Review

The blood-brain barrier in health, neurological diseases, and COVID-19

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A R T I C L E   I N F O

A B S T R A C T

The blood-brain barrier (BBB) is a protective interface between the central nervous system (CNS) and the circulating blood, and is critical in controlling the movement of ions, molecules and cells to maintain CNS homeostasis. The disruption of BBB is a key event responsible for the pathology in a number of neurological diseases and has also been shown to be involved in the severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) infections recently. In this review, we discuss the cellular and molecular components orchestrating BBB formation and function maintenance across species. How this barrier can be modulated for efficient drug delivery into the brain, and how BBB breakdown participates in neurological diseases are discussed. Finally, we highlight the recent work identifying the possible mechanisms by which SARS-CoV-2 invades CNS by crossing BBB in Corona Virus Disease 2019 (COVID-19) patients.

1. Introduction

The BBB is coordinated by a series of physiological processes that limit vascular permeability and acts as a barrier between peripheral blood circulation and the CNS. It protects the CNS from pathogens, toxins, and other harmful substances. A functional BBB is necessary for maintaining the special physiological processes of the brain, such as nutrient supply, immune infiltration and metabolism. The barrier properties of BBB, maintained mainly by interactions between endothelial cells (ECs) and other neurovascular units (NVUs), are primarily determined by the paracellular endothelial tight junctions (TJs), influx and efflux transporters, and metabolic properties of ECs. The breakdown of the BBB accelerates degenerative changes in the CNS, which are believed to underlie a range of neurodegenerative diseases, including multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), Alzheimer disease (AD), Parkinson disease (PD), and Huntington disease (HD) [1].

On the other hand, the strict restrictions of material transport by the BBB have become one of the major challenges in drug delivery into the brain. For a very long time, many attempts have been made to conquer the difficulties of opening the BBB for drug delivery, including but not limited to the temporary open of endothelial junctions, osmotic pressure, micro bubbles, and ultrasound [2].

In the recent two years, mounting evidence from the patients suffering corona virus disease 2019 (COVID-19) which is characterized by

acute respiratory symptoms and caused by severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) indicates that SARS-CoV-2 can invade the CNS and cause CNS infection [3]. Since a breached BBB is also required for pathogens entering the CNS, an invasion pathway across BBB might be involved in the infecting process of SARS-CoV-2 into the CNS.

Here, we review the physiological structure and function of BBB, and pathological changes of BBB in neurological diseases as well as in COVID-19. Insight into the maintenance of selective passages through the BBB will enable development of drug delivery strategies. Understanding the disruption of the BBB under pathological conditions and the underlying mechanisms will help in developing potential treatment approaches.

2. Cellular components of blood-brain barrier

The BBB is defined as a multicellular cerebral vascular structure centrally located in the NVUs. Its core anatomical element is the brain microvascular endothelial cells (BMECs) sealed by TJs. The abluminal surface of BMECs is covered by a basement membrane where pericytes are embedded (Fig. 1). This intercellular crosstalk between BMECs and pericytes is crucial for BBB maturation. Astrocytes, a major glia cell population, extend foot processes to encircle the brain capillaries and maintain BBB integrity (Fig. 1). The BBB is surrounded by or closely associated
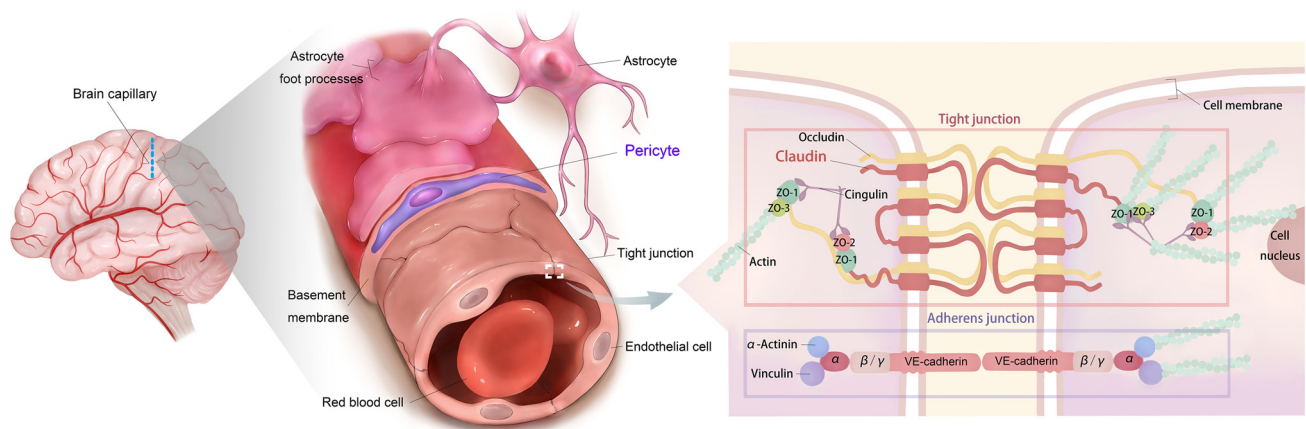

Fig. 1. The cellular and molecular components of the BBB. BMECs are sealed mainly by the TJs and AJs proteins. In the TJs, a complex of transmembrane proteins, including Claudins and Occludin, are connected to the actin cytoskeleton via the ZO proteins ZO-1, ZO-2, and ZO-3, thus creating a high-resistance paracellular barrier to molecules and ions. AJs proteins such as VE-Cadherin also contribute to BBB properties. Pericytes cover the endothelium's abluminal surface via basement membrane and communicate with BMEC to promote BBB maturation. Astrocytes extend foot processes that encircle the abluminal side of the vessel to maintain BBB function. Abbreviation: BBB, blood-brain barrier; BMECs, brain microvascular endothelial cells; TJs, tight junctions; AJs, adherens junctions; ZO, zonula occludens; VE-Cadherin, vascular endothelial-cadherin.

with other cell types in the NVUs, including neurons, microglia, and, optionally, blood-borne immune cell populations that also contribute to the modulation of BBB functions [4].

2.1. Brain microvascular endothelial cells

BBB formation in mammals initiates at the early embryonic period when endothelial progenitor cells enter the cortex and vascularize the CNS [5]. The exact timing is species-dependent and varies regionally. For instance, in mice the angiogenesis of brain capillary begins at embryonic day 10 (E10) [5], whereas in human this process starts at gestational week 8. By interacting with the CNS microenvironment, the BMECs exhibit unique BBB properties compared to ECs in the peripheral tissues. Firstly, the BMECs are held together by continuous intercellular TJs (Fig. 1), which create a high-resistance paracellular barrier restricting the crossing of ions and molecules. Secondly, BMECs undergo extremely low rates of transcytosis and lack fenestrations, which greatly limit transcellular transport through the EC layer. Thirdly, BMECs express a series of transporters, including highly specific nutrient transporters and efflux transporters, thus strictly regulating the movement of required substances into the brain and toxic/waste substances outside the brain; and fourthly, these ECs have low expression of leukocyte adhesion molecules (LAMs) to reduce the invasion of immune cells into the healthy CNS, therefore regulating CNS immune surveillance [6]. These properties allow BMECs to tightly control the passage of ions, molecules, and cells between the blood and the brain.

Research over the past decade showed that the formation of BMECs-specific characteristics is a sequential and well-orchestrated process [7]. It commences when neural progenitor cells secret factors such as VEGF and Wnt to induce angiogenesis, mature vessel morphology, and many BBB properties of BMECs, including the expression of TJ proteins and nutrient transporters. Subsequently, the functions of the BBB gradually develop with the coverage and recruitment of pericytes to the endothelial walls, which improves the barrier function of BMECs by stabilizing TJs, decreasing transcytosis, and suppressing the expression of LAMs. Finally, astrocytes appear and aid in regulating the function of the barrier in adulthood. Thus, although key properties of the BBB are manifested within the BMECs, they are induced and regulated through proper communication among multiple cellular components in the NVUs [7].

2.2. Pericytes

Pericytes are a population of mural cells surrounding the ECs of the microvascular network comprising capillaries, precapillary arterioles,

and postcapillary venules (Fig. 1). The distribution of pericytes is heterogeneous in different organs, among which the CNS shows the highest pericyte coverage, in agreement with the functional importance of pericytes in the BBB. The density and morphology also vary among different brain microvascular segments [8], suggesting the diversity of their functions. Pericytes in the capillary beds typically exhibit long processes that traverse the microvasculature in single strands or pairs that twist in a helical fashion and maybe more important for maintaining the BBB. In the postcapillary venules, pericytes extend more circumferential processes to cover the BMECs and might regulate immune cell entry to the brain parenchyma. Finally, pericytes in the precapillary arterioles express more contractile proteins such as α-SMA and are likely to be preferentially involved in regulating cerebral blood flow [9]. The brain vessels thus appear to harbor specialized pericytes to perform various neurovascular tasks.

Critical insights into the specific roles of pericytes in the brain vasculature have come from mouse models deficient in pericytes through genetic manipulation of the PDGF-B/PDGFRβ signaling pathway, which is essential for the proliferation, migration, and recruitment of pericytes to the vascular wall [10]. Homozygous deletion of the *Pdgfb* or *Pdgfrb* gene results in a complete lack of CNS pericytes and is embryonic lethal [11]. Varying the strength of PDGF-B/PDGFRβ signaling leads to viable mouse models with different pericyte deficiencies [12]. The neonatal, adult and aging mice showed increased BBB permeability, the extent of which inversely correlated with the density of brain pericytes. Also, these studies demonstrate that pericytes function at the BBB in multiple ways, including the formation of TJs, vesicle trafficking in BMECs, regulating brain microcirculation, and providing signals that limit CNS immune surveillance [13].

Pericytes and BECs are engaged in reciprocal communication, which is essential for BBB integrity but is incompletely understood. PDGF-B secreted by BECs binds to PDGFRβ in pericytes, leading to pericyte recruitment. The attached pericytes in turn secret paracrine signaling molecules including angiopoietin-1, TGF-β, and Sphingosine-1-phosphate to act on their receptors in BECs, promoting stabilization of ECs and BBB maturation [10]. The failure of cell-cell interactions between BECs and pericytes has been frequently implicated in a variety of human diseases including stroke, Alzheimer's diseases, and cancer. Our recent work showed that the dynamic expression of CD146 (MCAM) plays a critical role in the interplay of ECs and pericytes and orchestrates BBB development spatiotemporally [14]. On the other hand, the finding that CD146 is significantly elevated in BECs to promote neuroinflammation under several pathological conditions suggests CD146 as a potential

therapeutic target for cerebrovascular disorders. However, an in-depth understanding of the BEC-pericytes interaction is warranted. Little is known about the molecular mechanisms coordinating the pericyte-EC behavior and communication during the gradual process of BBB development. It is also not clear how and why this crosstalk fails in many pathological conditions.

2.3. Astrocytes

Astrocytes, occupying ∼50% of the brain parenchymal volume, have a close spatial relationship with BMECs (Fig. 1). Early studies have proposed astrocytes as important mediators of BBB formation and function. This hypothesis is based on transplantation and *in vitro* coculture studies where astrocytes were shown to increase the barrier properties of non-CNS blood vessels [15]. However, recent work analyzing the BBB in dissected rodent embryos showed that the timing of astrocyte generation and BBB formation is controversial. Astrocytes are first generated directly after birth and extend processes that contact vessels during the first postnatal week, whereas the BBB is formed during embryogenesis [13]. Therefore, astrocytes are not required to initially induce the BBB and reports suggest that they are involved in the maintenance of the BBB TJs by secreting sonic hedgehog and angiotensin II that signal to their receptors on BMECs. In addition, these cells provide almost complete coverage of cerebral micro-vessels with multiple end-feet, which can release vasoactive substances that regulate blood flow in response to neuronal activity [16].

2.4. Other cellular components

The BBB requires support from other cellular components within the NVUs, especially neurons and microglia. Every neuron is positioned within 15 μm of a blood vessel. This neurovascular coupling ensures that the regional blood flow is finely regulated to supply nutrients and remove metabolic waste depending on the local neuronal activity. Conversely, changes in BBB permeability can in turn influence neural function by disrupting nutrient and waste exchange, as well as by increasing the influx of other neuroactive molecules to CNS [17]. Microglia cells are the resident immune cells of the brain, the activation of which is believed to play a central role in neuroinflammation. Under physiological conditions, microglia regulate brain function by removing dying neurons, pruning non-functional synapses, producing ligands that support neuronal survival, and controlling neuronal activity by sensing and catabolizing extracellular ATP [18]. Recent studies have revealed that these cells also contribute to the maintenance of the BBB functions [19].

3. Molecular components contributing to blood-brain barrier function

BBB formation and maintenance are complex processes requiring both interactions among cellular components and retainment of physiological properties which are regulated by molecular components in NVUs. Paracellular transports are mainly limited by the TJs, adherens junctions (AJs), junctional adhesion molecules (JAMs), and gap junctions, while transcellular transports by the carrier-mediated transport (CMT) and vesicular transport mainly include adsorptive- and receptor-mediated transcytosis (AMT, RMT) [20].

3.1. Junctional components

TJs are important junction complexes between ECs or epithelial cells (Fig. 1). It is formed by TJ-related membrane proteins including transmembrane proteins such as Claudins, TJ-associated MARVEL proteins (TAMP) families containing Occludin, Tricellulin, and MarvelD3 under the synergistic assist of periplasmic scaffolding proteins such as Zonula Occludens (ZO) family, membrane lipids, and mechanical forces [21]. TJs play an essential role in maintaining the paracellular permeability

and the cell polarity by tightening the paracellular gap, establishing the electrochemical gradient and serving as scaffolds for polarity signaling proteins. Disruption of the TJ layer, which leads to BBB breakdown, is observed in a range of neurological disorders, including stroke, infections, brain tumors, multiple sclerosis (MS), and Alzheimer's disease [7].

Claudins are polymerized on the plasma membrane of the ECs and constitute the main components of TJ strand structure (Fig. 1). Twenty-seven members of this family have been described in mammals. They can be distributed to the most apical region between cell-cell membrane in cerebrovascular ECs, maintaining selective intercellular permeability of ions or solutes and appropriating apical and basolateral membrane domains. Claudin-5, as a main component of the TJ between BMECs, is closely related to the BBB permeability by sealing the paracellular pathway through the BBB. Claudin-5 was previously known to have the exclusive function of regulating the paracellular permeability. However, a recent study has revealed a novel function of Claudin-5 in enhancing adhesive forces between arterial ECs [22]. Other BBB-related TJs proteins such as Claudin-1, -3 are weakly expressed in the BBB. Knockdown of Claudin-5 is accompanied by increased Claudin-1 expression, suggesting a compensatory role of Claudin-1 under pathological conditions. Claudin-25 is found to be responsible for proper TJ strand morphology [23]. Further characterization for functions of Claudins in BBB is still under exploration.

ZO proteins are scaffolding molecules for various proteins correlated with TJ assembly and maintenance. It has been confirmed that ZO proteins can selectively recruit and orderly organize TJ-related membrane proteins including Claudins and Occludins (Fig. 1). But not all TJ-related proteins can enter the "ZO droplet", such as JAM-A [24]. Dynamic change of ZO proteins causes reorganization of TJ, thus increasing macromolecule permeability induced by reorganized actomyosin under inflammation. Additionally, phase separation of ZO proteins is likely to cause TJ-related membrane proteins to cluster, resulting in lipid microdomain formation, which might, in turn, strengthen the TJ formation [21].

Recent studies have shown that membrane lipids and mechanical forces are also important in TJ assembly and formation. Studies suggest that cholesterol and very-long-chain ceramides play a role in TJ formation. Composition changes of membrane lipid in *α-catenin*-knocked out (KO) EpH4 cells can lead to pathological Claudins endocytosis. Mechanical forces are reported to regulate the phase separation of ZO proteins, thus regulating the TJ assembly [21].

As well as TJs, membrane apposition is responsible for the endothelial barrier function. These are two distinct structures between ECs maintaining paracellular barrier. While TJs are closely correlated with Claudins, membrane apposition formation does not seem to require involvement of Claudins [25]. Membrane apposition is reported to be related to JAMs, AJs containing vascular endothelial cadherin (VE-Cadherin) and platelet EC adhesion molecules-1 (PECAM), Occludin and Tricellulin [25]. In the absence of TJ strand, Claudin quintuple-KO MDCK II cells still possess macromolecule permeability (>4kD) barrier while micromolecule permeability barrier is destroyed [25]. Elimination of JAM-A leads to widen intracellular gap in MDCK II cells, resulting in a breakdown of macromolecule permeability barrier [25]. However, further studies into the role of the membrane apposition in BBB formation are needed.

3.2. Other junctional components

Other cellular junctions including AJs and gap junctions are enriched in the BBB and also contribute to the barrier properties of BBB. AJ is a form of cell-cell adhesion structure that lies in the gap (about 10-20 nm) between adjacent cells. This structure is common in a variety of cell types including ECs, epithelial cells, mesenchymal and neural cells. It has a similar organization to TJ and is created when transmembrane proteins, including cadherins, PECAM, and nectin, are anchored to cytoplas-

mic proteins, particularly the catenin family members (p120-catenin, β-catenin, and α-catenin) and afadin. The cytoplasmic bridging proteins further bind to cytoskeletal components, such as actin filaments and microtubules. Together, these molecular complexes form the primary architecture of AJs and maintain the physical association between neighboring cells. Interestingly, there is now emerging experimental evidence suggesting intimate crosstalk between AJs and TJs in BBB maintenance. AJs are clearly distinguished from TJs in epithelial cells, whereas the localization of these two junctional complexes in BECs appears intermingled. Formation of TJs is considered to generally require the existence of AJs, and VE-cadherin is observed to induce the expression of molecular components of TJs such as Claudin-5 [26]. Future work is required to study more aspects of AJ-TJ crosstalk and how it will regulate barrier properties and signaling pathways of the BBB. Gap junctions are formed by a group of proteins termed connexins and are widely distributed in nearly every system in the body. At the gap junction, connexins exist as intercellular hemichannels with an approximately 2 nm gap between adjacent cells. Among the 21 human connexins, Cx37, Cx40, Cx43, and Cx45 are expressed in vascular cells. They are essential for direct intercellular communications and signal transduction via the passage of ions and small molecules smaller than 1 kDa, thereby coordinating vascular function and communication. Beyond these functions, recent studies have demonstrated that connexins have additional roles which are independent of involvement in the well-established gap junction. For instance, Cx43 has been shown to influence the endothelial cell motility and migration via its C-terminal cytoplasmic tail, which is the main site of posttranslational changes such as phosphorylation, acetylation, and S-nitrosylation [27].

3.3. Glucose transporters

The mammalian CNS depends on the aerobic metabolism of glucose as its main source of energy. The normal adult human brain accounts for ~2% of the bodyweight but consumes ~20% of glucose in the body (about 5.6 mg glucose per 100 g human brain tissue per minute). The glucose metabolism in the brain is centrally cooperated by BMECs, glia cells, and neurons, which express different types of glucose transporters (GLUTs) that facilitate the transport of glucose across membranes [28]. The GLUTs, encoded by SCL2A, are a wide group of 14 membrane proteins named GLUT1-14. Among them, GLUT1-6 and GLUT8 are observed in different brain areas and cells, and currently, GLUT1 and 3 have been reported to be critically involved in mediating glucose transport. Disturbed GLUTs expression and function in the brain are associated with many diseases. GLUT1 deficiency syndrome is a genetic disorder caused by mutations in the *SCL2A1* gene. These patients present low glucose and a variety of symptoms such as epilepsy, movement disorders, and cognitive impairment. Impaired GLUT1 levels and glucose uptake also contribute to the pathobiology of Alzheimer's disease by initiating BBB breakdown and accelerating amyloid β-peptide pathology [29]. During ischemic stroke and traumatic brain injury, the expression of GLUT1 and GLUT3 is changed and may be involved in the disease progression [30].

3.4. Other endothelial transporters

A number of other transporters are localized on both luminal and abluminal sides of endothelial membrane and crucially regulate CNS homeostasis in response to internal and external stimuli [7]. A series of ATP-binding cassette transporters are highly expressed in the luminal membrane of ECs, actively limiting the entry of a large number of exogenous compounds and drugs into the CNS. In this way, these transporters contribute to brain detoxification, overall normal CNS physiology, and the BBB bottleneck for therapeutic drug delivery to the brain. The CMT system is a large superfamily containing more than 300 transporters such as GLUT1. These transporters recognize specific substrates, including carbohydrate, monocarboxylic acids, hormones, fatty acids,

nucleotides, organic anions, amines, choline, and vitamins, and facilitate their transport. RMT and AMT provide the means for large macromolecules across the BBB via transcytosis. The former involves a specific interaction with receptors expressed on BECs, thereby transporting a limited set of proteins or peptides such as the low-density lipoprotein, insulin, and iron-transferrin. AMT is charge-dependent adsorptive transcytosis, which is triggered by nonspecific electrostatic interaction between cationic molecules and anionic microdomains on the cytoplasm membrane of the BECs. Both RMT and AMT have also been utilized to develop efficient drug delivery to the brain, although the RMT has been used more actively based on the preferable specificity and high expression of transferrin receptor, insulin receptor, and other RMT receptors in targeted cells.

4. Phylogeny of the BBB across species

The physiological functions of BBB is evolutionarily conserved across species of maintaining and protecting the homeostasis of the CNS [31]. Although the first experimental evidence of cerebral barrier has already been obtained on several animals in the early 20th centuries, it was not until the 1980s that the anatomical investigations of BBB on cellular levels were firstly analyzed by Abbott and others [32]. Earlier studies found that in Cephalopod mollusks (e.g., octopus, squid, cuttlefish), the only members of the phylum Mollusca processing a closed circulatory system, a cerebrovascular network is necessary for the nutrients delivered into the brain and a perivascular glia-contributed BBB regulates the influx and efflux changes in the primitive brain (Table 1). In *Drosophila*, which belongs to the class of insects and is a widely used model animal, the subperineurial glia are surrounded by a layer of perineurial glial cells and function to separate the circulating hemolymph from the neuropil, therefore forming the hemolymph-brain barrier with analogous functions to the BBB in vertebrates (Table 1) [33]. The subperineurial glial cells are connected by septate junctions which are functionally related to the TJs in vertebrates [33,34].

Although the BBB is functionally conserved across species, the cellular compositions and the NVUs contributing to the barrier function vary. Combining specific tracer leakage and imaging assays, scientists could systematically analyze the cellular constituents and the development of BBB, from embryos to adults, in lots of typical species during the past decades. In invertebrates, such as the fruit fly mentioned above, the barrier function is contributed by perivascular or boundary glial cells [32]. In contrast, in most vertebrates including tetrapods (amphibia, reptiles, birds, and mammals), the BBB function is mainly determined by the cerebral capillary ECs which are connected by paracellular TJs and are typically surrounded by pericytes and astrocytic glial cells (Table 1). According to the view of Bundgaard and Abbott, the ectoderm-derived glial cells likely form the primitive BBB in the ancestral vertebrate, which is still retained in elasmobranchs and sturgeons [31]. During the convergent evolution, the glial-contributed barrier has been replaced by the mesoderm-derived endothelial BBB several times in evolution, which provided enough selective advantage in the evolution [31,35] Table 1. summarizes and enumerates the cellular constituents contributing to BBB functions in representative species of different classes, from octopus of Mollusca to mammals.

5. Transport across blood-brain barrier and drug delivery strategies

In CNS, the BBB separates the peripheral blood and neural tissues by restricting the entry of toxins, pathogens from the circulating blood. It strictly regulates the influx of water, ions, oxygen and nutrients, and the efflux of metabolites, therefore maintaining the cerebral homeostasis under normal physiological conditions. In mammals, the cerebral ECs facing the luminal circulations play the most essential role in the barrier functions. The ECs are tightly connected by TJs and AJs to limit the

Table 1
Phylogeny of the BBB across representative species.

Representative Species	Class	Main characters of cerebral barrier or BBB	References
Octopus (*Enteroctopus dofleini*)	Cephalopoda	perivascular glial	[76]
Drosophila melanogaster (*Pseudoobscura*)	Insecta	Subperineurial cells connected by septate junctions to establish a hemolymph-brain barrier	[34]
Sharks, skates and rays(Elasmobranch fish)	Chondrichthyes	glial	[77]
Sturgeon (*Acipenser*)	Actinopteri	glial	[31]
Zebrafish (*Danio rerio*)	Actinopteri	endothelial	[78]
Bichir (*Polypterus senegalus*)	Cladistia	endothelial	[31]
Lungfish (Protopterus annectens)	Dipnotetrapodomorpha	endothelial	[31]
Chicken (*Gallus gallus domesticus*)	Aves	endothelial	[79]
Mouse (*Mus musculus*)	Mammalia	endothelial	[80]
Chimpanzees (*Pan troglodytes*)	Mammalia	endothelial	[35]

movement of substances through the paracellular space (Fig. 1). Meanwhile, the innermost ECs interact with and are regulated by other cellular components in NVU, such as pericytes and astrocytes, dynamically controlling the BBB permeability. Therefore, the barrier function of BBB is mainly achieved by the brain capillary endothelium.

Due to the existence of paracellular TJs instead of large fenestration between adjacent ECs, the paracellular transport of molecules through the BBB is strictly limited under normal physiological conditions (Fig. 2). Only some gas and small lipophilic molecules with a molecular weight <600 Da could diffuse through the paracellular route to enter the brain [36]. Although targeting and disruption of the TJ integrity by several methods could be potential strategies to temporarily open the BBB for drug delivery, these strategies present high risks during the opening of TJs, such as invasion of immune cells like leukocytes, uncontrolled influx of toxins or molecules from the peripheral circulation into the brain [37].

Another typical feature of brain capillary ECs is their low levels of transcytotic vesicles and reduced pinocytosis compared to peripheral ECs and an absence of fenestrae. These limit the efficient transcellular transport or transcytosis across the brain endothelium forming the BBB [38]. Some non-polar solutes may passively diffuse through the ECs of BBB. Besides that, two main systems involved in the transcytosis process across CNS ECs are vesicular transport and the CMT [20]. During these processes, two well-known endocytosis routes are clathrin-coated vesicles and caveolae transporters, which function as facilitative cargos cytosolically [38].

CMT can transport many essential polar molecules, such as glucose, amino acids, nucleosides, and small peptides, into the CNS [20]. By far the most well-known endothelial membranous carriers are facilitative glucose transporters (GLUTs). Among the GLUTs family, GLUT1 is identified to localize in cerebral ECs, astrocytes, and neurons [39]. In brain ECs, GLUT1 is expressed on both the luminal membrane and abluminal membrane of the ECs, facilitating the continuous glucose transcytosis and meeting the high energy demands of the brain (Fig. 2). In recent years, GLUT1 is also applied as an ideal target for drug delivery into the brain for the treatment of CNS diseases. By modifying the nanocarrier surface with a precise density of glucose, GLUT1 binds to and enables the transcytosis of nanocargo into the brain through the endothelium [40].

Vesicular mechanisms, mainly including AMT and RMT, are responsible for regulating the transcytosis of certain macromolecules including peptides and proteins through BBB. AMT seems to be activated nonspecifically by positively charged macromolecules during transcytosis while the underlying mechanisms are still poorly understood so far [20]. In RMT, ligands in capillary ECs specifically bind to membranous receptors to mediate the endothelial endocytosis, such as in the case of insulin, transferrin (Tf), and omega-3 fatty acid [41]. Typically, Tf in the blood is transported via RMT across the BBB by Tf receptor (TfR) localized in the capillary endothelium (Fig. 2) [42]. Since TfR is expressed on both the luminal and the abluminal membranous of brain ECs, the TfR could

mediate not only the influx of holotransferrin from peripheral blood into the brain, but also the reverse transcytosis of apotransferrin from the brain into the blood circulation [43]. As a newly identified essential inhibitor of BBB transcytosis, major facilitator superfamily domain-containing protein 2a (Mfsd2a) functions as a lipid transporter for the omega-3 fatty acid docosahexaenoic acid (Fig. 2) [41,44,45]. Loss of Mfsd2a in mice led to increased permeability of BBB due to upregulated transcytosis [45]. The most recent structural definition of Mfsd2a increases the potential to modulate the BBB permeability by targeting Mfsd2a for efficient drug delivery [44].

During the therapy of CNS diseases, many effective drugs fail to cross the BBB due to the limited permeability between peripheral blood and CNS. Therefore, reducing the physiological barrier function of the BBB, such as reversibly opening TJ barrier or improving the influx transporters, may help to improve the delivery efficiency. Besides, design and modification of pro-drugs could also optimize their properties for better facilitating the delivery into the CNS, such as lipid modification, glycosylation modification, being coupled with nano-carriers [2]. Therefore, in-depth research on mechanisms for molecular transport across BBB will help and enable the development of drug delivery strategies.

However, an effective drug delivery into brain is a complex and challenging process which requires better preciseness rather than just crossing the BBB. A successful reach to the lesion site and the clearance of the drug residues from the site should also be in consideration for drug efficacy and safety, where the targeting, therapeutic, stability, and safety of the molecules need to be considered comprehensively.

6. Blood-brain barrier dysfunction in central nervous system disorders

Dysfunction of BBB is a central feature in a range of neurological conditions, including stroke, MS, ALS, and epilepsy. In animal models of these diseases, the properties of BBB have altered, which are largely supported by neuroimaging in patients with neurological disorders. However, the molecular factors regulating BBB dysfunction, the cellular consequences of BBB dysfunction, and the relative contribution of BBB dysfunction to the pathophysiology of these diseases have remained elusive.

6.1 Stroke

Stroke, a cerebrovascular disease, is the leading cause of death and serious long-term disability across the world. It occurs when the cerebral blood flow is locally interrupted or reduced, resulting in insufficient nutrient and oxygen supply and leading to the death of brain cells. During the pathological process of stroke, the integrity of BBB is damaged with increased permeability, which may cause various inflammatory reactions and secondary cerebral edema. For instance, activated microglia in the infarct area phagocytose cell fragments, showing anti-inflammation effects during the early stage of stroke. However, it subsequently increases the release of ROS, cytokines, tumor
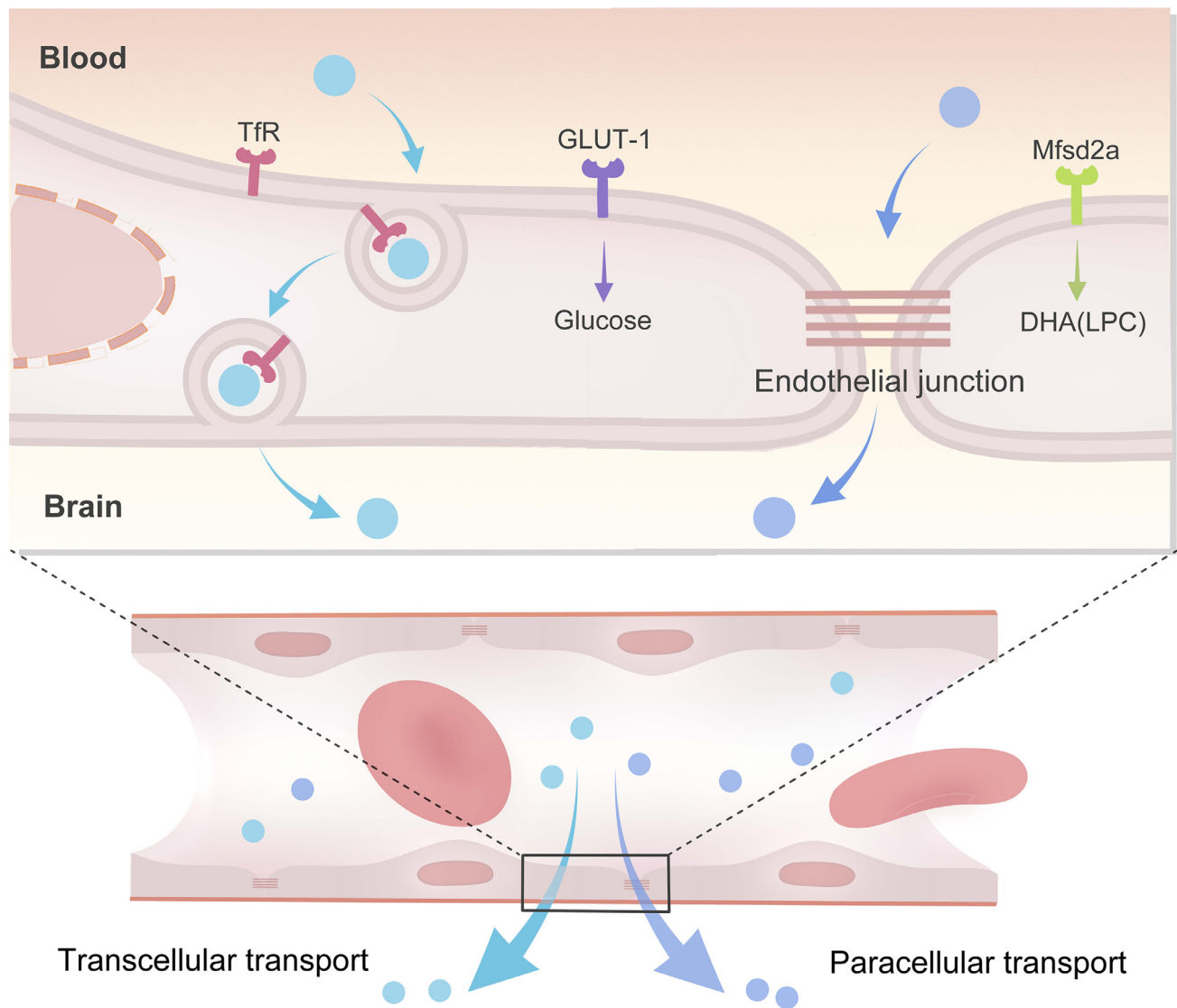
Fig. 2. The potential pathway for materials exchange across the BBB. The BBB function that strictly limits the diffusion of the molecules is maintained by both transcellular and paracellular transport restrictions. Some gas and small lipophilic molecules with a molecular weight <600 Da could diffuse through the paracellular route to enter the brain. Non-polar solutes may cross the BBB by passive transport. Polar molecules including glucose, amino acids, nucleosides, and small peptides may favor vesicular transport (including AMT and RMT) and the CMT facilitated by corresponding receptors, including GLUT-1, TfR, Mfsd2a. Abbreviation: BBB, blood-brain barrier; AMT, adsorptive mediated transcytosis; RMT, receptor-mediated transcytosis; CMT, carrier-mediated transcytosis; GLUT-1, Glucose transporters-1; TfR, transferrin receptor; Mfsd2a, major facilitator superfamily domain-containing protein 2a.

necrosis factor-α (TNF-α), and matrix metalloproteinases, resulting in TJs brokendown via degradation of TJ proteins such as Claudin-5 and Occludin [46]. Meanwhile, activated astrocytes with increased protrusion cause physical separation of astrocytic endfeet from the ECs, leading to leaky BBB. Moreover, activated astrocytes will release vascular endothelial growth factor A (VEGF-A) to mediate TJ proteins degradation [47]. Several other mechanisms such as cytoskeletal rearrangements, matrix metalloproteinase-9 activation, oxidative stress, and pro-inflammatory cytokines have also been verified contributing for BBB damage [48]. These effects all together cause BBB breakdown and brain edema, which are associated with poor clinical prognosis in stroke. Brain edema is broadly classified as either cytotoxic or vasogenic and is the leading cause of mortality in 60-80% of the patients. After stroke, cytotoxic edema forms early due to impaired cellular metabolism, followed by disruption of the BBB and extravasation of water and plasma proteins into the brain interstitial compartment, which causes vasogenic edema and the ultimate total brain swelling, neurological dysfunction, and death. Thus, BBB breakdown worsens

stroke damage, and restoring BBB integrity is likely important to reduce brain swelling and death in stroke. A major hurdle for this strategy is the limited understanding of the molecular mechanisms regulating BBB disruption in stroke, although they have been partly elucidated as described above [48]. In our recent studies, we demonstrated that redistribution of membranous Claudin-5 accompanied by autophagy activation is essential for hypoxia-induced BBB injury after stroke [49,50]. These findings further strengthen the roles of BBB in the pathophysiology of stroke and facilitate the exploration of new therapies to target BBB dysfunction.

6.2. Epilepsy

Epilepsy is characterized by recurrent seizures, affecting 65-70 million people worldwide. These patients showed BBB leakage visible with contrast-enhanced MRI, increased parenchymal albumin, and regional reduction in Glucose transporter 1, all of which indicate a clear association between epilepsy and BBB dysfunction. Furthermore, patients with

osmotic disruption of BBB, BBB-GLUT1 deficiency, or BBB breakdown-associated diseases such as stroke and traumatic brain injury develop seizures [51]. Experimental evidence suggests that BBB microvascular dysfunction is involved both in the induction of seizures and in the progression to epilepsy. The identified cellular events linking microvascular pathology to epilepsy can be mediated by multiple pathways. For instance, seizures induce enhanced vascular expression of LAMs, which promotes the leukocyte-endothelial adhesion and contributes to the pathogenesis of seizures and epilepsy. Inhibiting BBB breakdown by blockade of leukocyte-endothelial interaction can exert preventive and therapeutic effects in experimental animal models of epilepsy [52]. A recent study showed that BMECs could release chemokines like chemokine (C-X-C motif) ligand 1, which decreases glutamate transporter 1-mediated glutamate reuptake by astrocytes, affecting the balance between synaptic excitation and inhibition and leading to epileptogenesis [53]. The leakage of albumin into the brain after BBB disruption is also suggested to play a role in epileptogenesis through different mechanisms including activation of transcription of inflammatory genes, accumulation of extracellular potassium, and enhancement of neuronal excitability [54].

6.3. Amyotrophic lateral sclerosis

ALS is a fatal disease in which the motor neurons progressively degenerate in the brain and spinal cord, leading to muscle atrophy, paralysis, and death typically within three to five years from diagnosis. Recent studies in both ALS patients and animal models expressing mutant SOD1 provide compelling evidence of BBB disruption, including EC damage and pericyte degeneration, microvascular leakage, intra- and extracellular edema, reduced TJ protein expression, and microhemorrhages [55]. Importantly, these BBB alterations were exacerbated with disease progression, highlighting that they may aggravate ALS pathogenesis. Currently, the particular mechanisms responsible for these damages still need to be determined. It has been suggested that oxidative stress and activation of matrix metalloproteinases (MMPs), especially MMP-2 and MMP-9 may impair BBB functions in ALS [56]. Also, the exact roles of BBB disruption in this disease are unclear. Some reports have shown that the BBB breakdown, as indicated by decreased levels of IgG, TJ proteins, GLUT1, and deposits of perivascular hemosiderin, occurs prior to motor neuron loss and inflammation degeneration, suggesting that it could trigger and also aggravate the disease progression [57].

6.4. Multiple sclerosis

MS is an autoimmune disorder that affects the CNS. In healthy brains, BMECs express low LAM and prevent peripheral leukocytes from crossing the BBB, thereby maintaining immune homeostasis. However, a key step in the pathophysiology of MS is the dysfunction of BBB, which facilitates the infiltration of extensive pathogenic immune cells into the CNS. These infiltrating cells result in inflammatory lesions in the CNS parenchyma, ultimately leading to demyelination, axonal loss, neurodegeneration, and neurological disability. During this process, there is enhanced expression of many LAMs in the BMECs including, but not limited to, α4-integrin, vascular cell adhesion molecule-1, intercellular adhesion molecules, and CD146 [58]. Furthermore, these molecules are found to be important for tethering, rolling, gripping of circulating immune cells over the BMECs and the subsequent transmigration across the BBB in the MS pathogenesis [59]. Notably, therapeutic approaches that center on blocking the perivascular immune cells trafficking across the BBB by manipulating these molecules have been proven to be effective in treating MS. For instance, natalizumab, a humanized monoclonal antibody against the cell adhesion molecule α4-integrin, greatly reduces the inflammatory immune cells passing through BBB and lesion formation and is FDA-approved for the treatment of MS [60].

7. Blood-brain barrier and COVID-19

Despite that the main symptoms observed in COVID-19-infected patients are in the respiratory system, SARS-CoV-2 is reported to cause multiple extrapulmonary symptoms, including symptoms in the brain, olfactory neurons, eyes, vasculature, pancreas and gastrointestinal tract symptoms, and also injuries of kidney, liver and heart [61]. The entry of SARS-CoV-2 into infected cells depends on ACE2 and TMPRSS2 which are widely expressed in the brain, vascular endothelium, liver, heart, kidney, intestine, and several other extrapulmonary organs, more than only in the lung [61]. This may explain the extrapulmonary symptoms caused by SARS-CoV-2. Here, we focus on CNS breakdown resulting from SARS-CoV-2 infections. Existing studies on neurological infection of SARS-CoV-2 suggest it to be a combination of neural cell infections, endothelial damage (which leads to BBB breakdown in CNS), and immune system disorders. SARS-CoV-2 infection in CNS contributes to multiple neurological manifestations, including ischemic stroke, encephalitis, mental symptoms, epilepsy, and controversially MS, PD, AD [62]. In the recent work of Yang et al., it was found that very similar cellular subpopulations of microglia and astrocyte with pathological features were identified in severe COVID-19 patients and patients with neurodegenerative diseases [63], suggesting that SARS-CoV-2 infection in CNS may contribute to the occurrence of neurodegenerative diseases. Additionally, individuals with neurodegenerative disease or inflammatory-mediated neurological disorder are potentially more susceptible to be infected by SARS-CoV-2 [62]. Brain symptoms indicate that SARS-CoV-2 somehow has the ability to cross the BBB. ACE2 and TMPRSS2 in the ECs of cerebral blood vessels possibly provide access for viral entry into the CNS, thus resulting in endothelium and brain dysfunction. On the other hand, though no traces of SARS-CoV-2 was found in the brain, Yang et al. showed that peripheral inflammation can still infiltrate into the brain [63], indicating a potential relationship between inflammation and COVID-19-associated neurological symptoms.

In addition, researchers have explored and demonstrated a role of cytokine storm syndrome in SARS-CoV-2 infection recently [64]. Cytokine storm is a systemic excessive inflammatory response. So far, the exact cause of this phenomenon has not been fully understood. When the body is infected, the release of primary pro-inflammatory cytokines, including interferon-gamma (IFN-γ) and tumor necrosis factor-alpha (TNF-α), leads to the activation of more immune cells, including macrophages, dendritic cells, neutrophils, effector T cells, B cells, mast cells, and ECs. These immune cells further secrete cytokines, including interleukin-6 (IL-6), interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), chemokine (c-c-motif) ligand 2 (CCL2), granulocyte macrophage colony-stimulating factor (GM-CSF), and chemokine (C-X-C motif) ligand 10 (CXCL10). IL-6 secreted by macrophages and ECs further activated immune cells in a positive feedback manner [64]. A large number of cytokines are produced in a short period of time, and over activation of immune cells in the system will lead to deterioration of the patient's condition or even death. This further explains the extrapulmonary symptoms caused by SARS-CoV-2 infection, including various degrees of damage in the liver, kidney, intestine, heart, brain, blood vessels, and other organs or tissues. Short-term use of dexamethasone, a synthetic corticosteroid, has been shown to reduce the severity of inflammation and lung injury, suggesting an important role of cytokine storm in pathogenic processes of SARS-CoV-2 [64]. Immune cells activated by cytokine storm will damage vascular ECs, which will lead to dysfunction of vascular barrier and increase the exudation of inflammatory factors, and possibly accounts for coagulation dysfunction in patients with COVID-19. The secondary inflammatory reaction caused by EC injury, in turn, damages vascular ECs, causing worse vascular damage and increasing vascular permeability, which leads to the destruction of BBB. Pathogens in the lesion site are therefore more likely to enter the blood vessels. Interestingly, it is reported that SARA-CoV-2 enters the brain through BBB with basement membrane disruption but not TJs [65]. Infections of SARS-CoV-2 in ECs
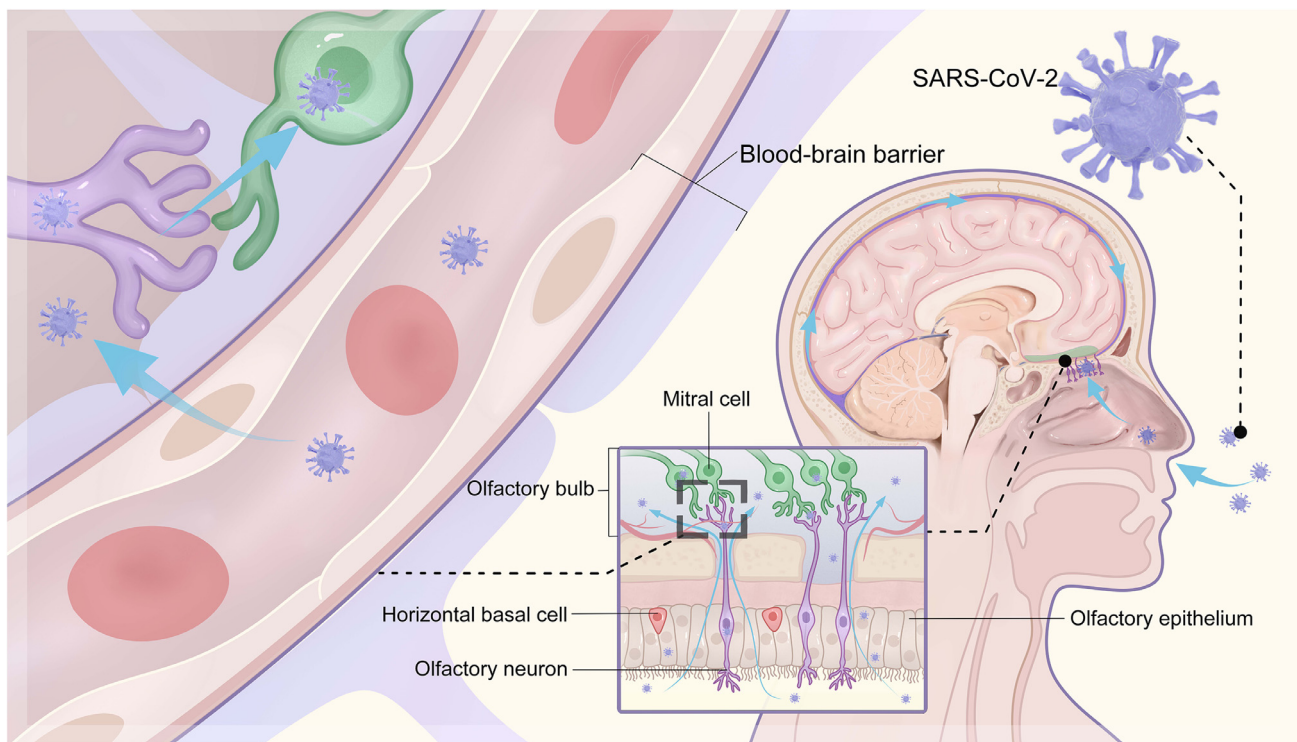
Fig. 3. A Schematic diagram shows possible pathways through which SARS-CoV-2 may enter the CNS. SARS-CoV-2 infects the CNS through the nose/OBs and hematogenous pathways. The differentiating olfactory neurons, termed horizontal basal cells, can be infected by SARS-CoV-2 since ACE2 receptors are expressed in them. Mature olfactory neurons spread the viruses to the OBs through synaptic connections. Subsequently, the viruses are able to spread throughout the brain since the OBs shares many connections with the brain parenchyma. SARS-CoV-2 can also infect the ACE2 expressing ECs, which causes the release of inflammatory cytokines and possibly the following cytokine storm. The cascaded inflammation disrupts the TJs, thus increasing the BBB permeability, which allows the viruses to cross the BBB and infect the CNS. CNS infection can potentially be attributed to viral spread through cerebrospinal fluid and lymphatic drainage. Abbreviation: SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; CNS, central nervous system; OBs, olfactory bulbs; ACE2, angiotensin-converting enzyme 2; ECs, endothelial cells; TJs, tight junctions; BBB, blood-brain barrier.

and brain parenchyma, together with inflammatory cytokines released by the neural cells and surrounding immune cells, lead to breakdown of BBB.

Furthermore, it is reported that smell dysfunction is found in patients with novel coronavirus [66], which means that SARS-CoV-2 infection may affect the olfactory nerve. In fact, as early as the early 20th century, scientists found that the olfactory region can be one of the ways for viruses including mouse hepatitis virus (MHV) and SARS-CoV-1 to enter the brain and drug delivery through the olfactory nerve pathway into the brain is shown to be effective. Additionally, TMPRSS2 and high intensity of ACE2 expression are found in the olfactory epithelium, which indicates that SARS-CoV-2 may target olfactory epithelium to enter and infect the human body [67]. Later research has revealed that SARS-CoV-2 could infect the olfactory nerve and brain in hamsters. In humans, the ACE2 expressing horizontal basic cells, surrounded by olfactory epithelium can mature into olfactory neurons. Because olfactory bulbs (OBs) share many connections with olfactory neurons and the brain, olfactory neurons, OBs, and the brain together form the pathway through which the virus enters the CNS [68]. This allows the virus to break through the BBB and infect the CNS. In addition, the tissue damage caused by SARS-CoV-2 infection may disable the barrier in the olfactory area, which may make the virus more likely to infect the brain through this pathway (Fig. 3) [68]. However, since viral RNA was also found in medulla oblongata [69], which is not directly connected with the OBs, involvement of other pathways in viral invasion to the brain was suggested. But later autopsy study suggested that brainstem infection may be attributed to viral infection through the nose via axonal transport (Fig. 3) [70]. A very recent study however, showed that no evidence of SARS-COV-2 infecting the OBs parenchyma could be found. But the presence

of viral RNA in the leptomeninges surrounding the OBs was found in eleven out of thirty patients, suggesting that even though SARS-CoV-2 cannot infect the CNS through the nose/olfactory bulb pathway, it can possibly through the circulating cerebrospinal fluid (Fig. 3). But SARS-COV-2 is, after all, not a neurotropic virus like rabies virus. The limited autopsy results showing no evidence of SARS-COV-2 infecting OBs parenchyma did not necessarily confirm that the virus could not infect OBs parenchyma. More evidence is needed to determine whether SARS-CoV-2 can infect the CNS through this pathway [71]. Additionally, recent study has suggested the potential role of lymphatic drainage in SARS-CoV-2 infection into the brain [72]. Taken together, SARS-CoV-2 may enter the brain through the nose, olfactory nerve, and defective BBB, cerebrospinal fluid and lymphatic drainage (Fig. 3).

However, definite evidence for neural injury due to SARS-CoV-2 is still sorely lacking. Available evidence comes from limited autopsy studies, which discover signs of SARS-CoV-2 infection in the brain. Nevertheless, whether the neurological symptoms result from direct infection of the SARS-CoV-2 remains unclear. Early research has found very low levels of viral RNA without viral protein detected in 5 out of 18 patients with neurological symptoms [73], which is inconsistent with the fact that the sufferers endure severe neurological symptoms including stroke, epilepsy, encephalitis. Curiously, the coronavirus that causes the common cold has also been found in the brain, but it is rarely accompanied by serious neurological symptoms as SARS-CoV-2 does [74]. These discoveries suggest that severe neurological symptoms found in COVID-19 patients are possibly not caused by direct infection of the SARS-CoV-2. The neurological symptoms in COVID-19 patients are likely to be correlated with systematic inflammation. Later studies found viral protein in the brain, which is in contradiction with previous autopsy studies [69].

Since a long postmortem interval is almost inevitable for autopsy studies, it is speculated that viruses may have already been eliminated by the time the autopsy was performed. Note that, those results of autopsy studies come from severely ill patients, whether it can be generalized to patients with moderate symptoms still needs further exploration.

As the world is entering the third year of the pandemic, the mounting COVID-19 cases and accompanied surge of mortality are chilling. Though scientists from all over the world have made outstanding contributions to the development of potential therapeutic strategies, more researches are still needed for the final conquer of this challenging plague. During this process, a accessibility of proper animal models is crucial and indispensable for the related scientific studies. Unfortunately, currently available animal models for brain infections of SARS-CoV-2, such as mice and Syrian hamsters, are still limited. Though SARS-CoV-2 does not cause severe neurological symptoms in mice as SARS-CoV does, the establishment of mouse model is still an appropriate and significant choice for the studies of COVID-19 [75].

8. Conclusion and future perspectives

The BBB is important in protecting the proper function of the brain under both physiological and pathology circumstance. Molecular determinants of the regulation of BBB permeability include pericellular junctions and membranous receptors or carriers responsible for chemical transportation into the brain. Though transportation through BBB is strictly restricted, the SARS-CoV-2 could somehow find its specific way invading into the brain. Existing evidences favor nose/olfactory pathway, hematogenous spread, and possibly lymphatic drainage and cerebrospinal fluid circulation as the possible routes of SARS-CoV-2 infection.

Decades of scientific studies have led us to a more comprehensive understanding on the BBB function and regulation. However, there are still major challenges in the exploration of BBB research filed. In the future, in-depth researches are required: (1) how the different NVUs from distinct blastoderms function and interact with each other during the development of the BBB; (2) the disruption and protection mechanisms of BBB in CNS diseases; (3) drug delivery strategies across BBB during the therapy of CNS diseases. Efforts on conquering above challenges will enable the delineation of mechanisms maintaining the BBB in the changing internal environment and the development of treatment targeting the CNS.

Declaration of Competing Interest

The authors declared that they have no conflicts of interest in this work.

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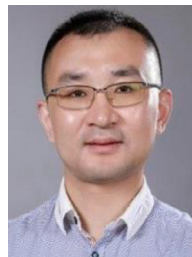
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