to the eukaryotic enzymes involved in the kynurenine pathway and can therefore also convert tryptophan into kynurenine and other downstream derivatives[115]. Kynurenine has been shown to protect barrier function in a DSS-induced mouse model of colitis[116]. Moreover, kynurenine and tryptophan can cross the BBB through the large neutral amino acid transporter *SLC7A5* or L-type amino acid transporter 1 (LAT1) and thereby influence neurotransmitter production[115].

More than 90% of 5-HT is synthesized in the gut by enterochromaffin cells in humans and mice, and resident microorganisms play a key role in modulating this production[117]. Though gut serotonin can reach systemic circulation but cannot cross the BBB, it has the potential to influence brain function via the microbiota–gut–brain axis. Given the well-established effects of tryptophan-derived metabolites in modulating gut barrier integrity (Table 2 and Fig. 3), further investigation of the role of indole metabolites in modulating brain barriers is warranted.

Secondary bile acids. Gut microorganisms also metabolize host-derived compounds that are present in the gastrointestinal tract, such as bile acids, which are released into the duodenum to aid the absorption of dietary lipids. Primary bile acids, cholic acid and chenodeoxycholic acid (CDCA), can be further metabolized by gut microorganisms to generate secondary bile acids, such as deoxycholic acid (DCA), ursocholic acid, ursodeoxycholic acid and lithocholic acid (LCA)[118]. Our current knowledge regarding secondary bile acids modulating barrier function is relatively scarce. DCA and CDCA have been shown to have disruptive effects on the gut barrier in vivo and in vitro[119–121], whereas LCA seems to have a protective role[121]. A better understanding of microbial modulation of secondary bile acids and of the mechanisms by which these metabolites modulate gut barrier permeability will be essential to the identification of potential therapeutic strategies to balance the gut barrier. As for the brain barriers, CDCA and DCA have also shown disruptive effects in the BBB in animal models[122], suggesting common mechanisms of barrier disruption across barriers (Table 1 and Fig. 3).

Polyamines. Polyamines, such as spermine, putrescine and spermidine, are essential metabolites that can be produced in the host by cytoplasmic enzymes ornithine decarboxylase or *S*-adenosyl-methionine decarboxylase, predominantly from the amino acids ornithine and methionine, and to a lesser degree, arginine and lysine[123]. Gut microorganisms produce polyamines in the gut lumen, especially in the large intestine, where they can be taken up by gut epithelial cells[124]. Thus, gut microorganisms can influence polyamine levels in the host. Polyamines have been shown to modulate gut epithelial barrier function in in vitro models (Table 1 and Fig. 3), but their role in modulating brain barriers has not been explored. Interestingly, although polyamines in general are known to have limited transport across the BBB, spermidine has been shown to cross the BBB and to improve cognition through increasing mitochondrial function in the hippocampus in mice[125].

Microbial structural components and microbial membrane vesicles. Although not strictly considered microbial metabolites, it is important to acknowledge the importance of structural components derived from bacterial cell walls and of bacterial membrane vesicles, and their effect on host physiology. Structural components are frequently termed microorganism-associated molecular patterns (MAMPs). Host receptors specialized in recognizing these microbial structural elements, known as pattern-recognition receptors, have been demonstrated to have crucial roles in the host's functions that extend beyond innate immunity[126]. Moreover, the presence of structural components from bacterial walls in the systemic circulation, such as LPS derived from Gram-negative bacteria, has long been appreciated. Excessive circulating LPS levels are usually associated with compromised gut barrier function and elevated inflammation, and pathogenic LPS is often used for barrier disruption in in vitro and in vivo preclinical studies[88,94,96,105] (Table 2). However, low levels of LPS also reach the systemic circulation in healthy individuals[127], in whom gut barrier function is presumably not compromised. Importantly, LPS is also present in the cell wall of commensal Gram-negative bacteria, and the presence of circulating LPS in healthy individuals suggests specialized mechanisms of crossing an intact gut barrier[76,128]. Structural differences in LPS from commensal versus pathogenic species seems to be a key factor in its effects in the host. Peptidoglycans are MAMPs present in the cell wall of Gram-positive and, to a lesser degree, Gram-negative bacteria. There is strong evidence for physiological roles of peptidoglycans in host physiology, including signalling at the microbiota–gut–brain axis (reviewed elsewhere[126]). Overall, understanding how structurally different MAMPs from commensal versus pathogenic species affect signalling at the microbiota–gut–brain axis, and how barriers play a part in this context, requires further investigation.

Bacterial membrane vesicles are lipid bilayer capsules released from the outer membranes of both Gram-negative and Gram-positive bacteria. Similar to eukaryotic extracellular vesicles, bacterial membrane vesicles transport and protect a wide array of cargoes, including proteins, DNA, RNA, metabolites, enzymes, peptidoglycans, polysaccharides and toxins[77,129]. Importantly, bacterial membrane vesicles can also traverse cell membranes and enter eukaryotic cells from the host[77,129]. Gut microbial membrane vesicles can even cross the intestinal barrier, enter the bloodstream and cross the BBB, and therefore

Table 2 | Selected studies highlighting barrier dysfunction in neurodevelopmental and neurocognitive disorders

Condition or disease	Species or model	Gut barriers	Brain barriers
Neurodevelopmental disorders			
ASD	Humans	Altered mRNA and protein levels of tight junction proteins[164]	Altered mRNA and protein levels of tight junction proteins[164]
		Increased levels of circulating ZO-1; positive correlation between ZO-1 concentrations and ASD severity[165]	No information
	Maternal immune activation ASD mouse model (also relevant as a model of schizophrenia)	Altered gut permeability; altered levels of tight junction mRNA and protein levels[166]	Altered BBB (prenatal to adult)[48]; altered BCSFB (prenatal)[167]
	Prenatal valproic acid ASD rodent model	No information	Altered BBB permeability; prevented by minocycline treatment[168]
	Maternal obesity ASD mouse model	Altered gut barrier function in offspring (increased serum 4 kDa FITC–dextran levels) and decreased claudin 1, claudin 3, occludin and ZO-1 mRNA expression in offspring at 3 weeks[206]	Altered BBB structure and function at the median eminence[207]
	Genetic ASD mouse model (*Shank3*$^{-/-}$)	Altered gut barrier function and altered levels of ZO-1 mRNA[170]	No information
Schizophrenia	Humans	Increased gut permeability[231,232]	22q11.2 deletion contains claudin 5 gene; increased risk of rs10314 variant of claudin 5 allele[233]
		No information	BBB-like endothelium differentiated from human 22qDS^{+} schizophrenia-induced pluripotent stem cells exhibit impaired barrier integrity and decreased claudin 5 mRNA and protein levels[234]
	Humans	No information	Choroid plexus enlargement[235]; altered transcriptome[236]
	Genetic schizophrenia mouse models	No information	Disrupted BBB in 22q11.2 deletion syndrome mice[234]
ADHD	Humans	No information	Increased serum zonulin and claudin 5 levels (indicators of BBB disruption)[237]
Epilepsy	Humans	No information	Patients with treatment-resistant epilepsy show diminished claudin 5 protein levels and BBB disruption[238]
	Mouse model of genetic claudin 5 deficiency	No information	Claudin 5 deficiency induces exacerbated or lowered threshold to epileptic seizures; moreover, stabilization of BBB integrity attenuated seizures and decreased neural damage in kainic acid-induced epilepsy[238]
Mood disorders			
Major depressive disorder	Humans	Increased LBP (a marker of gut barrier increased permeability) in women[212]	Reduced claudin 5 mRNA levels in nucleus accumbens of patients with major depressive disorder[185]; reduced claudin 5 protein in hippocampus grey and increased occludin mRNA levels in occipital cortex of these patients[188]; reduced claudin 5 mRNA and protein levels in medial prefrontal cortex of female patients[187]
	CSDS and chronic variable stress mouse depression models	Changes in tight junctions in jejunum and elevated circulating LBP levels[212]	CSDS-susceptible mice showed reduced BBB integrity with reduced claudin 5 expression in the nucleus accumbens promoting IL-6 infiltration[185] and in the medial prefrontal cortex (specifically in female mice)[187]
Bipolar disorder	Humans	Increased gut permeability (measured by increased serum zonulin levels)[239]	Increased claudin 5 mRNA levels in the cerebellum and occipital cortex in bipolar disorder[188]; alteration in CSF composition reflecting brain barriers dysfunction[240]; altered choroid plexus transcriptome[236] and enlarged ventricles[235]
Anxiety disorders			
Anxiety	Mice	No information	Early-life isolation induced anxiety and decreased claudin 5 levels in amygdala in female mice[241]; choroid plexus vascular barrier closure induced anxiety-like behaviour in mice[39]; targeted disruption of the BBB in the female prefrontal cortex induced anxiety-like and depression-like behaviours[187]
Neurodegenerative disorders			
Alzheimer disease	Humans	No information	APOE4 carriers showed BBB breakdown in the hippocampus and medial temporal lobe, independent of amyloid and tau accumulation in the brain[242]; alterations in choroid plexus transcriptomics, including genes relevant to BCSFB function[243]; BBB disruption (assessed by fibrinogen and IgG infiltration) in patients Alzheimer disease[244,245]

Table 2 (continued) | Selected studies highlighting barrier dysfunction in neurodevelopmental and neurocognitive disorders

Condition or disease	Species or model	Gut barriers	Brain barriers
Neurodegenerative disorders (continued)			
Alzheimer disease (continued)	Mice (injection of amyloid-β oligomers)	No information	Disruption of BCSFB integrity by matrix metalloproteinases[246]
	AppNL-G-F mutant Alzheimer disease mouse model	No information	BCSFB showed disrupted integrity of occludin, ZO-1 and claudin 1 in the BCSFB[96]
PD	Humans	Increased colonic barrier permeability (sucralose excretion), reduced LBP levels and decreased ZO-1 integrity[247,248]	Increased BBB permeability (determined by dynamic contrast-enhanced MRI)[249]
		Lower *Ocln* mRNA and altered distribution of occludin and ZO-1 proteins in colonic biopsies of patients with PD; no differences in paracellular and transcellular permeability (Ussing chambers)[199]	No information
	Rotenone PD mouse model	Decreased ZO-1 intensity; *tlr4*$^{-/-}$ partially protected[247]	No information
	A53T PD mouse model	Altered gut barrier function (circulating LBP) and levels of ZO-1 and occludin in colonic tissue at various ages[250]	Decreased ZO-1, claudin 5 and occludin levels, mediated by astrocytic VEGF secretion[251]
Huntington disease	Humans	No information	iPSC-derived Huntington disease brain endothelial cells: impaired barrier properties (increased transcytosis and paracellular permeability) mediated by Wnt signalling dysregulation[252] iPSC-derived brain endothelial cells from juvenile patients with Huntington disease: reduced barrier function (reduced TEER) and decreased levels of tight junction protein ZO-1[253] Mutant huntingtin aggregated in the NVU of patients; BBB disruption (decreased levels of tight junction proteins occludin and claudin 5, and increased extravascular fibrin)[254]
	R6/2 mouse model of Huntington disease	Increased gut permeability (plasma levels of FITC–dextran), with no alterations in colonic tight junction proteins occludin and ZO-1[255]	Mutant huntingtin aggregates in the NVU of R6/2 mouse model; evidence of BBB disruption (decreased levels of tight junction proteins occludin and claudin 5) and increased trasncytosis[254]

ADHD, attention deficit hyperactivity disorder; ASD, autism spectrum disorder; BBB, blood–brain barrier; BCSFB, blood–cerebral spinal fluid barrier; CSDS, chronic social defeat stress; CSF, cerebrospinal fluid; FITC, fluorescein isothiocyanate; iPSC, induced pluripotent stem cells; LBP, lipopolysaccharide-binding protein; NVU, neurovascular unit; PD, Parkinson disease; TEER, transepithelial–transendothelial electrical resistance; VEGF, vascular endothelial growth factor; ZO-1, zonula occludens 1.

constitute a key component of the microbiota–gut–brain axis[77,129]. Interestingly, bacterial membrane vesicles have been shown to regulate gut barrier function through modulation of mucosal innate immune cells such as macrophages and dendritic cells[130]. Overall, there is growing emphasis on exploring the role of bacterial membrane vesicles derived from commensal or probiotic bacterial strains, and their potential to improve host health[77].

Most studies on microbial modulation of barriers have focused on exploring the protective effects of specific microbial metabolites on barrier function against various insults and how the lack of these metabolites contributes to barrier dysfunction per se. However, few studies have also focused on determining detrimental effects of some metabolites on barrier integrity. Some in vitro studies have shown how some microbial metabolites protect barrier function from the disruptive effects of LPS derived from pathogenic bacteria[88,94,107]. Moreover, it is important to consider that barriers in live organisms are simultaneously exposed to a vast and complex collection of microbial and host metabolites, and that the relative amounts of these will greatly vary depending on factors such as circadian rhythms, age, dietary patterns, stress levels and microbiota composition, among others[79,131] Thus, the balance between these metabolites is likely to be as important as the presence or absence of some of them.

Beyond tight junction proteins, P-glycoprotein (P-gp) efflux transporter expression and function in the gastrointestinal epithelium has also been shown to be modulated by the gut microbiome and by the microbial metabolites SCFAs and secondary bile acids in a synergistic manner[30,31]. This finding raises the interesting possibility that microbial modulation extends to P-gp in the BBB, which could potentially lead to harnessing microbial products as therapeutic modulators of P-gp function. Modelling the complexity of synergistic effects of microbial signals is challenging. The use of mixtures of known metabolites or of fluids with complex metabolomic compositions such as sterile-filtered caecal extract, plasma, or CSF together with more elaborate in vitro or ex vivo systems, such as combinations of cell types or organoids, may be promising tools to advance our understanding of microbial modulation of barrier physiology. The direct contribution of the gut microbiota to gut barrier function has been extensively studied, but less so in relation to the function of brain barriers (Table 1). Intriguingly,

paracellular permeability at the colonic gut barrier has been reported to be decreased (thus, more restrictive) in germ-free mice as compared with conventionally-housed mice[17], suggesting a key role of the microbiota in maintaining physiological levels of gut barrier permeability. Importantly, barrier function should not be regarded as 'the tighter the better'. Barrier permeability is dynamic and needs to be tightly orchestrated and constantly adapted to maintain homeostasis. Gut microorganisms seem to play a major part in achieving this goal. In addition, enteric mucosal development and maintenance depends on gut microbial signals[132], establishing a link between microbial modulation of the gut epithelial barrier through mucosal glia.

Interestingly, germ-free and antibiotic-treated mice show region-specific differences in tight junction gene expression[50,51], suggesting that microbial modulation of BBB function might be region-specific. BBB modulation by microbial metabolites affecting other cells in the NVU such as astrocytes, pericytes or even microglia, remains to be investigated. Astrocytes have been shown to be modulated by microbial signals, both directly by tryptophan metabolites[133] and SCFAs[134] and indirectly by microglia[135]. Moreover, microbial regulation of microglial properties has been widely described[136,137], as has their role in modulating BBB function[48,49].

In contrast to the gut barrier, brain barriers (BBB and BCSFB) have been shown to be more permeable in germ-free mice[93,96,138], suggesting that microbial modulation of barriers can be somewhat barrier-specific. Apart from barrier function in adulthood, the role of the microbiota in barrier function at the extremes of life is a current topic of active investigation (Box 2). In this context, germ-free mice already show a disrupted BBB during embryonic stages, pointing to a role of the maternal microbiota in BBB maturation[93]. A study exploring the enduring effects of a low-dose penicillin from embryonic day 12 (E12) to postnatal day 21 (P21) in mice revealed persistent effects on barriers during adulthood. Although colonic barrier function and tight junction protein levels were unaffected, brain tight junctions (occludin and claudin 5) were dysregulated at the mRNA and protein levels in a sex-specific and region-specific manner. Remarkably, concurrent maternal supplementation with the probiotic bacterial strain *Lacticaseibacillus rhamnosus* (formerly *Lactobacillus rhamnosus*) JB-1 prevented some of these alterations[139]. Moreover, a study showed that perturbation of maternal microbiota during a critical perinatal window (E13 to P3) on administration of ampicillin induced alterations in mRNA levels of BBB-related tight junctions in the prefrontal cortex in the offspring, with some differences between male and female mice[140]. Among microbial metabolites, different tryptophan metabolites have been reported to have a protective role in the gut barrier and in the BBB, but some are also disruptive, such as indoxyl sulfate (Table 1), which has been shown to induce gut and BBB barrier disruption in vitro and in vivo[110,141].

Barrier dysfunction in disease

Unsurprisingly, malfunction of barriers has long been appreciated as a factor that has a negative effect on host physiology. The term 'leaky' has commonly been used to refer to an impaired barrier function, but, despite its widespread use, the term is vague, and we should move away from referring to a barrier as leaky as it oversimplifies a complex and dynamic process that is the modulation of barrier permeability. Notably, most of the available information involving barrier dysfunction in disease comes from preclinical studies due to the technical limitations in human studies.

Given the high molecular and cellular similarities among barriers, it is likely that pathology-associated barrier disruption happens at the level of several barriers across the microbiota–gut–brain axis, compromising its bidirectional communication. In this context, alterations in gut microbiota could contribute to barrier disruption at various levels: an altered microbiota involves alterations in microbial-derived products (such as decreased levels of SCFAs), which could affect gut and brain barrier function; microbiota-led gut dysfunctional barriers (epithelial and/or vascular barriers) would become more permissive to microbial-derived products, which could in turn reach and potentially alter brain barriers; and alterations in gut microbiota could influence barrier function through modulation of gut and brain neuroimmune signals, which are well known to be modulated by the gut microbiota[142]. Notably, other barrier aspects apart from physical integrity can be dysfunctional, such as transporter functions[29,38,103,143]. Thus, despite a major focus on barrier disruption, we should explore the potential of microbial signals to modulate transport across gut and brain barriers (Fig. 4).

Gastrointestinal disorders

Inflammatory bowel disease (IBD) comprises two chronic gut inflammatory disorders: Crohn's disease, which involves inflammation in any part of the intestine, and ulcerative colitis, in which inflammation is restricted to the rectum and colon[28]. Studies have demonstrated that an impaired intestinal barrier occurs years before clinical diagnosis of IBD in humans[144,145]. However, during later stages of IBD, increased permeability is most likely driven by tissue damage in the gut mucosa (through the unrestricted pathway, discussed below)[144–146]. Studies have also shown dysregulated expression and distribution of tight junction proteins in colonic biopsies from patients with active Crohn's disease[147]. Gut barrier disruption in preclinical models of IBD has also been extensively researched. For instance, mouse models of IBD, such as the genetic *Il10*-knockout model, also show increased gut barrier permeability even before disease onset[148]. Moreover, one of the most used mouse model of colitis uses DSS as a chemical agent that induces colitis[149]. DSS-induced colitis in mice has been shown to induce changes in phosphorylation of colonic claudins, which are thought to modulate gut barrier permeability[150]. As mentioned earlier, malfunction in transport systems across barriers has also been associated with gut disorders. In this context, alterations in ABC transporters in the gut epithelial barrier have been shown to be involved in the pathophysiology of IBD[29]. Furthermore, as discussed, a dysfunctional mucus barrier is often associated with inflammatory conditions such as Crohn's disease or ulcerative colitis. The gut microbiota has a key role in mucus production, although the exact mechanisms are not fully understood. Moreover, dietary factors such as a Western diet low in fibre and high in fats, refined sugars and emulsifiers have been also shown to disrupt the mucus layer. The mucus layer and its bidirectional interaction with gut microorganisms is discussed in detail elsewhere[12,151].

Interestingly, circadian, dietary and microbiota patterns modulate gut barrier function[26]. Thus, disruption of any of these factors can negatively influence gut barrier function. An important study showed that a subset of small-intestine epithelial cells show circadian variations in MHC-II expression, which is governed by circadian dietary timing and the gut microbiome, and plays a key part in regulating the small-intestinal barrier through IL-10 production[152]. Conversely, when this exquisitely regulated mechanism is disrupted by changes in the circadian clock, diet or gut microbiota, gut barrier function was impaired leading to exacerbated Crohn's-like enteritis in mice[152]. These findings put the modulation of gut barrier function through diet and the gut microbiota as potential therapeutic strategies in IBD.

Box 2

Barriers across the lifespan

Barriers across the gut–brain axis adapt to the evolving changes associated with different life stages. Changes in the microbial ecosystem are accompanied by changes in barrier function and maturation (see the figure, coloured lines), suggesting an intricate interplay across the lifespan. Overall, physiological variances specific to each life stage, which are adapted to that particular period, might render individuals more vulnerable to various external factors that can disrupt homeostasis, including gut–brain communication.

Embryonic life

There is often the misconception that fetal and early postnatal barrier mechanisms are poorly developed. However, specific barrier mechanisms develop appropriately for each stage of brain development[266].

- Microbial metabolites and bacterial structural components derived from the maternal gut microbiota cross maternal and embryonic barriers and influence the sterile embryo[104,267,268].
- The maternal microbiome is essential for embryonic blood–brain barrier (BBB) function[93].

Blood–placental barrier

- The placenta establishes the blood–placental barrier, facilitating maternal–fetal communication[269].
- Syncytiotrophoblasts utilize tight junction proteins (claudins, occludins, zonula occludens 1 (ZO-1) and ZO-2) to regulate placental transport and barrier functions[270].

Brain barriers

- The BBB is functional early in embryonic development[56,60].
- The choroid plexus is a key structure orchestrating neurodevelopment[67,68,271]. Whilst information on embryonic blood–cerebrospinal fluid barrier (BCSFB) functionality is limited, tight junctions are expressed in embryonic stages in mice and larval stages in zebrafish[167,258].
- The cerebrospinal fluid (CSF)–brain barrier is a transient barrier during early neurodevelopment formed by strap junctions between adjacent neuroepithelial cells of the developing brain, which restricts passage of most molecules into the brain; it reduces progressively and disappears during development[266].

Gut barriers

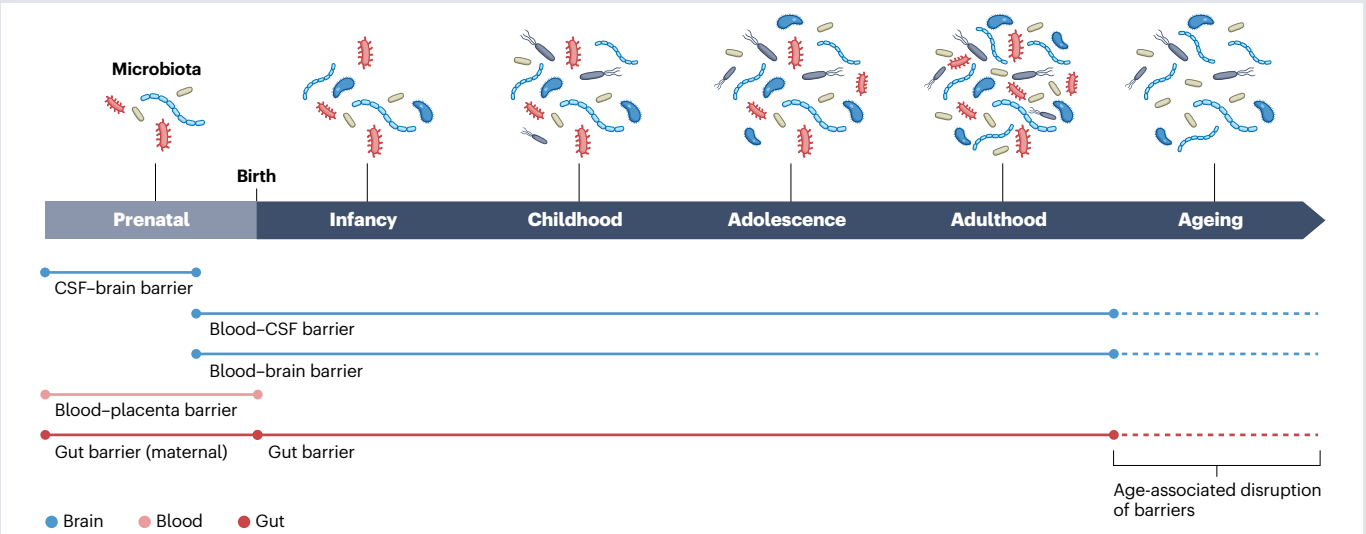
- In embryonic life, there is a high degree of macromolecular transfer (immunoglobulins) via adsorptive endocytosis[272]. 'Gut closure' refers to the restriction of this enhanced endocytosis shortly after birth[272].
- Adaptations to the extrauterine environment and microbiota colonization occur at birth. Impairments of this initial colonization have been related to immune, structural and vascular deficits in the gastrointestinal tract[17,273–277].

Postnatal to adult life

Brain and gut barriers are functional and respond and adapt to environmental and physiological changes such as diet, stress and biological rhythms[26,54].

Brain barriers

- During early postnatal life mice, the BBB structure continues to develop and mature[278]. Incorporation of brain astrocytes increases the complexity in the neurovascular unit, as astrocytic end-feet gradually ensheath brain vasculature[278].
- In humans, astrocytes differentiate during embryonic development, contrasting with mice in which astrocytes differentiate around birth, emphasizing species-specific maturation differences[279].



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- In adulthood, astrocytes and pericytes have crucial roles in finely regulating the expression of BBB tight junction molecules[38].
- A subset of glial cells and pericytes are present in the choroid plexus, but their potential contributions to BCSFB and/or choroid plexus vascular barrier function remain to be explored[72].

Gut barriers

- Gastrointestinal barrier permeability in healthy mice is increased during postnatal ages versus adulthood[280], which suggests, as in the case of brain barriers, that gut barriers also have specific mechanisms that are appropriate for each stage of development.
- Similar to astrocytes of the central nervous system, enteric glia contribute to the maintenance of gut barrier function[281].

Ageing

Ageing is associated with progressive deleterious changes in barrier function, which has been described for gastrointestinal and brain barriers across multiple species.

Brain barriers

- Disruption of the BBB has been reported during healthy ageing in humans[163] and mice[282]. The latter show a transition from receptor-mediated to non-specific transcytosis[53,283].
- With ageing, changes have been identified in BBB pericytes (decreases in number and contacts with the endothelium), astrocytes (hypertrophy and increased pro-inflammatory genes), vascular basement membrane and glycocalyx in mice and humans[53,283].
- Ageing-associated changes in the BBB might be a response to age-associated conditions (for example, sleep disturbances) and could also increase susceptibility to age-associated brain disorders[283].
- Changes in choroid plexus epithelium have been described in mice, including morphological and structural changes in the epithelium, interrupted tight junction and changes in mitochondrial morphology and density[284].

Gut barriers

- Gut barrier disruption associated with ageing, such as increased epithelial apoptosis, reduced mucus layer thickness, and changes in gut microbiota composition, has been extensively described in humans[26,285,286], mice[287] and fruit fly[288].
- Further negative hits to barrier function during ageing can aggravate the naturally occurring decline in physiological processes[26].

Coeliac disease, an immune-mediated disorder related to gluten consumption in the diet, has also been shown to show gut barrier disruption[26]. Moreover, gluten-free diets in patients with coeliac disease can lead to barrier restoration[153]. Distinct gut microbiota changes in patients before coeliac disease onset and after the onset of the disease have been identified, pointing to an altered trajectory of the gut microbial ecosystem that precedes the break in tolerance to gluten[154]. Further, another study found that children developing coeliac disease show characteristic changes in cytokine levels and a distinct gut microbiota composition, accompanied by a twofold increase in plasma microbiota-derived secondary bile acid taurodeoxycholic acid[155]. Whether these microbiota changes are a cause or a consequence of a pro-inflammatory status and altered gut barrier function should be further explored. Interestingly, the more recently discovered gut vascular barrier has also been shown to be disrupted in patients with coeliac disease with elevated transaminase levels as a marker of liver damage occurring independently of gut epithelial barrier integrity[15]. With the discovery of the gut vascular barrier, it became clear that breakdown of the gut epithelial barrier is not sufficient for microorganisms to access systemic circulation, but it is probably sufficient for molecules of <70 kDa to cross[15]. Future studies will further clarify the role of the gut vascular barrier in the pathophysiology of gastrointestinal disorders as well as their comorbidity with disorders of the brain (Fig. 4).

Irritable bowel syndrome (IBS) is a gastrointestinal disorder marked by abdominal pain, bloating and irregular stool patterns in otherwise healthy individuals[156]. However, growing evidence suggests that factors such as diet, gut microbiota and gut barrier function can be important contributors to IBS symptomatology through modulation of the immune system, the limbic system, the hypothalamic–pituitary–adrenal axis and the enteric nervous system[157,158]. Interestingly, a systematic review published in 2021 showed a positive association between increased gut barrier permeability and IBS symptoms in a subset of patients with predominant diarrhoea bowel patterns (IBS-D) or post-infectious IBS, a form of IBS that develops in some patients after viral or bacterial gastrointestinal infection[159]. Moreover, patients with IBS-D have also been found to show structural abnormalities such as disruption of the apical junctional complex in the jejunal epithelial barrier[160]. In another study, colonic biopsies from patients with IBS (subtype not specified) also showed impaired colonic function characterized by significantly increased permeability compared with that in healthy individuals[161]. At a molecular level, studies have shown that patients with IBS have dysregulated expression of tight junction proteins, which differs among the different subtypes, with IBS-D the most affected in terms of dysregulated expression of tight junction proteins ZO-1 and occludin[161,162].

CNS disorders

As described above, brain barriers play a key part in maintaining homeostasis of the brain's microenvironment across the lifespan. Thus, barrier disruption beyond physiological variation is likely to lead to some degree of brain dysfunction. BBB disruption has been described as an early marker of cognitive decline associated with normal ageing[163] and neurovascular deficits have been found in a wide range of neurocognitive disorders (Table 2 and Fig. 4). Neurodevelopmental disorders, such as ASD or schizophrenia, have been found in clinical and preclinical studies to be associated with brain barrier disruption[48,164–170], as well as gut microbiota alterations[34,166,171–174]. Interestingly, mouse models of genetic and of environmental ASD or schizophrenia show gut and brain barrier dysfunction (Table 2 and Fig. 4), suggesting that barrier disruption is a common feature among the complex aetiology of these neurodevelopmental disorders. Among the genetic models,

Shank3 is a known genetic risk factor for ASD. *Shank3* encodes a scaffolding protein at glutamatergic synapses and mouse and zebrafish mutants with *Shank3* knockout display ASD-like behaviours such as social behaviour deficits and repetitive behaviours[169,175,176]. Interestingly, *shank3* is expressed in other organs such as the gut, where it seems to display pleiotropic actions such as gut barrier modulation in mice and gut transit in zebrafish[170,177]. *Shank3*-mutant mice have also been shown to have gut microbiota alterations. For instance, a decrease in the abundance of *Limosilactobacillus reuteri* and other bacterial species from the Firmicutes phylum have been observed[169,178,179]. How this host genetic mutation leads to microbiome changes is not clear, but it highlights the complex interaction between host genetics and the microbiota in modulating complex phenotypes[180].

An example of a non-genetic risk of neurodevelopmental disorders is maternal infection, which has been linked with a markedly increased risk of neurodevelopmental and psychiatric disorders in the offspring, such as schizophrenia and ASD in humans[181–183]. In accordance, preclinical animal models exposed to maternal immune activation, in which an infection is mimicked during pregnancy by injecting an immune-challenging substance, recapitulate these effects by inducing ASD-like behaviours in the offspring[166,171,184]. Once again, this environmental ASD or schizophrenia model has also been found to show gut and brain barrier disruption[48,166,167]. Remarkably, this model has also been shown to have alterations in gut microbiota composition[166,171]. One of these studies also demonstrated a causal relationship between microbial alterations and gut barrier dysfunction, as alterations in gut barrier permeability and in colonic tight junction genes observed in offspring with maternal immune activation were shown to recover following treatment with *Bacteroides fragilis*[166]. Interestingly, gut and brain barrier dysfunction have been reported in patients with schizophrenia (Table 2). The exploration of whether these barrier deficits exist during neurodevelopmental stages and if alterations in gut microbiota could contribute to such barrier dysregulation is essential as they might negatively affect ongoing neurodevelopmental processes, potentially influencing the onset of conditions such as schizophrenia and ASD. Further investigation into these areas is warranted to deepen our understanding of these complex relationships.

Links between brain and gut barriers dysfunction and mood disorders such as major depressive disorder have also been observed[185–188]. Maladaptive responses to chronic stress or stress during early-life are major risk factors for developing mood disorders[189,190] and stress is a well-known disruptor of gut microbiota composition in animal models and humans[191,192]. Stress could alter gut microbiota-mediated brain and gut barrier function through changes in microbial bioactive output. For example, HDAC1 has been identified as a mediator of stress susceptibility through downregulation of claudin 5 in mice[186], and SCFAs are well-known inhibitors of HDAC activity[82]. Thus, SCFA levels could be modifiers of stress susceptibility.

Barrier dysfunction can extend to impairment in barrier-associated transport systems. In this regard, abnormal function of ABC transporters at the BBB have been related to neurological disorders, such as Alzheimer disease or epilepsy[38]. Notably, both of these disorders also show brain barrier disruption in humans as well as in animal models (Table 2). Likewise, both BBB disruption and impaired BCAA transport through LAT1 transporter have been linked to ASD[103] (Table 2). Moreover, brain endothelial LAT1 is also responsible for transporting circulating kynurenine into the brain, inducing depressive-like symptoms in mice[143].

Many studies have demonstrated the relationship between the gut microbiota and brain disorders on the one hand and with barrier dysfunction on the other, but the links among these relationships, whereby the gut microbiota promotes brain dysfunction through barrier disruption or dysregulation of transport systems, is an emerging topic (Fig. 4).

Gut and neurological comorbidities

Mounting evidence shows that compromised gut barrier function is relevant for a wide range of CNS disorders, including neurodevelopmental, psychiatric and neurological disorders[34], such as ASD, schizophrenia and depression[34] (Fig. 4). Interestingly, gut microbiota alterations and brain barrier dysfunction have been observed in all these brain disorders[98,174,193] (Table 2). For some of these disorders, microbial changes have been functionally linked to their pathophysiology (Table 2), which makes it relevant to expand our view of some brain disorders into whole-body disorders in which the microbiota–gut–brain axis has a key role. Parkinson disease (PD) is probably one of the brain disorders in which gastrointestinal barrier dysfunction has been most widely described (Table 2). According to Braak's hypothesis, idiopathic forms of PD start with a pathogen in the gut that crosses the gut barriers and accesses the CNS via postganglionic enteric neurons[194]. Moreover, α-synuclein pathology has been detected in patients with PD during the early stages, and there is evidence in humans and mice that α-synuclein fibrils can spread from the gut to the brain, which has been shown to depend on vagus nerve integrity in mice[195,196]. This finding led to the hypothesis of the 'brain-first' and 'body-first' forms of PD. The brain-first variant is characterized by the initial emergence of α-synuclein pathology in the brain, followed by secondary spreading to the peripheral autonomic nervous system; and in the body-first variant, the pathology originates in the enteric or peripheral autonomic nervous system and subsequently spreads to the brain[196]. PD-related gastrointestinal symptoms, such as dysfunctional gastrointestinal barriers and changes in gut microbiota, are well established and precede neurological symptoms[197–199].

ASD is a complex developmental condition involving challenges with social communication, restricted interests and repetitive behaviours. The degree of severity and coexistence of symptoms in ASD is highly variable, and its aetiology involves complex interactions of genetic and environmental factors[200]. Gastrointestinal dysfunction is often reported in patients with ASD[201] and changes in microbial composition, though sometimes controversial, have been well characterized in patients with ASD[193] as well as in infants at elevated risk of developing ASD[172]. Beyond correlative reports, some studies have also established functional links between microbiota changes and ASD gut and brain pathophysiology in preclinical experimental models[166,169,173,202–205]. Furthermore, barrier dysfunction across the microbiota–gut–brain axis in ASD has been found in animal models[48,166–168,170,206,207] and humans[164,165], in which both the gut and brain barriers have been shown to be dysfunctional (Table 2).

Many patients with IBS present with psychiatric comorbidities. In a cohort of 150 individuals diagnosed with IBS, >50% showed symptoms of anxiety and depression[158]. Importantly, individuals in this group exhibited more pronounced gastrointestinal symptoms and lower quality of life than those without any psychiatric comorbidities[158].

Notably, stress (especially chronic stress or stress during early life) has been identified as a major predisposing factor to the development of IBS and psychiatric disorders, including anxiety and depression[208]. Stress is also known to induce changes in gut microbiota

Box 3

Outstanding research questions

- Is gut microbial modulation of barriers causally related to neurodevelopmental and neurocognitive disorders? In this context, an interesting aspect is to establish whether microbial modulation of barrier function during neurodevelopment has critical windows, in which dysregulated microbiota composition negatively influencing barrier development cannot be completely rescued in later life. Would this process confer a different susceptibility to develop other disorders later in life?
- What is the role of bacterial structural components and of extracellular vesicles derived from commensal and/or probiotic bacterial strains in barrier modulation across the microbiota–gut–brain axis?
- Is microbial modulation of the gut barrier different along the biogeography of the gastrointestinal tract? Similarly, does blood–brain barrier heterogeneity result in region-specific modulation by microbial signals?
- How do microbial signals modulate epithelial and vascular barriers and their interplay at the gastrointestinal tract and the choroid plexus?
- The gut microbiota from laboratory animals strongly differs from that in wild counterparts, which might be more accurate as models for microbiome studies with potential to translate to humans. How does barrier function modulation by the microbiome differ between wild and laboratory animals? On a similar theme, what are the contributions of non-bacterial gut microorganisms (that is, virus, fungi and archaea) to barrier function?
- Can we target the barriers for therapeutic benefit across the lifespan? The microbiota is highly amenable to modulation by factors such as diet, probiotics, prebiotics and symbiotics. Can we harness nutrition to influence barrier–microbiota–gut–brain axis interactions, for example by boosting short-chain fatty acid (SCFA) production with dietary fibre in combination with probiotics that can produce SCFAs.
- Though not covered in this Review, brain meninges constitute another complex barrier in the brain. Although a gut–meningeal–immune axis that also involves the gut microbiota has been established[289], it remains a key outstanding question whether these barrier-forming cells are also modulated by microbial signals.

composition[191]. Consequently, stress-induced microbiota changes could potentially underlie IBS and psychiatric comorbidities through disruption of the microbiota–gut–brain axis, including impairing gut and brain barrier function. Furthermore, patients with IBD are also known to show an increased risk of anxiety and depression, but the exact magnitude and underlying mechanisms of their co-occurrence remain to be further clarified[209]. Patients with IBD have been shown to have a less diverse gut microbiota, a feature that is also found in patients with major depressive disorder[210,211]. However, whether the gut microbiome changes are a cause or a consequence in IBD and in depression remains to be clarified.

In support of how stress–microbiome interactions could be underlying gastrointestinal comorbidities involving barrier disruption, a study in mice revealed that psychological stress induces inflammatory enteric glia and transcriptional immaturity in enteric neurons through chronic glucocorticoid signalling[142]. Furthermore, psychosocial stress induced brain and gut barrier disruption alongside depressive-like behaviours in stress-susceptible mice[187,212] (Fig. 4).

Overall, there is no clear understanding of how functional alteration of gut and brain barriers are mechanistically linked with CNS and gastrointestinal pathologies. An altered gut barrier would allow abnormal translocation of microbial metabolites and structural components into the bloodstream, which could reach the brain barriers. Moreover, the uncontrolled translocation of microbial components could also elicit an inflammatory response leading to neuroinflammation and subsequent brain dysfunction. However, as mentioned above, barriers across the microbiota–gut–brain axis establish an interconnected system of epithelial and endothelial barriers that interact and cooperate to maintain homeostasis[40]. Thus, gut barrier dysfunction could contribute to CNS disorders by promoting alterations in brain barrier function (Fig. 4). Further supporting the notion of inter-barrier communication across the microbiota–gut–brain axis, an influential study demonstrated that the choroid plexus vascular barrier closes upon gut vascular barrier opening associated with intestinal inflammation[39], which could be a mechanism to protect the brain from circulating inflammatory mediators. This closure occurs by upregulation of the Wnt–β-catenin signalling pathway. Interestingly, the authors also showed that a genetic mouse model of vascular barrier closure leads to impairment of episodic memory and anxiety-like behaviour[39], suggesting that choroid plexus vascular barrier permissive function is important for cognitive function, and that mental symptoms related to gut inflammatory disorders might therefore be the consequence of a dysregulated gut–brain vascular axis.

Finally, it is important to highlight certain prevalent lifestyle factors mostly associated with industrialized countries (such as obesity, physical inactivity, poor dietary habits, stress and gut microbiota disruption) can promote a state of low-grade systemic chronic inflammation. This condition is characterized by a chronic non-infectious activation of immune components[213]. This persistent state of chronic inflammation can give rise to various diseases ranging from metabolic syndrome, neurodegenerative disorders and depression, which collectively stand as primary contributors to disability and mortality on a global scale[213]. Given the increasing prevalence and the inflammatory nature of these conditions, coupled with the involvement of the gut microbiota and various factors that possess the potential to influence both the microbiota and barrier integrity (Fig. 4), it becomes imperative to understand the role of inter-barrier communication and the microbiota–gut–brain axis. Such understanding could pave the way for targeted interventions and therapeutic strategies aimed at mitigating chronic low-grade inflammation and its associated health risks.

Outstanding questions and future directions

Several outstanding questions remain (Box 3) and there are also some limitations and challenges that need to be clarified and overcome to advance the field. Microbial metabolites are dynamic and part of a complex mixture of host and microbial metabolites. Thus, investigating individual metabolites, although informative, has limited value for complex systems, as the net effect of microbial metabolites is likely to depend on their relative levels.

Importantly, preclinical models are useful but always face the challenge of translation to humans. Dominant microbial genera and their relative abundance differ between rodents and humans. However, efforts should probably focus mostly on the functional potential of these microorganisms, which might be more conserved than microbial species. Moreover, differences in gastrointestinal tract and brain anatomy between species might make comparisons more difficult. Brain and gut barriers have been well characterized in diverse species, revealing high degree of functional conservation across the animal kingdom. However, human barriers have their own species-specific adaptations reflected in differences in cellular composition at the barriers. It remains to be clarified whether these differences affect barrier function. For example, human astrocytes differentiate during embryonic life, as opposed to astrocytes in rodents, in which gliogenesis begins just before birth and occurs mostly during postnatal life[214]. The potential consequences of these and other differences on BBB function and modulation remain to be unravelled.

Modelling brain and gut disorders in rodent models or other model organisms constitutes an additional challenge. These models aim to replicate symptoms of complex disorders, which often encompass symptoms common to multiple disorders. For example, maternal immune activation models aspects of neurodevelopmental disorders such as ASD and schizophrenia, but no model can recapitulate the complexity in a human patient. All in all, preclinical models are instrumental in advancing our understanding of the complex interactions between host and microorganisms within the holobiont, as they enable study of these interactions at molecular and cellular levels to a degree that we could never reach in humans. We should, therefore, aim to refine preclinical models and utilize, where possible, a cross-species approach in which we leverage particular advantages of different species. Combining preclinical models with in vitro models such as human induced pluripotent stem cells to model barrier function associated with different conditions could provide added advantages to our current limitations. Finally, translating findings in humans back to preclinical models could help us dissect complex disorders into the underlying malfunctional processes.

Conclusions

The realization that the gut microbiome is a critical element regulating brain and behaviour across the lifespan has been a long road and we need to uncover more about the routes of communication. Gastrointestinal and brain barriers are dynamic and adaptive structures that have evolved to be crucial 'secret' gates that enable key aspects of this communication to occur. Although barriers have traditionally had a negative connotation in our language, we now appreciate that they have played an instrumental role in the evolution of holobionts in a microbially-dominated world.

We discuss how barriers have key similarities at the structural and functional levels, but also how each has particularities that are essential for their individual function and for cross-barrier communication. The BBB has long been considered the main gateway to the brain, and a variety of microbial products have been identified as key modulatory signals of their function. Remarkably, with the emergence of the BCSFB as another gate of communication of microbial signals to the brain, we are observing that the same main microbial metabolites that modulate the BBB are also key for BCSFB integrity. However, further investigation of the complex interaction between microbial signals and brain barriers, and their common and specific modulation, is warranted.

We also discussed epithelial and vascular barriers, and their close interaction especially in the gastrointestinal tract and the choroid plexus, structures that both show remarkable similarities in their barrier structure. Vascular barriers are more permissive than their epithelial counterparts, but they have been shown to adapt this permissiveness to physiological as well as pathological circumstances. How microbial signals modulate specifically epithelial and vascular barriers, and their interplay, remain to be further explored.

Given the importance of barriers in maintaining homeostasis across the microbiota–gut–brain axis, we discussed how their malfunction can disrupt its communication and therefore be at the basis of gastrointestinal and neurological comorbidities. In conclusion and returning to Tolkien, this adventure is far from having an end at this stage and we must carry on the story.

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References

1. Cryan, J. F. et al. The microbiota-gut-brain axis. *Physiol. Rev.* **99**, 1877–2013 (2019).
2. Tolkien, J. R. R. The Lord of the Rings (George Allen & Unwin, 1954–1955).
3. Theis, K. R. et al. Getting the hologenome concept right: an eco-evolutionary framework for hosts and their microbiomes. *mSystems* **1**, e00028-16 (2016).
4. Nagpal, J. & Cryan, J. F. Microbiota-brain interactions: moving toward mechanisms in model organisms. *Neuron* **109**, 3930–3953 (2021).
5. Izumi, Y. & Furuse, M. Molecular organization and function of invertebrate occluding junctions. *Semin. Cell Dev. Biol.* **36**, 186–193 (2014).
6. Chomicki, G., Werner, G. D. A., West, S. A. & Kiers, E. T. Compartmentalization drives the evolution of symbiotic cooperation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **375**, 20190602 (2020).
7. Peterson, L. W. & Artis, D. Intestinal epithelial cells: regulators of barrier function and immune homeostasis. *Nat. Rev. Immunol.* **14**, 141–153 (2014).
8. Schröder, K. & Bosch, T. C. G. The origin of mucosal immunity: lessons from the holobiont Hydra. *MBio* **7**, e01184-16 (2016).
9. Horowitz, A., Chanez-Paredes, S. D., Haest, X. & Turner, J. R. Paracellular permeability and tight junction regulation in gut health and disease. *Nat. Rev. Gastroenterol. Hepatol.* **20**, 417–432 (2023).
10. Bevins, C. L. & Salzman, N. H. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. *Nat. Rev. Microbiol.* **9**, 356–368 (2011).
11. Chen, K., Magri, G., Grasset, E. K. & Cerutti, A. Rethinking mucosal antibody responses: IgM, IgG and IgD join IgA. *Nat. Rev. Immunol.* **20**, 427–441 (2020).
12. Gustafsson, J. K. & Johansson, M. E. V. The role of goblet cells and mucus in intestinal homeostasis. *Nat. Rev. Gastroenterol. Hepatol.* **14**, 3171–3183 (2022).
13. Perez-Lopez, A., Behnsen, J., Nuccio, S.-P. & Raffatellu, M. Mucosal immunity to pathogenic intestinal bacteria. *Nat. Rev. Immunol.* **16**, 135–148 (2016).
14. Powell, N., Walker, M. M. & Talley, N. J. The mucosal immune system: master regulator of bidirectional gut-brain communications. *Nat. Rev. Gastroenterol. Hepatol.* **14**, 143–159 (2017).
15. Spadoni, I. et al. A gut-vascular barrier controls the systemic dissemination of bacteria. *Science* **350**, 830–834 (2015).
16. Vicentini, F. A. et al. Intestinal microbiota shapes gut physiology and regulates enteric neurons and glia. *Microbiome* **9**, 210 (2021).
17. Hayes, C. L. et al. Commensal microbiota induces colonic barrier structure and functions that contribute to homeostasis. *Sci. Rep.* **8**, 14184 (2018).
18. McCallum, G. & Tropini, C. The gut microbiota and its biogeography. *Nat. Rev. Microbiol.* https://doi.org/10.1038/s41579-023-00969-0 (2023).
19. Eckburg, P. B. et al. Diversity of the human intestinal microbial flora. *Science* **308**, 1635–1638 (2005).
20. Lieberkühn, J. N. De Fabrica et Actione villorum intestinorum tenuium Hominis. *J. Physiol. Biochem.* **64**, 393–414 (2008).
21. Kanaya, T., Williams, I. R. & Ohno, H. Intestinal M cells: tireless samplers of enteric microbiota. *Traffic* **21**, 34–44 (2020).
22. Schneider, C., O'Leary, C. E. & Locksley, R. M. Regulation of immune responses by tuft cells. *Nat. Rev. Immunol.* **19**, 584–593 (2019).

23. Gehart, H. & Clevers, H. Tales from the crypt: new insights into intestinal stem cells. *Nat. Rev. Gastroenterol. Hepatol.* **16**, 19–34 (2019).
24. Donaldson, G. P., Lee, S. M. & Mazmanian, S. K. Gut biogeography of the bacterial microbiota. *Nat. Rev. Microbiol.* **14**, 20–32 (2016).
25. Zihni, C., Mills, C., Matter, K. & Balda, M. S. Tight junctions: from simple barriers to multifunctional molecular gates. *Nat. Rev. Mol. Cell Biol.* **17**, 564–580 (2016).
26. Martel, J. et al. Gut barrier disruption and chronic disease. *Trends Endocrinol. Metab.* **33**, 247–265 (2022).
27. Otani, T. & Furuse, M. Tight junction structure and function revisited. *Trends Cell Biol.* **30**, 805–817 (2020).
28. Chelakkot, C., Ghim, J. & Ryu, S. H. Mechanisms regulating intestinal barrier integrity and its pathological implications. *Exp. Mol. Med.* **50**, 103 (2018).
29. Verma, N., Paul, J. & Kumari, R. ATP-binding cassette (ABC) transporters and their role in inflammatory bowel disease (IBD). Preprint at *Biomed. J. Sci. Tech. Res. Med.* **5**, https://doi.org/10.26717/BJSTR.2018.05.001141 (2018).
30. Foley, S. E. et al. Gut microbiota regulation of P-glycoprotein in the intestinal epithelium in maintenance of homeostasis. *Microbiome* **9**, 183 (2021).
31. Foley, S. E. et al. Microbial metabolites orchestrate a distinct multi-tiered regulatory network in the intestinal epithelium that directs P-glycoprotein expression. *mBio* **13**, e0199322 (2022).
32. Odenwald, M. A. & Turner, J. R. The intestinal epithelial barrier: a therapeutic target? *Nat. Rev. Gastroenterol. Hepatol.* **14**, 9–21 (2017).
33. Bischoff, S. C. et al. Intestinal permeability – a new target for disease prevention and therapy. *BMC Gastroenterol.* **14**, 189 (2014).
34. Pellegrini, C. et al. The intestinal barrier in disorders of the central nervous system. *Lancet Gastroenterol. Hepatol.* **8**, 66–80 (2023).
35. Zeisel, M. B., Dhawan, P. & Baumert, T. F. Tight junction proteins in gastrointestinal and liver disease. *Gut* **68**, 547–561 (2019).
36. Yu, Y.-B. & Li, Y.-Q. Enteric glial cells and their role in the intestinal epithelial barrier. *World J. Gastroenterol.* **20**, 11273–11280 (2014).
37. Seguella, L. & Gulbransen, B. D. Enteric glial biology, intercellular signalling and roles in gastrointestinal disease. *Nat. Rev. Gastroenterol. Hepatol.* **18**, 571–587 (2021).
38. Sweeney, M. D., Zhao, Z., Montagne, A., Nelson, A. R. & Zlokovic, B. V. Blood-brain barrier: from physiology to disease and back. *Physiol. Rev.* **99**, 21–78 (2019).
39. Carloni, S. et al. Identification of a choroid plexus vascular barrier closing during intestinal inflammation. *Science* **374**, 439–448 (2021).
40. Spadoni, I., Fornasa, G. & Rescigno, M. Organ-specific protection mediated by cooperation between vascular and epithelial barriers. *Nat. Rev. Immunol.* **17**, 761–773 (2017).
41. Saunders, N. R., Dziegielewska, K. M., Fame, R. M., Lehtinen, M. K. & Liddelow, S. A. The choroid plexus: a missing link in our understanding of brain development and function. *Physiol. Rev.* **103**, 919–956 (2023).
42. Ehrlich, P. *Das Sauerstoff-Bedürfniss des Organismus: Eine Farbenanalytische Studie* (August Hirschwald, 1885).
43. Saunders, N. R. et al. The rights and wrongs of blood-brain barrier permeability studies: a walk through 100 years of history. *Front. Neurosci.* **8**, 404 (2014).
44. Alves de Lima, K., Rustenhoven, J. & Kipnis, J. Meningeal immunity and its function in maintenance of the central nervous system in health and disease. *Annu. Rev. Immunol.* **38**, 597–620 (2020).
45. Weller, R. O., Sharp, M. M., Christodoulides, M., Carare, R. O. & Møllgård, K. The meninges as barriers and facilitators for the movement of fluid, cells and pathogens related to the rodent and human CNS. *Acta Neuropathol.* **135**, 363–385 (2018).
46. Langen, U. H., Ayloo, S. & Gu, C. Development and cell biology of the blood-brain barrier. *Annu. Rev. Cell Dev. Biol.* **35**, 591–613 (2019).
47. Kadry, H., Noorani, B. & Cucullo, L. A blood-brain barrier overview on structure, function, impairment, and biomarkers of integrity. *Fluids Barriers CNS* **17**, 69 (2020).
48. Zhao, Q. et al. Prenatal disruption of blood–brain barrier formation via cyclooxygenase activation leads to lifelong brain inflammation. *Proc. Natl Acad. Sci. USA* **119**, e2113310119 (2022).
49. Kang, R. et al. The dual role of microglia in blood-brain barrier dysfunction after stroke. *Curr. Neuropharmacol.* **18**, 1237–1249 (2020).
50. Schaeffer, S. & Iadecola, C. Revisiting the neurovascular unit. *Nat. Neurosci.* **24**, 1198–1209 (2021).
51. Dion-Albert, L., Dudek, K. A., Russo, S. J., Campbell, M. & Menard, C. Neurovascular adaptations modulating cognition, mood, and stress responses. *Trends Neurosci.* **46**, 276–292 (2023).
52. Profaci, C. P., Munji, R. N., Pulido, R. S. & Daneman, R. The blood-brain barrier in health and disease: important unanswered questions. *J. Exp. Med.* **217**, e20190062 (2020).
53. Knox, E. G., Aburto, M. R., Clarke, G., Cryan, J. F. & O'Driscoll, C. M. The blood-brain barrier in aging and neurodegeneration. *Mol. Psychiatry* **27**, 2659–2673 (2022).
54. Segarra, M., Aburto, M. R. & Acker-Palmer, A. Blood-brain barrier dynamics to maintain brain homeostasis. *Trends Neurosci.* **44**, 393–405 (2021).
55. Chow, B. W. & Gu, C. Gradual suppression of transcytosis governs functional blood-retinal barrier formation. *Neuron* **93**, 1325–1333.e3 (2017).
56. O'Brown, N. M., Megason, S. G. & Gu, C. Suppression of transcytosis regulates zebrafish blood-brain barrier function. *Elife* **8**, e47326 (2019).
57. Ayloo, S. & Gu, C. Transcytosis at the blood-brain barrier. *Curr. Opin. Neurobiol.* **57**, 32–38 (2019).
58. Fischer, H., Gottschlich, R. & Seelig, A. Blood-brain barrier permeation: molecular parameters governing passive diffusion. *J. Membr. Biol.* **165**, 201–211 (1998).
59. Andreone, B. J. et al. Blood-brain barrier permeability is regulated by lipid transport-dependent suppression of caveolae-mediated transcytosis. *Neuron* **94**, 581–594.e5 (2017).
60. Ben-Zvi, A. et al. Mfsd2a is critical for the formation and function of the blood-brain barrier. *Nature* **509**, 507–511 (2014).
61. Wilhelm, I., Nyúl-Tóth, Á., Suciu, M., Hermenean, A. & Krizbai, I. A. Heterogeneity of the blood-brain barrier. *Tissue Barriers* **4**, e1143544 (2016).
62. Banks, W. A. The blood-brain barrier as an endocrine tissue. *Nat. Rev. Endocrinol.* **15**, 444–455 (2019).
63. Dunton, A. D., Göpel, T., Ho, D. H. & Burggren, W. Form and function of the vertebrate and invertebrate blood-brain barriers. *Int. J. Mol. Sci.* **22**, 12111 (2021).
64. O'Brown, N. M., Pfau, S. J. & Gu, C. Bridging barriers: a comparative look at the blood-brain barrier across organisms. *Genes. Dev.* **32**, 466–478 (2018).
65. Lun, M. P., Monuki, E. S. & Lehtinen, M. K. Development and functions of the choroid plexus-cerebrospinal fluid system. *Nat. Rev. Neurosci.* **16**, 445–457 (2015).
66. Fame, R. M. & Lehtinen, M. K. Emergence and developmental roles of the cerebrospinal fluid system. *Dev. Cell* **52**, 261–275 (2020).
67. Lehtinen, M. K. et al. The cerebrospinal fluid provides a proliferative niche for neural progenitor cells. *Neuron* **69**, 893–905 (2011).
68. Johansson, P. A. The choroid plexuses and their impact on developmental neurogenesis. *Front. Neurosci.* **8**, 340 (2014).
69. Steinemann, A., Galm, I., Chip, S., Nitsch, C. & Maly, I. P. Claudin-1, -2 and -3 are selectively expressed in the epithelia of the choroid plexus of the mouse from early development and into adulthood while claudin-5 is restricted to endothelial cells. *Front. Neuroanat.* **10**, 16 (2016).
70. Wolburg, H., Wolburg-Buchholz, K., Liebner, S. & Engelhardt, B. Claudin-1, claudin-2 and claudin-11 are present in tight junctions of choroid plexus epithelium of the mouse. *Neurosci. Lett.* **307**, 77–80 (2001).
71. Bill, B. R. & Korzh, V. Choroid plexus in developmental and evolutionary perspective. *Front. Neurosci.* **8**, 363 (2014).
72. Dani, N. et al. A cellular and spatial map of the choroid plexus across brain ventricles and ages. *Cell* **184**, 3056–3074.e21 (2021).
73. Mortazavi, M. M. et al. The choroid plexus: a comprehensive review of its history, anatomy, function, histology, embryology, and surgical considerations. *Childs Nerv. Syst.* **30**, 205–214 (2014).
74. Spor, A., Koren, O. & Ley, R. Unravelling the effects of the environment and host genotype on the gut microbiome. *Nat. Rev. Microbiol.* **9**, 279–290 (2011).
75. Tosoni, G., Conti, M. & Diaz Heijtz, R. Bacterial peptidoglycans as novel signaling molecules from microbiota to brain. *Curr. Opin. Pharmacol.* **48**, 107–113 (2019).
76. Jiang, W. & Banks, W. A. Viewpoint: is lipopolysaccharide a hormone or a vitamin? *Brain Behav. Immun.* **114**, 1–2 (2023).
77. Haas-Neill, S. & Forsythe, P. A budding relationship: bacterial extracellular vesicles in the microbiota-gut-brain axis. *Int. J. Mol. Sci.* **21**, 8899 (2020).
78. Krautkramer, K. A., Fan, J. & Bäckhed, F. Gut microbial metabolites as multi-kingdom intermediates. *Nat. Rev. Microbiol.* **19**, 77–94 (2020).
79. Chen, L. et al. Influence of the microbiome, diet and genetics on inter-individual variation in the human plasma metabolome. *Nat. Med.* **28**, 2333–2343 (2022).
80. Caspani, G. & Swann, J. Small talk: microbial metabolites involved in the signaling from microbiota to brain. *Curr. Opin. Pharmacol.* **48**, 99–106 (2019).
81. Dalile, B., Van Oudenhove, L., Vervliet, B. & Verbeke, K. The role of short-chain fatty acids in microbiota-gut-brain communication. *Nat. Rev. Gastroenterol. Hepatol.* **16**, 461–478 (2019).
82. O'Riordan, K. J. et al. Short chain fatty acids: microbial metabolites for gut-brain axis signalling. *Mol. Cell. Endocrinol.* **546**, 111572 (2022).
83. den Besten, G. et al. Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *Am. J. Physiol. Gastrointest. Liver Physiol.* **305**, G900–G910 (2013).
84. Roediger, W. E. Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology* **83**, 424–429 (1982).
85. Demigné, C., Yacoub, C. & Rémésy, C. Effects of absorption of large amounts of volatile fatty acids on rat liver metabolism. *J. Nutr.* **116**, 77–86 (1986).
86. Mitchell, R. W., On, N. H., Del Bigio, M. R., Miller, D. W. & Hatch, G. M. Fatty acid transport protein expression in human brain and potential role in fatty acid transport across human brain microvessel endothelial cells. *J. Neurochem.* **117**, 735–746 (2011).
87. Vijay, N. & Morris, M. E. Role of monocarboxylate transporters in drug delivery to the brain. *Curr. Pharm. Des.* **20**, 1487–1498 (2014).
88. Hoyles, L. et al. Microbiome-host systems interactions: protective effects of propionate upon the blood-brain barrier. *Microbiome* **6**, 55 (2018).
89. Soliman, M. L. & Rosenberger, T. A. Acetate supplementation increases brain histone acetylation and inhibits histone deacetylase activity and expression. *Mol. Cell. Biochem.* **352**, 173–180 (2011).
90. Schroeder, F. A., Lin, C. L., Crusio, W. E. & Akbarian, S. Antidepressant-like effects of the histone deacetylase inhibitor, sodium butyrate, in the mouse. *Biol. Psychiatry* **62**, 55–64 (2007).
91. Fischer, A., Sananbenesi, F., Wang, X., Dobbin, M. & Tsai, L.-H. Recovery of learning and memory is associated with chromatin remodelling. *Nature* **447**, 178–182 (2007).
92. Valvassori, S. S. et al. Sodium butyrate, a histone deacetylase inhibitor, reverses behavioral and mitochondrial alterations in animal models of depression induced by early- or late-life stress. *Curr. Neurovasc. Res.* **12**, 312–320 (2015).

93. Braniste, V. et al. The gut microbiota influences blood-brain barrier permeability in mice. *Sci. Transl. Med.* **6**, 263ra158 (2014).
94. Knox, E. G. et al. Microbial-derived metabolites induce actin cytoskeletal rearrangement and protect blood-brain barrier function. *iScience* **25**, 105648 (2022).
95. Wishart, D. S. et al. Human metabolome database. *Nucleic Acids Res.* **35**, D521–D526 (2007).
96. Xie, J. et al. Gut microbiota regulates blood-cerebrospinal fluid barrier function and Aβ pathology. *EMBO J.* **42**, e111515 (2023).
97. Radjabzadeh, D. et al. Gut microbiome-wide association study of depressive symptoms. *Nat. Commun.* **13**, 7128 (2022).
98. Valles-Colomer, M. et al. The neuroactive potential of the human gut microbiota in quality of life and depression. *Nat. Microbiol.* **4**, 623–632 (2019).
99. Schönfeld, P. & Wojtczak, L. Short- and medium-chain fatty acids in energy metabolism: the cellular perspective. *J. Lipid Res.* **57**, 943–954 (2016).
100. Rose, S. et al. Butyrate enhances mitochondrial function during oxidative stress in cell lines from boys with autism. *Transl. Psychiatry* **8**, 42 (2018).
101. Park, Y. U. et al. Disrupted-in-schizophrenia 1 (DISC1) plays essential roles in mitochondria in collaboration with Mitofilin. *Proc. Natl Acad. Sci. USA* **107**, 17785–177890 (2010).
102. Picard, M. & Sandi, C. The social nature of mitochondria: implications for human health. *Neurosci. Biobehav. Rev.* **120**, 595–610 (2021).
103. Tărlungeanu, D. C. et al. Impaired amino acid transport at the blood brain barrier is a cause of autism spectrum disorder. *Cell* **167**, 1481–1494.e18 (2016).
104. Vuong, H. E. et al. The maternal microbiome modulates fetal neurodevelopment in mice. *Nature* **586**, 281–286 (2020).
105. Hoyles, L. et al. Regulation of blood-brain barrier integrity by microbiome-associated methylamines and cognition by trimethylamine N-oxide. *Microbiome* **9**, 235 (2021).
106. Zhu, W. et al. Gut microbial metabolite TMAO enhances platelet hyperreactivity and thrombosis risk. *Cell* **165**, 111–124 (2016).
107. Stachulski, A. V., Knausenberger, T. B.-A., Shah, S. N., Hoyles, L. & McArthur, S. A host-gut microbial amino acid co-metabolite, *p*-cresol glucuronide, promotes blood-brain barrier integrity in vivo. *Tissue Barriers* **11**, 2073175 (2022).
108. Rothhammer, V. & Quintana, F. J. The aryl hydrocarbon receptor: an environmental sensor integrating immune responses in health and disease. *Nat. Rev. Immunol.* **19**, 184–197 (2019).
109. Wiggins, B. G. et al. Endothelial sensing of AHR ligands regulates intestinal homeostasis. *Nature* **621**, 821–829 (2023).
110. Bobot, M. et al. Uremic toxic blood-brain barrier disruption mediated by AhR activation leads to cognitive impairment during experimental renal dysfunction. *J. Am. Soc. Nephrol.* **31**, 1509–1521 (2020).
111. Filbrandt, C. R., Wu, Z., Zlokovic, B., Opanashuk, L. & Gasiewicz, T. A. Presence and functional activity of the aryl hydrocarbon receptor in isolated murine cerebral vascular endothelial cells and astrocytes. *Neurotoxicology* **25**, 605–616 (2004).
112. Dolwick, K. M., Schmidt, J. V., Carver, L. A., Swanson, H. I. & Bradfield, C. A. Cloning and expression of a human Ah receptor cDNA. *Mol. Pharmacol.* **44**, 911–917 (1993).
113. Scott, S. A., Fu, J. & Chang, P. V. Microbial tryptophan metabolites regulate gut barrier function via the aryl hydrocarbon receptor. *Proc. Natl Acad. Sci. USA* **117**, 19376–19387 (2020).
114. Modoux, M. et al. Butyrate acts through HDAC inhibition to enhance aryl hydrocarbon receptor activation by gut microbiota-derived ligands. *Gut Microbes* **14**, 2105637 (2022).
115. Agus, A., Planchais, J. & Sokol, H. Gut microbiota regulation of tryptophan metabolism in health and disease. *Cell Host Microbe* **23**, 716–724 (2018).
116. Lanis, J. M. et al. Tryptophan metabolite activation of the aryl hydrocarbon receptor regulates IL-10 receptor expression on intestinal epithelia. *Mucosal Immunol.* **10**, 1133–1144 (2017).
117. Yano, J. M. et al. Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* **161**, 264–276 (2015).
118. Collins, S. L., Stine, J. G., Bisanz, J. E., Okafor, C. D. & Patterson, A. D. Bile acids and the gut microbiota: metabolic interactions and impacts on disease. *Nat. Rev. Microbiol.* **21**, 236–247 (2023).
119. Stenman, L. K., Holma, R., Eggert, A. & Korpela, R. A novel mechanism for gut barrier dysfunction by dietary fat: epithelial disruption by hydrophobic bile acids. *Am. J. Physiol. Gastrointest. Liver Physiol.* **304**, G227–G234 (2013).
120. Raimondi, F. et al. Bile acids modulate tight junction structure and barrier function of Caco-2 monolayers via EGFR activation. *Am. J. Physiol. Gastrointest. Liver Physiol.* **294**, G906–G913 (2008).
121. Sarathy, J. et al. The Yin and Yang of bile acid action on tight junctions in a model colonic epithelium. *Physiol. Rep.* **5**, e13294 (2017).
122. Quinn, M. et al. Bile acids permeabilize the blood brain barrier after bile duct ligation in rats via Rac1-dependent mechanisms. *Dig. Liver Dis.* **46**, 527–534 (2014).
123. Miller-Fleming, L., Olin-Sandoval, V., Campbell, K. & Ralser, M. Remaining mysteries of molecular biology: the role of polyamines in the cell. *J. Mol. Biol.* **427**, 3389–3406 (2015).
124. Kurihara, S. Polyamine metabolism and transport in gut microbes. *Biosci. Biotechnol. Biochem.* **86**, 957–966 (2022).
125. Schroeder, S. et al. Dietary spermidine improves cognitive function. *Cell Rep.* **35**, 108985 (2021).
126. Gonzalez-Santana, A. & Diaz Heijtz, R. Bacterial peptidoglycans from microbiota in neurodevelopment and behavior. *Trends Mol. Med.* **26**, 729–743 (2020).
127. Luo, Z. et al. Variation in blood microbial lipopolysaccharide (LPS) contributes to immune reconstitution in response to suppressive antiretroviral therapy in HIV. *EBioMedicine* **80**, 104037 (2022).
128. Mohr, A. E., Crawford, M., Jasbi, P., Fessler, S. & Sweazea, K. L. Lipopolysaccharide and the gut microbiota: considering structural variation. *FEBS Lett.* **596**, 849–875 (2022).
129. Xie, J., Haesebrouck, F., Van Hoecke, L. & Vandenbroucke, R. E. Bacterial extracellular vesicles: an emerging avenue to tackle diseases. *Trends Microbiol.* **31**, 1206–1224 (2023).
130. Liang, X. et al. Gut bacterial extracellular vesicles: important players in regulating intestinal microenvironment. *Gut Microbes* **14**, 2134689 (2022).
131. Dallmann, R., Viola, A. U., Tarokh, L., Cajochen, C. & Brown, S. A. The human circadian metabolome. *Proc. Natl Acad. Sci. USA* **109**, 2625–2629 (2012).
132. Kabouridis, P. S. et al. Microbiota controls the homeostasis of glial cells in the gut lamina propria. *Neuron* **85**, 289–295 (2015).
133. Rothhammer, V. et al. Type I interferons and microbial metabolites of tryptophan modulate astrocyte activity and central nervous system inflammation via the aryl hydrocarbon receptor. *Nat. Med.* **22**, 586–597 (2016).
134. Spichak, S. et al. Microbially-derived short-chain fatty acids impact astrocyte gene expression in a sex-specific manner. *Brain Behav. Immun. Health* **16**, 100318 (2021).
135. Rothhammer, V. et al. Microglial control of astrocytes in response to microbial metabolites. *Nature* **557**, 724–728 (2018).
136. Castillo-Ruiz, A. et al. Brain effects of gestating germ-free persist in mouse neonates despite acquisition of a microbiota at birth. *Front. Neurosci.* **17**, 1130347 (2023).
137. Erny, D. et al. Host microbiota constantly control maturation and function of microglia in the CNS. *Nat. Neurosci.* **18**, 965–977 (2015).
138. Knox, E. G. et al. The gut microbiota is important for the maintenance of blood-cerebrospinal fluid barrier integrity. *Eur. J. Neurosci.* **57**, 233–241 (2022).
139. Leclercq, S. et al. Low-dose penicillin in early life induces long-term changes in murine gut microbiota, brain cytokines and behavior. *Nat. Commun.* **8**, 15062 (2017).
140. Morel, C., Martinez Sanchez, I., Cherifi, Y., Chartrel, N. & Diaz Heijtz, R. Perturbation of maternal gut microbiota in mice during a critical perinatal window influences early neurobehavioral outcomes in offspring. *Neuropharmacology* **229**, 109479 (2023).
141. Huang, Y. et al. Indoxyl sulfate induces intestinal barrier injury through IRF1-DRP1 axis-mediated mitophagy impairment. *Theranostics* **10**, 7384–7400 (2020).
142. Schneider, K. M. et al. The enteric nervous system relays psychological stress to intestinal inflammation. *Cell* **186**, 2823–2838.e20 (2023).
143. Walker, A. K., Wing, E. E., Banks, W. A. & Dantzer, R. Leucine competes with kynurenine for blood-to-brain transport and prevents lipopolysaccharide-induced depression-like behavior in mice. *Mol. Psychiatry* **24**, 1523–1532 (2019).
144. Turpin, W. et al. Increased intestinal permeability is associated with later development of Crohn's disease. *Gastroenterology* **159**, 2092–2100.e5 (2020).
145. Torres, J. et al. Serum biomarkers identify patients who will develop inflammatory bowel diseases up to 5 years before diagnosis. *Gastroenterology* **159**, 96–104 (2020).
146. Mehandru, S. & Colombel, J.-F. The intestinal barrier, an arbitrator turned provocateur in IBD. *Nat. Rev. Gastroenterol. Hepatol.* **18**, 83–84 (2021).
147. Zeissig, S. et al. Changes in expression and distribution of claudin 2, 5 and 8 lead to discontinuous tight junctions and barrier dysfunction in active Crohn's disease. *Gut* **56**, 61–72 (2007).
148. Madsen, K. L. et al. Interleukin-10 gene-deficient mice develop a primary intestinal permeability defect in response to enteric microflora. *Inflamm. Bowel Dis.* **5**, 262–270 (1999).
149. Melgar, S. et al. Validation of murine dextran sulfate sodium-induced colitis using four therapeutic agents for human inflammatory bowel disease. *Int. Immunopharmacol.* **8**, 836–844 (2008).
150. Li, J. et al. Changes in the phosphorylation of claudins during the course of experimental colitis. *Int. J. Clin. Exp. Pathol.* **8**, 12225–12233 (2015).
151. Paone, P. & Cani, P. D. Mucus barrier, mucins and gut microbiota: the expected slimy partners? *Gut* **69**, 2232–2243 (2020).
152. Tuganbaev, T. et al. Diet diurnally regulates small intestinal microbiome-epithelial-immune homeostasis and enteritis. *Cell* **182**, 1441–1459.e21 (2020).
153. Duerksen, D. R., Wilhelm-Boyles, C. & Parry, D. M. Intestinal permeability in long-term follow-up of patients with celiac disease on a gluten-free diet. *Dig. Dis. Sci.* **50**, 785–790 (2005).
154. Leonard, M. M. et al. Microbiome signatures of progression toward celiac disease onset in at-risk children in a longitudinal prospective cohort study. *Proc. Natl Acad. Sci. USA* **118**, e2020322118 (2021).
155. Girdhar, K. et al. Dynamics of the gut microbiome, IgA response, and plasma metabolome in the development of pediatric celiac disease. *Microbiome* **11**, 9 (2023).
156. Ford, A. C., Sperber, A. D., Corsetti, M. & Camilleri, M. Irritable bowel syndrome. *Lancet* **396**, 1675–1688 (2020).
157. Black, C. J. & Ford, A. C. Global burden of irritable bowel syndrome: trends, predictions and risk factors. *Nat. Rev. Gastroenterol. Hepatol.* **17**, 473–486 (2020).
158. Stasi, C. et al. The complex interplay between gastrointestinal and psychiatric symptoms in irritable bowel syndrome: a longitudinal assessment. *J. Gastroenterol. Hepatol.* **34**, 713–719 (2019).
159. Hanning, N. et al. Intestinal barrier dysfunction in irritable bowel syndrome: a systematic review. *Ther. Adv. Gastroenterol.* **14**, 17562848211993586 (2021).
160. Martínez, C. et al. Diarrhoea-predominant irritable bowel syndrome: an organic disorder with structural abnormalities in the jejunal epithelial barrier. *Gut* **62**, 1160–1168 (2013).

161. Piche, T. et al. Impaired intestinal barrier integrity in the colon of patients with irritable bowel syndrome: involvement of soluble mediators. *Gut* **58**, 196–201 (2009).
162. Bertiaux-Vandaële, N. et al. The expression and the cellular distribution of the tight junction proteins are altered in irritable bowel syndrome patients with differences according to the disease subtype. *Am. J. Gastroenterol.* **106**, 2165–2173 (2011).
163. Montagne, A. et al. Blood-brain barrier breakdown in the aging human hippocampus. *Neuron* **85**, 296–302 (2015).
164. Fiorentino, M. et al. Blood-brain barrier and intestinal epithelial barrier alterations in autism spectrum disorders. *Mol. Autism* **7**, 49 (2016).
165. Esnafoglu, E. et al. Increased serum zonulin levels as an intestinal permeability marker in autistic subjects. *J. Pediatr.* **188**, 240–244 (2017).
166. Hsiao, E. Y. et al. Microbiota modulate behavioral and physiological abnormalities associated with neurodevelopmental disorders. *Cell* **155**, 1451–1463 (2013).
167. Cui, J. et al. Inflammation of the embryonic choroid plexus barrier following maternal immune activation. *Dev. Cell* **75**, 617–628.e6 (2020).
168. Kumar, H. & Sharma, B. Minocycline ameliorates prenatal valproic acid induced autistic behaviour, biochemistry and blood brain barrier impairments in rats. *Brain Res.* **1630**, 83–97 (2016).
169. Sgritta, M. et al. Mechanisms underlying microbial-mediated changes in social behavior in mouse models of autism spectrum disorder. *Neuron* **101**, 246–259.e6 (2019).
170. Wei, S.-C. et al. SHANK3 regulates intestinal barrier function through modulating ZO-1 expression through the PKCε-dependent pathway. *Inflamm. Bowel Dis.* **23**, 1730–1740 (2017).
171. Tartaglione, A. M. et al. Maternal immune activation induces autism-like changes in behavior, neuroinflammatory profile and gut microbiota in mouse offspring of both sexes. *Transl. Psychiatry* **12**, 384 (2022).
172. Zuffa, S. et al. Early-life differences in the gut microbiota composition and functionality of infants at elevated likelihood of developing autism spectrum disorder. *Transl. Psychiatry* **13**, 257 (2023).
173. Sharon, G. et al. Human gut microbiota from autism spectrum disorder promote behavioral symptoms in mice. *Cell* **177**, 1600–1618.e17 (2019).
174. Xu, R. et al. Altered gut microbiota and mucosal immunity in patients with schizophrenia. *Brain Behav. Immun.* **85**, 120–127 (2020).
175. Peça, J. et al. Shank3 mutant mice display autistic-like behaviours and striatal dysfunction. *Nature* **472**, 437–442 (2011).
176. Liu, C.-X. et al. CRISPR/Cas9-induced *shank3b* mutant zebrafish display autism-like behaviors. *Mol. Autism* **9**, 23 (2018).
177. James, D. M. et al. Intestinal dysmotility in a zebrafish (*Danio rerio*) *shank3a;shank3b* mutant model of autism. *Mol. Autism* **10**, 3 (2019).
178. Tabouy, L. et al. Dysbiosis of microbiome and probiotic treatment in a genetic model of autism spectrum disorders. *Brain Behav. Immun.* **73**, 310–319 (2018).
179. Osman, A. et al. Acetate supplementation rescues social deficits and alters transcriptional regulation in prefrontal cortex of Shank3 deficient mice. *Brain Behav. Immun.* **114**, 311–324 (2023).
180. Nagpal, J. & Cryan, J. F. Host genetics, the microbiome & behaviour – a 'Holobiont' perspective. *Cell Res* **31**, 832–833 (2021).
181. Canetta, S. E. & Brown, A. S. Prenatal infection, maternal immune activation, and risk for schizophrenia. *Transl. Neurosci.* **3**, 320–327 (2012).
182. Brown, A. S. et al. Prenatal rubella, premorbid abnormalities, and adult schizophrenia. *Biol. Psychiatry* **49**, 473–486 (2001).
183. Nudel, R. et al. Maternal pregnancy-related infections and autism spectrum disorder – the genetic perspective. *Transl. Psychiatry* **12**, 334 (2022).
184. Choi, G. B. et al. The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. *Science* **351**, 933–939 (2016).
185. Menard, C. et al. Social stress induces neurovascular pathology promoting depression. *Nat. Neurosci.* **20**, 1752–1760 (2017).
186. Dudek, K. A. et al. Molecular adaptations of the blood-brain barrier promote stress resilience vs. depression. *Proc. Natl Acad. Sci. USA* **117**, 3326–3336 (2020).
187. Dion-Albert, L. et al. Vascular and blood-brain barrier-related changes underlie stress responses and resilience in female mice and depression in human tissue. *Nat. Commun.* **13**, 164 (2022).
188. Greene, C., Hanley, N. & Campbell, M. Blood-brain barrier associated tight junction disruption is a hallmark feature of major psychiatric disorders. *Transl. Psychiatry* **10**, 373 (2020).
189. McEwen, B. S. Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. *Ann. N. Y. Acad. Sci.* **1032**, 1–7 (2004).
190. Ochi, S. & Dwivedi, Y. Dissecting early life stress-induced adolescent depression through epigenomic approach. *Mol. Psychiatry* **28**, 141–153 (2023).
191. Leigh, S.-J. et al. The impact of acute and chronic stress on gastrointestinal physiology and function: a microbiota-gut-brain axis perspective. *J. Physiol.* **601**, 4491–4538 (2023).
192. Bastiaanssen, T. F. S. et al. Volatility as a concept to understand the impact of stress on the microbiome. *Psychoneuroendocrinology* **124**, 105047 (2021).
193. Morton, J. T. et al. Multi-level analysis of the gut-brain axis shows autism spectrum disorder-associated molecular and microbial profiles. *Nat. Neurosci.* **26**, 1208–1217 (2023).
194. Braak, H., Rüb, U., Gai, W. P. & Del Tredici, K. Idiopathic Parkinson's disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. *J. Neural Transm.* **110**, 517–536 (2003).
195. Kim, S. et al. Transneuronal propagation of pathologic α-synuclein from the gut to the brain models Parkinson's disease. *Neuron* **103**, 627–641.e7 (2019).
196. Horsager, J. et al. Brain-first versus body-first Parkinson's disease: a multimodal imaging case-control study. *Brain* **143**, 3077–3088 (2020).
197. Wallen, Z. D. et al. Metagenomics of Parkinson's disease implicates the gut microbiome in multiple disease mechanisms. *Nat. Commun.* **13**, 6958 (2022).
198. Tan, A. H., Lim, S. Y. & Lang, A. E. The microbiome-gut-brain axis in Parkinson disease – from basic research to the clinic. *Nat. Rev. Neurol.* **18**, 476–495 (2022).
199. Clairembault, T. et al. Structural alterations of the intestinal epithelial barrier in Parkinson's disease. *Acta Neuropathol. Commun.* **3**, 12 (2015).
200. Lord, C. et al. Autism spectrum disorder. *Nat. Rev. Dis. Prim.* **6**, 5 (2020).
201. Madra, M., Ringel, R. & Margolis, K. G. Gastrointestinal issues and autism spectrum disorder. *Child. Adolesc. Psychiatr. Clin. N. Am.* **29**, 501–513 (2020).
202. Buffington, S. A. et al. Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. *Cell* **165**, 1762–1775 (2016).
203. Buffington, S. A. et al. Dissecting the contribution of host genetics and the microbiome in complex behaviors. *Cell* **184**, 1740–1756.e16 (2021).
204. Kim, E. et al. Maternal gut bacteria drive intestinal inflammation in offspring with neurodevelopmental disorders by altering the chromatin landscape of CD4$^+$ T cells. *Immunity* **55**, 145–158.e7 (2021).
205. Kim, S. et al. Maternal gut bacteria promote neurodevelopmental abnormalities in mouse offspring. *Nature* **549**, 528–532 (2017).
206. Xie, R. et al. Maternal high fat diet alters gut microbiota of offspring and exacerbates DSS-induced colitis in adulthood. *Front. Immunol.* **9**, 2608 (2018).
207. Haddad-Tóvolli, R. et al. Maternal obesity damages the median eminence blood-brain barrier structure and function in the progeny: the beneficial impact of cross-fostering by lean mothers. *Am. J. Physiol. Endocrinol. Metab.* **324**, E154–E166 (2023).
208. Agorastos, A. & Chrousos, G. P. The neuroendocrinology of stress: the stress-related continuum of chronic disease development. *Mol. Psychiatry* **27**, 502–513 (2022).
209. Bisgaard, T. H., Allin, K. H., Keefer, L., Ananthakrishnan, A. N. & Jess, T. Depression and anxiety in inflammatory bowel disease: epidemiology, mechanisms and treatment. *Nat. Rev. Gastroenterol. Hepatol.* **19**, 717–726 (2022).
210. Schirmer, M., Garner, A., Vlamakis, H. & Xavier, R. J. Microbial genes and pathways in inflammatory bowel disease. *Nat. Rev. Microbiol.* **17**, 497–511 (2019).
211. Bosch, J. A. et al. The gut microbiota and depressive symptoms across ethnic groups. *Nat. Commun.* **13**, 7129 (2022).
212. Doney, E. et al. Chronic stress exposure alters the gut barrier: sex-specific effects on microbiota and jejunum tight junctions. *Biol. Psychiatry Glob. Open Sci.* https://doi.org/10.1016/j.bpsgos.2023.04.007 (2023).
213. Furman, D. et al. Chronic inflammation in the etiology of disease across the life span. *Nat. Med.* **25**, 1822–1832 (2019).
214. Kriegstein, A. R., Subramanian, L., Obernier, K. & Alvarez-Buylla, A. in *Patterning and Cell Type Specification in the Developing CNS and PNS* 2nd edn Ch. 31 (eds Rubenstein, J., Rakic, P., Chen, B. & Kwan, K. Y.). 775–806 (Academic Press, 2020).
215. Shimada, Y. et al. Commensal bacteria-dependent indole production enhances epithelial barrier function in the colon. *PLoS ONE* **8**, e80604 (2013).
216. Nielsen, D. S. G. et al. Effect of butyrate and fermentation products on epithelial integrity in a mucus-secreting human colon cell line. *J. Funct. Foods* **40**, 9–17 (2018).
217. Peng, L., He, Z., Chen, W., Holzman, I. R. & Lin, J. Effects of butyrate on intestinal barrier function in a Caco-2 cell monolayer model of intestinal barrier. *Pediatr. Res.* **61**, 37–41 (2007).
218. Peng, L., Li, Z.-R., Green, R. S., Holzman, I. R. & Lin, J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J. Nutr.* **139**, 1619–1625 (2009).
219. Feng, Y., Wang, Y., Wang, P., Huang, Y. & Wang, F. Short-chain fatty acids manifest stimulative and protective effects on intestinal barrier function through the inhibition of NLRP3 inflammasome and autophagy. *Cell. Physiol. Biochem.* **49**, 190–205 (2018).
220. Wang, H.-B., Wang, P.-Y., Wang, X., Wan, Y.-L. & Liu, Y.-C. Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. *Dig. Dis. Sci.* **57**, 3126–3135 (2012).
221. Paparo, L. et al. Butyrate as a bioactive human milk protective component against food allergy. *Allergy* **76**, 1398–1415 (2021).
222. Diao, H., Jiao, A. R., Yu, B., Mao, X. B. & Chen, D. W. Gastric infusion of short-chain fatty acids can improve intestinal barrier function in weaned piglets. *Genes. Nutr.* **14**, 4 (2019).
223. Li, J. et al. Indole-3-propionic acid improved the intestinal barrier by enhancing epithelial barrier and mucus barrier. *J. Agric. Food Chem.* **69**, 1487–1495 (2021).
224. Venkatesh, M. et al. Symbiotic bacterial metabolites regulate gastrointestinal barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. *Immunity* **41**, 296–310 (2014).
225. Hu, J. et al. Gut microbiota-derived 3-phenylpropionic acid promotes intestinal epithelial barrier function via AhR signaling. *Microbiome* **11**, 102 (2023).
226. Singh, R. et al. Enhancement of the gut barrier integrity by a microbial metabolite through the Nrf2 pathway. *Nat. Commun.* **10**, 89 (2019).
227. Guo, X. et al. Polyamines are necessary for synthesis and stability of occludin protein in intestinal epithelial cells. *Am. J. Physiol. Gastrointest. Liver Physiol.* **288**, G1159–G1169 (2005).
228. Guo, X. et al. Regulation of adherens junctions and epithelial paracellular permeability: a novel function for polyamines. *Am. J. Physiol. Cell Physiol.* **285**, C1174–C1187 (2003).

229. Chen, S.-Y. et al. A novel trimethylamine oxide-induced model implicates gut microbiota-related mechanisms in frailty. *Front. Cell. Infect. Microbiol.* **12**, 803082 (2022).
230. Travier, L. et al. Neonatal susceptibility to meningitis results from the immaturity of epithelial barriers and gut microbiota. *Cell Rep.* **35**, 109319 (2021).
231. Ishida, I. et al. Gut permeability and its clinical relevance in schizophrenia. *Neuropsychopharmacol. Rep.* **42**, 70–76 (2022).
232. Gokulakrishnan, K. et al. Altered intestinal permeability biomarkers in schizophrenia: a possible link with subclinical inflammation. *Ann. Neurosci.* **29**, 151–158 (2022).
233. Greene, C. et al. Dose-dependent expression of claudin-5 is a modifying factor in schizophrenia. *Mol. Psychiatry* **23**, 2156–2166 (2018).
234. Crockett, A. M. et al. Disruption of the blood-brain barrier in 22q11.2 deletion syndrome. *Brain* **144**, 1351–1360 (2021).
235. Lizano, P. et al. Association of choroid plexus enlargement with cognitive, inflammatory, and structural phenotypes across the psychosis spectrum. *Am. J. Psychiatry* **176**, 564–572 (2019).
236. Kim, S., Hwang, Y., Lee, D. & Webster, M. J. Transcriptome sequencing of the choroid plexus in schizophrenia. *Transl. Psychiatry* **6**, e964 (2016).
237. Aydoğan Avşar, P. et al. Serum zonulin and claudin-5 levels in children with attention-deficit/hyperactivity disorder. *Int. J. Psychiatry Clin. Pract.* **25**, 49–55 (2021).
238. Greene, C. et al. Microvascular stabilization via blood-brain barrier regulation prevents seizure activity. *Nat. Commun.* **13**, 2003 (2022).
239. Kılıç, F., Işık, Ü., Demirdaş, A., Doğuç, D. K. & Bozkurt, M. Serum zonulin and claudin-5 levels in patients with bipolar disorder. *J. Affect. Disord.* **266**, 37–42 (2020).
240. Orlovska-Waast, S. et al. Cerebrospinal fluid markers of inflammation and infections in schizophrenia and affective disorders: a systematic review and meta-analysis. *Mol. Psychiatry* **24**, 869–887 (2019).
241. Wu, X. et al. Childhood social isolation causes anxiety-like behaviors via the damage of blood-brain barrier in amygdala in female mice. *Front. Cell Dev. Biol.* **10**, 943067 (2022).
242. Montagne, A. et al. APOE4 leads to blood-brain barrier dysfunction predicting cognitive decline. *Nature* **581**, 71–76 (2020).
243. Stopa, E. G. et al. Comparative transcriptomics of choroid plexus in Alzheimer's disease, frontotemporal dementia and Huntington's disease: implications for CSF homeostasis. *Fluids Barriers CNS* **15**, 18 (2018).
244. Ryu, J. K. & McLarnon, J. G. A leaky blood-brain barrier, fibrinogen infiltration and microglial reactivity in inflamed Alzheimer's disease brain. *J. Cell. Mol. Med.* **13**, 2911–2925 (2009).
245. Sengillo, J. D. et al. Deficiency in mural vascular cells coincides with blood-brain barrier disruption in Alzheimer's disease. *Brain Pathol.* **23**, 303–310 (2013).
246. Brkic, M. et al. Amyloid β oligomers disrupt blood–CSF barrier integrity by activating matrix metalloproteinases. *J. Neurosci.* **35**, 12766–12778 (2015).
247. Perez-Pardo, P. et al. Role of TLR4 in the gut-brain axis in Parkinson's disease: a translational study from men to mice. *Gut* **68**, 829–843 (2019).
248. Forsyth, C. B. et al. Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. *PLoS ONE* **6**, e28032 (2011).
249. Al-Bachari, S., Naish, J. H., Parker, G. J. M., Emsley, H. C. A. & Parkes, L. M. Blood-brain barrier leakage is increased in Parkinson's disease. *Front. Physiol.* **11**, 593026 (2020).
250. Pellegrini, C. et al. Enteric α-synuclein impairs intestinal epithelial barrier through caspase-1-inflammasome signaling in Parkinson's disease before brain pathology. *NPJ Parkinsons Dis.* **8**, 9 (2022).
251. Lan, G. et al. Astrocytic VEGFA: an essential mediator in blood-brain-barrier disruption in Parkinson's disease. *Glia* **70**, 337–353 (2022).
252. Lim, R. G. et al. Huntington's disease iPSC-derived brain microvascular endothelial cells reveal WNT-mediated angiogenic and blood-brain barrier deficits. *Cell Rep.* **19**, 1365–1377 (2017).
253. Linville, R. M. et al. Brain microvascular endothelial cell dysfunction in an isogenic juvenile iPSC model of Huntington's disease. *Fluids Barriers CNS* **19**, 54 (2022).
254. Drouin-Ouellet, J. et al. Cerebrovascular and blood-brain barrier impairments in Huntington's disease: potential implications for its pathophysiology. *Ann. Neurol.* **78**, 160–177 (2015).
255. Stan, T. L. et al. Increased intestinal permeability and gut dysbiosis in the R6/2 mouse model of Huntington's disease. *Sci. Rep.* **10**, 18270 (2020).
256. Quiñonez-Silvero, C., Hübner, K. & Herzog, W. Development of the brain vasculature and the blood-brain barrier in zebrafish. *Dev. Biol.* **457**, 181–190 (2020).
257. Chen, J., Poskanzer, K. E., Freeman, M. R. & Monk, K. R. Live-imaging of astrocyte morphogenesis and function in zebrafish neural circuits. *Nat. Neurosci.* **23**, 1297–1306 (2020).
258. Li, Y. et al. Claudin-5a is essential for the functional formation of both zebrafish blood-brain barrier and blood-cerebrospinal fluid barrier. *Fluids Barriers CNS* **19**, 40 (2022).
259. Parab, S., Quick, R. E. & Matsuoka, R. L. Endothelial cell-type-specific molecular requirements for angiogenesis drive fenestrated vessel development in the brain. *Elife* **10**, e64295 (2021).
260. Balmaceda-Aguilera, C. et al. Glucose transporter 1 and monocarboxylate transporters 1, 2, and 4 localization within the glial cells of shark blood-brain-barriers. *PLoS ONE* **7**, e32409 (2012).
261. Liu, X., Hodgson, J. J. & Buchon, N. *Drosophila* as a model for homeostatic, antibacterial, and antiviral mechanisms in the gut. *PLoS Pathog.* **13**, e1006277 (2017).
262. Flores, E. M., Nguyen, A. T., Odem, M. A., Eisenhoffer, G. T. & Krachler, A. M. The zebrafish as a model for gastrointestinal tract-microbe interactions. *Cell. Microbiol.* **22**, e13152 (2020).
263. Asano, A., Asano, K., Sasaki, H., Furuse, M. & Tsukita, S. Claudins in *Caenorhabditis elegans*: their distribution and barrier function in the epithelium. *Curr. Biol.* **13**, 1042–1046 (2003).
264. Günzel, D. & Yu, A. S. L. Claudins and the modulation of tight junction permeability. *Physiol. Rev.* **93**, 525–569 (2013).
265. Stamatovic, S. M., Johnson, A. M., Keep, R. F. & Andjelkovic, A. V. Junctional proteins of the blood-brain barrier: new insights into function and dysfunction. *Tissue Barriers* **4**, e1154641 (2016).
266. Saunders, N. R., Dziegielewska, K. M., Møllgård, K. & Habgood, M. D. Physiology and molecular biology of barrier mechanisms in the fetal and neonatal brain. *J. Physiol.* **596**, 5723–5756 (2018).
267. Kimura, I. et al. Maternal gut microbiota in pregnancy influences offspring metabolic phenotype in mice. *Science* **367**, eaaw8429 (2020).
268. Kennedy, K. M. et al. Questioning the fetal microbiome illustrates pitfalls of low-biomass microbial studies. *Nature* **613**, 639–649 (2023).
269. Maltepe, E., Bakardjiev, A. I. & Fisher, S. J. The placenta: transcriptional, epigenetic, and physiological integration during development. *J. Clin. Invest.* **120**, 1016–1025 (2010).
270. Miranda, J. et al. Syncytiotrophoblast of placentae from women with zika virus infection has altered tight junction protein expression and increased paracellular permeability. *Cells* **8**, 1174 (2019).
271. Johansson, P. A. et al. The transcription factor Otx2 regulates choroid plexus development and function. *Development* **140**, 1055–1066 (2013).
272. Vukavic, T. Timing of the gut closure. *J. Pediatr. Gastroenterol. Res.* **3**, 700–703 (1984).
273. Round, J. L. & Mazmanian, S. K. The gut microbiota shapes intestinal immune responses during health and disease. *Nat. Rev. Immunol.* **9**, 313–323 (2009).
274. Abrams, G. D., Bauer, H. & Sprinz, H. Influence of the normal flora on mucosal morphology and cellular renewal in the ileum. A comparison of germ-free and conventional mice. *Lab. Invest.* **12**, 355–364 (1963).
275. Stappenbeck, T. S., Hooper, L. V. & Gordon, J. I. Developmental regulation of intestinal angiogenesis by indigenous microbes via Paneth cells. *Proc. Natl Acad. Sci. USA* **99**, 15451–15455 (2002).
276. Reinhardt, C. et al. Tissue factor and PAR1 promote microbiota-induced intestinal vascular remodelling. *Nature* **483**, 627–631 (2012).
277. Willms, R. J., Jones, L. O., Hocking, J. C. & Foley, E. A cell atlas of microbe-responsive processes in the zebrafish intestine. *Cell Rep.* **38**, 110311 (2022).
278. Coelho-Santos, V. & Shih, A. Y. Postnatal development of cerebrovascular structure and the neurogliovascular unit. *Wiley Interdiscip. Rev. Dev. Biol.* **9**, e363 (2020).
279. Gömöri, E. et al. Fetal development of membrane water channel proteins aquaporin-1 and aquaporin-4 in the human brain. *Int. J. Dev. Neurosci.* **24**, 295–305 (2006).
280. Gleeson, J. P. et al. The enhanced intestinal permeability of infant mice enables oral protein and macromolecular absorption without delivery technology. *Int. J. Pharm.* **593**, 120120 (2021).
281. Gulbransen, B. D. & Sharkey, K. A. Novel functional roles for enteric glia in the gastrointestinal tract. *Nat. Rev. Gastroenterol. Hepatol.* **9**, 625–632 (2012).
282. Yang, A. C. et al. Physiological blood-brain transport is impaired with age by a shift in transcytosis. *Nature* **583**, 425–430 (2020).
283. Banks, W. A., Reed, M. J., Logsdon, A. F., Rhea, E. M. & Erickson, M. A. Healthy aging and the blood-brain barrier. *Nat. Aging* **1**, 243–254 (2021).
284. Scarpetta, V. et al. Morphological and mitochondrial changes in murine choroid plexus epithelial cells during healthy aging. *Fluids Barriers CNS* **20**, 19 (2023).
285. Kelly, J. R. et al. Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. *Front. Cell. Neurosci.* **9**, 392 (2015).
286. Man, A. L. et al. Age-associated modifications of intestinal permeability and innate immunity in human small intestine. *Clin. Sci.* **129**, 515–527 (2015).
287. Thevaranjan, N. et al. Age-associated microbial dysbiosis promotes intestinal permeability, systemic inflammation, and macrophage dysfunction. *Cell Host Microbe* **21**, 455–466.e4 (2017).
288. Clark, R. I. et al. Distinct shifts in microbiota composition during *Drosophila* aging impair intestinal function and drive mortality. *Cell Rep.* **12**, 1656–1667 (2015).
289. Di Marco Barros, R., Fitzpatrick, Z. & Clatworthy, M. R. The gut–meningeal immune axis: priming brain defense against the most likely invaders. *J. Exp. Med.* **219**, e20211520 (2022).

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Author contributions

Both authors researched data for the article, contributed substantially to discussion of the content and reviewed and/or edited the manuscript before submission. M.R.A. wrote the article.

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