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Review Article

The role of the blood–brain barrier during neurological disease and infection

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A healthy brain is protected by the blood–brain barrier (BBB), which is formed by the endothelial cells that line brain capillaries. The BBB plays an extremely important role in supporting normal neuronal function by maintaining the homeostasis of the brain microenvironment and restricting pathogen and toxin entry to the brain. Dysfunction of this highly complex and regulated structure can be life threatening. BBB dysfunction is implicated in many neurological diseases such as stroke, Alzheimer's disease, multiple sclerosis, and brain infections. Among other mechanisms, inflammation and/or flow disturbances are major causes of BBB dysfunction in neurological infections and diseases. In particular, in ischaemic stroke, both inflammation and flow disturbances contribute to BBB disruption, leading to devastating consequences. While a transient or minor disruption to the barrier function could be tolerated, chronic or a total breach of the barrier can result in irreversible brain damage. It is worth noting that timing and extent of BBB disruption play an important role in the process of any repair of brain damage and treatment strategies. This review evaluates and summarises some of the latest research on the role of the BBB during neurological disease and infection with a focus on the effects of inflammation and flow disturbances on the BBB. The BBB's crucial role in protecting the brain is also the bottleneck in central nervous system drug development. Therefore, innovative strategies to carry therapeutics across the BBB and novel models to screen drugs, and to study the complex, overlapping mechanisms of BBB disruption are urgently needed.

Introduction

The blood–brain barrier (BBB) formed by brain capillary endothelial cells is the dynamic physiological structure that protects the brain to maintain normal neuronal function. These highly specialised endothelial cells have intercellular tight junctions such as claudins, occludin, zonula occludens, and junctional adhesion molecules that control the movement of molecules through the paracellular pathway ('gate function') by showing size and charge selectivity. An array of specific transporters, receptors and enzymes controls the molecular traffic via the transcellular route and permit the passage of nutrients and removal of waste products across the BBB. Tight junctions also act as a 'fence' to segregate these transporters to the apical and basal domains, so that the endothelium can act as a polarised barrier to prevent free movement of the transporters. The presence of complex intercellular tight junctions results in high transendothelial electrical resistance (TEER) in brain microvessels, compared with peripheral microvessels. In addition, brain endothelial cells lack fenestrations, have very few pinocytotic vesicles, and reduced expression of adhesion molecules, which limit immune cell infiltration (for a comprehensive review on the BBB see [1]).

Disruption of the BBB can leave the brain vulnerable to damage. Understanding the detailed cellular and molecular mechanisms of BBB disruption has been a long-standing interest in the field. However, in many neuropathological conditions, whether BBB dysfunction is a causative or

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consequence of the pathology remains incompletely understood. Investigating these mechanisms can be challenging, due to the complexities with direct assessment of BBB function. However, recent developments in the field have provided some insights. This review aims to evaluate some of these latest developments and summarises the findings with a particular focus on the effects of inflammation and flow disturbances on the BBB during neurological disease and infection, and discusses whether timing of BBB disruption plays a role.

An intact BBB is extremely important for regulating central nervous system (CNS) homeostasis to maintain normal neuronal function, and to protect the brain from fluctuations in plasma neurotransmitter levels, circulating pathogens and toxins. However, the BBB does not function independently, but is in continuous 'cross-talk' with other cells [2] such as astrocytes [3,4], pericytes [5,6], neurones [7,8], perivascular macrophages [9], microglia [10] and immune cells [11]. The unique relationship between these cells and the BBB gave rise to the concept of the neurovascular unit (NVU) (Figure 1).

Role of astrocytes and pericytes in supporting the BBB

The NVU is a relatively new concept. However, the critical importance of astrocytes in the induction and maintenance of BBB structure and function has long been established (reviewed in [12]). Brain capillaries are surrounded by perivascular endfeet of astrocytes and therefore astrocytes occupy a strategic position between brain capillaries and neurons. This close proximity and the ability of astrocytes to secrete soluble factors [13,14] allow them to induce BBB phenotype in brain endothelial cells. Most *in vitro* BBB models use astrocytes in co-culture to take advantage of the astrocyte-secreted factors to increase the tightness/TEER of brain endothelial cells [15,16]. These *in vitro* studies have provided a great deal of information to support the role of astrocytes in up-regulating many BBB features, including low paracellular permeability and up-regulation of tight junctions [14,17,18], transporters [19–22] and enzymes [23]. Although astrocytes are important for the development and maintenance of the BBB, recent evidence suggests that it is pericytes that are critical for the formation and induction of the BBB and are implicated in contributing to the progression of CNS disease.

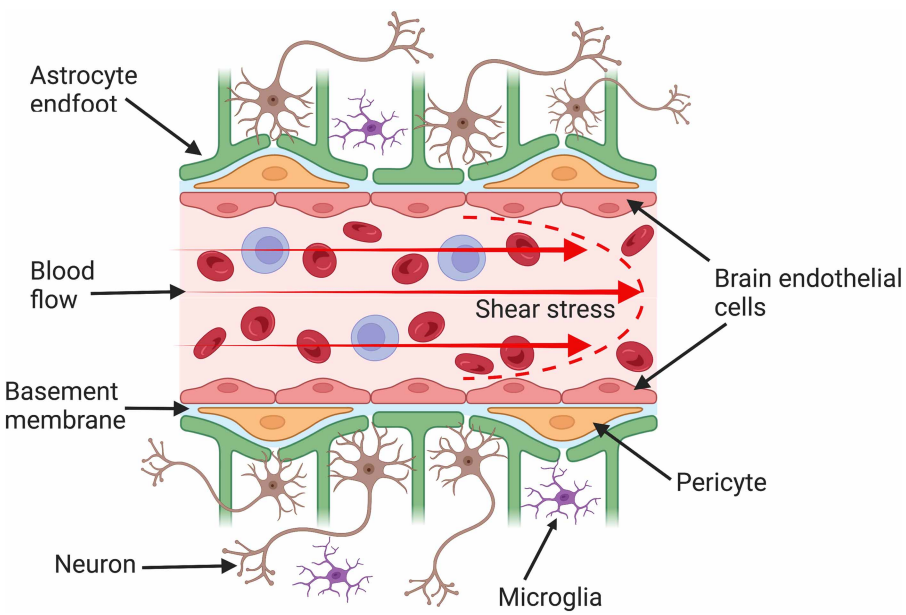

Figure 1. Structure of the blood–brain barrier (BBB).

The BBB is formed by brain endothelial cells that line cerebral capillaries and is the main protective physiological barrier preventing the entry of toxins and pathogens into the brain. These endothelial cells are supported by other cells such as astrocytes and pericytes of the neurovascular unit, which are important in inducing and maintaining BBB characteristics. Brain endothelial cells differ from those of peripheral tissues by having more complex intercellular tight junctions that restrict the paracellular permeation of molecules through the junctional cleft. Furthermore, endothelial cells *in vivo* are continuously exposed to shear stress (the frictional force generated by blood flow), which affects endothelial cell structure and function.

Pericytes are embedded in the basement membrane in the abluminal surface of brain microvessels. Unlike astrocytes, they only partially cover the microvessel, but their processes can span several endothelial cells. Due to a lack of pericyte-specific markers, pericytes can be difficult to differentiate from other mesenchymal cells such as vascular smooth muscle cells that also sit in the basement membrane [24]. Pericytes extend their processes along and around pre-capillary arterioles, capillaries and post-capillary venules, and may have different morphological and functional features depending on their position along the vascular tree [25]. The heterogeneity of pericyte morphology and the lack of specific pericyte markers have led to controversy and confusion about their functions [26,27]. Nevertheless, pericytes have been shown to have diverse functions including regulating BBB permeability and integrity [6,28], cerebral blood flow regulation and neurovascular coupling [29,30], secreting neuroinflammatory mediators [31], involvement in fibrosis following neuronal injury [32], cognitive decline and Alzheimer's disease [33,34] clearance of neurotoxins [35], providing neurotrophic support [36], maintaining white matter structure and function [37], potential to transform into multipotent stem cells [38,39] and are critically important for angiogenesis [40,41] and induction of the BBB.

Convincing evidence for the role of pericytes in BBB induction came from studies using mice that were genetically modified to have defects in pericyte generation by targeting the platelet-derived growth factor (PDGF)-B/PDGF receptor-β (PDGFR-β) signalling pathway. These studies showed that many BBB properties such as expression of occludin, claudin-5, zonula occludens-1 and BBB influx transporter Glut-1 were present a week earlier than astrogliogenesis [5], at the time of pericyte recruitment by endothelial cells via PDGF-B secretion during early embryogenesis [42]. Pericyte recruitment coincided with BBB sealing, as the pericyte-deficient mice had increased BBB permeability and increased expression of leukocyte adhesion molecules. They showed that the extent of the pericyte coverage determined BBB permeability. Remarkably, the basis for this increased permeability was an up-regulation of endothelial transcytosis and not a lack of tight junctions [5,6]. Thus, pericytes are critical during BBB development, and maintain BBB properties by inhibiting the expression of 'leaky' BBB features (such as transcytosis and expression of leukocyte adhesion molecules) to stabilise vessels, as they do not directly affect tight junction protein expression. Interestingly, this study also showed that pericytes express cues for guiding astrocyte endfeet attachment to endothelial cells, and the absence of pericytes led to abnormal endfeet polarisation [6]. Therefore, pericytes seem to play an important role in orchestrating the proper formation of the BBB and the NVU. Given that astrocytes are unlikely to be required for BBB induction, they are expected to be important for maintaining the fully differentiated BBB phenotype during adulthood and involved in BBB response to disease.

Mechanisms of BBB disruption

Destabilisation of the BBB occurs under or leads to several pathological conditions including stroke [43,44], Alzheimer's disease [45], multiple sclerosis [46,47], epilepsy [48–50], viral encephalitis [51–53], COVID-19 [54–56], Neuro Aids [57], malaria [58–60] and sequelae of traumatic brain injury [61] as well as peripheral diseases such as atrial fibrillation (AF) [62] (Figure 2). While some of the mechanisms of BBB disruption are CNS-derived (e.g. microglia, glutamate), others are peripheral in origin. Dysfunction of the BBB during most neurological diseases can lead to increased permeability (due to disrupted tight junctions and increased transcytosis) [63–65], leading to immune cell infiltration, [46] neuroinflammation [66] and oedema [67,68]. Leukocytes, interleukins, and other soluble factors are known to disrupt the integrity of the BBB [69–71]. Therefore, anti-inflammatory manoeuvres could prevent or repair the BBB [72–74]. In addition, disruption in ion regulation and transporter function in BBB [43,75–77] has also been reported, which severely impact neuronal function [78]. The initiation of this damage can be either due to a direct assault on the BBB, for example during infections, or due to secondary neurological damage, which leads to activation of neuroinflammatory pathways as is the case of ischaemic stroke.

Increased BBB permeability leading to cerebral oedema [68,79] and haemorrhagic transformation [80,81] are common complications of ischaemic stroke, which can impact the outcome of these patients with potential serious and life-threatening consequences [82]. A series of factors or events in combination or in sequence can lead to BBB disruption in ischaemic stroke. For example, several factors including reactive oxygen species (ROS) [83], pro-inflammatory cytokines [67,84] and matrix metalloproteinases (MMPs) [85–88] have been implicated in BBB damage after stroke. Neuronal injury following ischaemia leads to the release of proinflammatory mediators such as tumour necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), IL-1α, IL-1β and interferon-γ (IFN-γ) that can activate microglia, astrocytes and endothelial cells, promoting the expression of molecules that can contribute to NVU breakdown [83,89]. This local inflammation leads to the up-regulation

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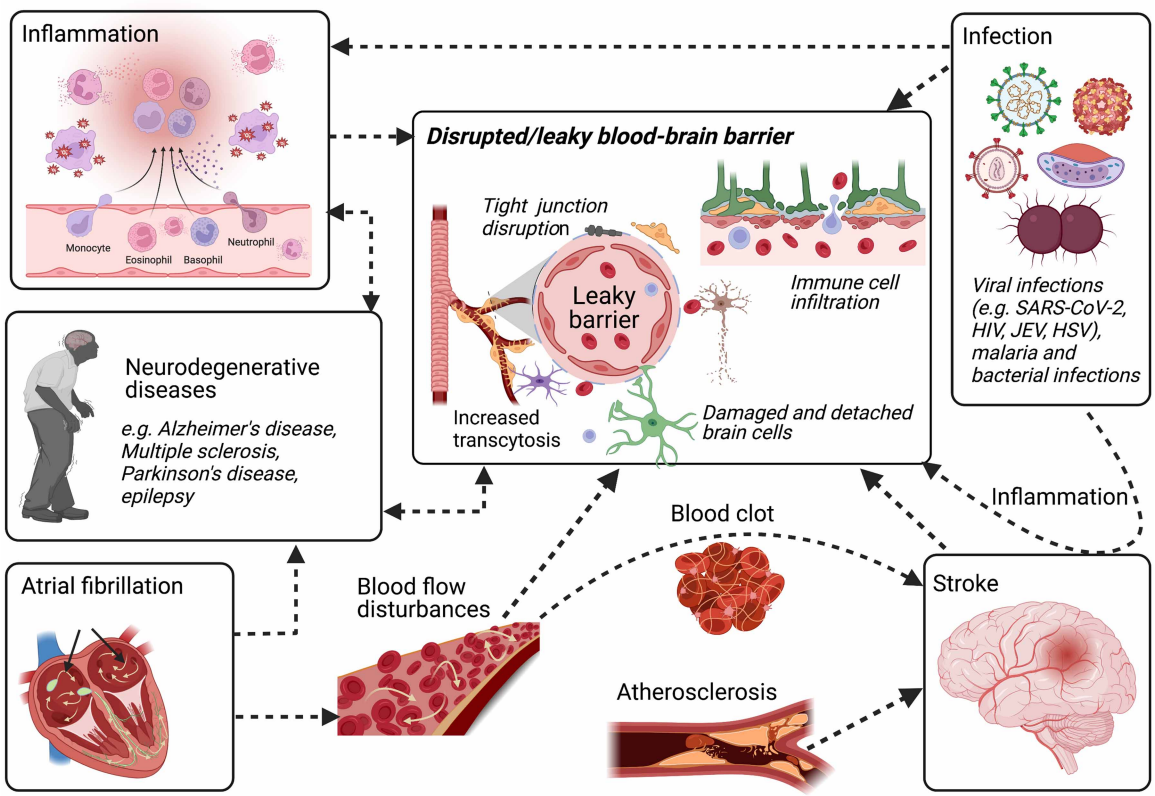

Figure 2. Blood–brain barrier (BBB) disruption during neurological infections and diseases.

BBB disruption is implicated in many neurological infections and diseases, including neurodegenerative diseases, ischaemic stroke as well as viral infections such as SARS-CoV-2, and peripheral diseases such as atrial fibrillation. Disruption of the BBB may involve the opening of tight junctions, damage to the endothelium, increased transport of molecules across the BBB (transcytosis) and alterations in transport systems. Inflammation is a final common pathway in many neurological diseases, allowing immune cell and pathogen entry to the brain, which leaves the brain vulnerable to damage. Furthermore, abnormal flow patterns or flow cessation can lead to changes in shear stress or pulsatility. This can deteriorate the brain endothelium and lead to barrier impairment. Deterioration in neuroprotective BBB function plays a major role in the pathogenesis of disease since the BBB dynamically responds to many events associated with flow disturbances, oxidative stress and proinflammatory cytokine generation. Any condition that affects the functional integrity of the BBB will cause secondary effects on cerebral blood flow and vascular tone, resulting in further damage to the brain.

of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), P-selectin in endothelial cells and secretion of MMPs such as MMP2 and MMP9 [86,88]. The enhanced expression of adhesion molecules in brain endothelial cells attracts leukocytes and platelets, and promotes the tethering/rolling, firm adhesion and transmigration of these cells across the BBB [90]. Adhesion of leukocytes causes further damage due to the activation of signalling pathways and release of ROS and inflammatory cytokines and MMPs from other cells of the NVU. This creates a proinflammatory environment, which further activates the endothelium and leads to increased BBB permeability. Increased permeability due to inflammation has been demonstrated using magnetic resonance imaging (MRI) at both the acute and chronic phases of stroke in patients with ischaemic stroke [91,92] and in animal models [93,94].

BBB damage due to inflammation is a final common pathway in many brain infections as well, allowing pathogens and immune cells access to the brain, and leading to CNS damage. For example, an inflammatory cytokine-mediated pathway is implicated for the BBB disruption seen in encephalitis caused by viruses such as Japanese encephalitis virus (JEV) [51,95], herpes simplex virus (HSV) [52,53], West Nile virus (WNV) [96,97] and human immunodeficiency virus (HIV) [98,99] and in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [56,100]. Several studies have provided evidence for an important role for inflammation in BBB

disruption and disease outcome. In clinical samples from patients with Japanese encephalitis, increased levels of pro-inflammatory cytokines and chemokines in cerebrospinal fluid (CSF) were shown to be associated with poor outcome [101]. Another study using human autopsy material showed that perivascular inflammation and a damaged BBB are associated with widespread perivascular oedema [102]. Further studies using *in vitro* human BBB models have provided evidence for JEV-induced inflammation causing increased BBB permeability [51,103]. Pro-inflammatory cytokine milieu seems to be main the driving force contributing to increased BBB permeability in JEV [51]. This was also demonstrated in a mouse model where the virus was detected in the brain and the onset of inflammation occurred prior to BBB disruption [95]. It is now established that JEV does cross the BBB, causing neurological damage primarily due to the resulting inflammatory response.

Mechanisms of neuroinvasion of other viruses such as SARS-CoV-2 are still under investigation. SARS-CoV-2 has been detected in the brains of patients with severe disease who present with neurological symptoms [104–106]. Among others, the most common neurological symptoms presented in hospital by patients infected with SARS-CoV-2 are stroke, seizures and encephalitis or meningitis [107]. BBB disruption is generally implicated in these neurological manifestations. A post-mortem study of patients with COVID-19 demonstrated microvascular damage and fibrinogen leakage, indicating a disrupted BBB. However, the study failed to detect SARS-CoV-2 in the brain, possibly due to the virus being cleared at the time of death or detection limitations in the assay used [108]. Another study by the same authors showed a significant increase in serum protein leakage, platelet accumulation as well as increased expression of platelet endothelial cell adhesion molecule-1 (PECAM-1) and von Willebrand factor in patients that died from COVID-19 compared with control subjects [109]. Furthermore, macrophages and some CD8+ T cells infiltration were also detected, similar to other neurological diseases where a leaky BBB leads to immune cell infiltration. The results suggest that SARS-CoV-2 infection could cause BBB disruption via activation of the endothelium leading to increased BBB permeability. Indeed, a recent study in COVID-19 patients has demonstrated that patients with neurological complications had the highest levels of biomarkers associated with BBB disruption, such as MMP9 [110].

The angiotensin-converting enzyme 2 (ACE2) has been shown to be the host receptor responsible for binding SARS-CoV-2 [111]. ACE2 is present in brain vascular endothelial cells [112] and the mechanisms of SARS-CoV-2 infection have been implicated in brain endothelial dysfunction and BBB damage, which may explain the neurological manifestations compounded by the effects of the systemic inflammatory response [113]. Convincing evidence from clinical/post-mortem studies is still lacking to provide a definite answer to whether BBB disruption due to SARS-CoV-2 infection is due to a direct infection of endothelial cells and/or the resulting inflammation. Results from animal and *in vitro* studies have provided some clues. SARS-CoV-2 can be detected in the brain [114] and that the virus is able to infect brain endothelial cells [115] and cause BBB disruption [116]. In addition, SARS-CoV-2 spike protein has been shown to increase BBB permeability, trigger a pro-inflammatory response (increased expression of cell adhesion molecules) and up-regulate the expression of MMPs in 2D static and 3D microfluidic *in vitro* human BBB models [56]. SARS-CoV-2 spike proteins have also been shown to induce brain endothelial dysfunction [117]. Another study using K18-hACE2 transgenic mice and Syrian hamsters demonstrated that SARS-CoV-2 can cross the BBB with direct infection of brain microvascular endothelial cells in both *in vivo* and *in vitro* models [118]. Infection led to an increase in BBB permeability with disrupted basement membrane via MMP9 mediated pathway. However, alterations in tight junctions were not observed. In agreement with other studies, pro-inflammatory modulators were also up-regulated. Others have reported conflicting findings, where SARS-CoV-2 replication in human brain endothelial cells was shown to be weak, and infection of the cells did not affect BBB integrity or lead to an increase in inflammation despite inoculating the cells with a high virus load [119]. It is possible that BBB disruption in COVID-19 is due to inflammation triggered by circulating pro-inflammatory cytokines in response to the systemic disease. Furthermore, the resulting leaky BBB could increase neuroinvasion of the virus, which could further exacerbate inflammation via triggering pro-inflammatory responses of other cells of the NVU.

Finally, it is also worth noting that stroke and COVID-19 pathophysiology exhibit overlapping molecular mechanisms that contribute to BBB disruption. In particular, in both conditions, inflammation plays a major role in the pathophysiology of CNS disease via vascular dysfunction. While in COVID-19, peripheral inflammation seems to be the major trigger of BBB disruption, in stroke, the inflammatory response of the cells forming the NVU plays a major role. Astrocyte activation in ischaemic stroke has a major impact on brain damage and repair mechanisms [120]. Similarly, Sánchez and Rosenberg propose that astrocyte dysfunction may play a major role in contributing to stroke development in COVID-19 patients, and that further understanding of the

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molecular pathways could provide potential insights into new therapies to combat neurological dysfunction due to SARS-CoV-2 infection [121].

Timing and extent of BBB disruption during neurological disease

BBB disruption can manifest as a transient event with minor consequences on normal brain function or lead on to a chronic or total breakdown of the barrier properties, resulting in significant and/or irreversible brain damage. In neurological diseases such as stroke [122–126], traumatic brain injury [127,128] and Alzheimer's disease [129], as well as in some neurological infections [130], BBB disruption has been demonstrated to be biphasic in nature. For example, experimental studies in ischaemic stroke have provided extensive evidence that demonstrates an early opening of the BBB within the first 4–6 h of a stroke is followed by a refractory period and then a late opening ∼48–72 h [131,132]. However, there is also evidence to suggest BBB recovery is not complete following the initial opening, and that BBB continues to be leaky [133,134]. Some studies have also shown further disruption to the BBB where BBB permeability is increased after 7 days or more post-reperfusion [135,136]. Conflicting data from these studies could be the result of using different stroke models and methods for detecting BBB disruption, but could also be due to the heterogenous nature of the mechanisms responsible for BBB disruption and stroke severity.

While BBB tight junction protein disassembly and reassembly have been shown to be responsible for BBB disruption following ischaemia [125], mainly via degradation of tight junction proteins by MMPs [137–140], other mechanisms such as an increase in the number of endothelial caveolae, up-regulation of endothelial transcytosis [141] and disruption of the glycocalyx [126] have also been suggested to play an important role depending on the phase of BBB disruption. For example, Knowland et al.'s study using a novel transgenic mouse strain with Claudin-5 labelled with eGFP demonstrated that tight junction disruption only appeared at the late phase, ∼48–58 h post-stroke, while the rate of transcytosis and the number of endothelial caveolae increased in the early phase at 6 h post-reperfusion [141]. Traditional view of BBB tight junction disassembly during the early phase of reperfusion has been questioned by several studies, which strongly suggest that transcellular permeability rather than paracellular permeability is increased during this phase, given that these studies demonstrate normal tight junction morphology despite elevated vascular permeability/transcytosis at the BBB [126,142–146]. Therefore, maintaining low rates of transcytosis appear to be important in maintaining barrier properties following stroke, and therapeutics that target up-regulated transcytosis, which precedes tight junction abnormalities will have significant potential in preventing BBB disruption in stroke.

There is now also strong evidence to demonstrate that BBB breakdown could be considered as an early biomarker of neurological disease. For example, in individuals with early cognitive impairment, brain capillary damage and BBB disruption can be seen independently of Alzheimer's Aβ and/or tau biomarker changes [147]. Another study has demonstrated that in carriers of the main susceptibility gene for Alzheimer's disease (E4 variant of apolipoprotein E), breakdown of the BBB contributes to cognitive decline independently of Alzheimer's disease pathology [33]. In addition, age-dependent early BBB breakdown has also been implicated in the hippocampus, which worsened with mild cognitive impairment [148]. In patients with early Alzheimer's disease, increased BBB permeability is associated with cognitive decline [149]. These studies suggest that compromised BBB is an early indication of developing cognitive deficits and dementia.

Role of shear stress in BBB

One of the distinctive features of endothelial cell physiology is their exposure to pulsatile shear stress [150,151]. This is a most neglected aspect for *in vitro* modelling of endothelial cells, where the majority of systems used lack a physiological level of shear stress [152]. When exposed to shear stress to levels comparable to *in vivo*, endothelial cells respond by morphological, transcriptional, and functional levels. In addition, it is becoming increasing accepted that altered shear stress promotes pathological changes in vascular function. At the morphological level, it has been shown that endothelial cells align with flow [153–155]. This is likely due to calcium influx-mediated cytoskeletal rearrangement [151,156]. Atherosclerosis-prone regions of larger arteries have endothelial cells exposed to irregular, complex flow patterns with a non-physiological magnitude of shear stress (low levels and rapidly changing direction) [157]. After complete flow cessation, endothelial cells re-enter cell cycle and 'pile up' onto each other [158–160]. This is commonly observed in traditional, no-flow models *in vitro* [160]. Transcriptional changes in endothelial cells exposed to shear stress were first reported by the

Janigro group [158,159]. The changes in mRNA levels were primarily related to cytoskeletal remodelling, abolishment of cell cycle (mitotic arrest and differentiation), glucose metabolism and nicotinamide adenine dinucleotide (NADH) production (shift of glycolytic efficiency towards reduction mechanism to counter oxidative stress), and transporter levels and membrane positioning [20,161]. From the functional viewpoint, the main effect of shear is the control of vascular tone by a mechanism mediated by calcium entry and nitric oxide (NO) production by endothelial nitric oxide synthase (eNOS) [162–164]. NO released by endothelial cells causes smooth muscle relaxation and increased blood flow to the organ.

Pathophysiology of BBB due to flow disturbances

At the pathological level, turbulent flow (altered shear stress) or flow cessation/reperfusion have been shown to alter vascular function by impeding many of the physiological mechanisms listed above. In addition, low NO levels have a prothrombotic effect, triggering the formation of emboli and ultimately causing stroke. A paradigm of pathological changes due to altered flow is evident during AF. AF, the most common cardiac dysrhythmia, is associated with poor outcomes, including stroke [165–167]. The incidence of stroke attributable to AF increases from 1.5% at age 50–59 years to 23.5% at age 80–89 years [168]. Over 12 million people worldwide have a stroke yearly [169], of which at least 25% have been directly attributed to clinically diagnosed AF [170]. The adverse effects of AF are due to haemodynamic changes with multiple factors leading to a prothrombotic state [171]. AF also affects the BBB (For a comprehensive review on the effects of AF on BBB, see [62]) and elevates biomarkers of cerebral injury [172]. A link between AF, the BBB and cognitive impairment has been proposed [62]. NO has been recognised as a key component in the regulation of vascular tone and in mediating the prothrombotic state in AF [171]. NO production by endothelial cells is reduced in AF [173,174]; reduced NO affects vessel diameter and increases the probability of thrombus formation [175]. Reversal of AF to normal rhythm also reverses endothelial cell dysfunction [176]; AF-like pacing reduces NO production by endothelial cells in an animal model and *in vitro* [177]. Finally, wall shear stress, the frictional force of blood flow tangential to an artery lumen, has been demonstrated in multiple studies to influence aneurysm formation and risk of rupture [178,179].

If one looks more specifically at the brain vasculature and the BBB, most of the findings on systemic vessels hold true. Intriguingly, Aryal and Patabendige have suggested that AF may be causative of cognitive decline by a mechanism involving altered shear stress [62]. This was also suggested in seminal translational articles on aging and the vascular unit [180,181]. However, only sparse data [157,158] are available addressing BBB-specific changes triggered by altered shear stress, since most of the literature has focused on endothelial sequelae after embolic or cardiogenic stroke and reperfusion. Novel tools will be required to study the effects of altered vascular perfusion on NO production and TEER in cultured brain endothelial cells exposed to pulsatile flow (but see [164,182–184]). Ideally, to study AF on brain vasculature endothelial cells one should devise a system where shear stress can be modulated beyond the usual parameters of increasing or decreasing steady-state perfusion (and shear), flow cessation/reperfusion, and static conditions. In particular, a system where AF-derived abnormal heart rhythms to trigger perfusion events would be useful to study acute and chronic effects on endothelial cell cultures exposed to pathological levels of intermittent shear and turbulence.

Concluding remarks

A healthy brain is protected by the BBB, and is extremely important for the normal functioning of neurons. However, during many neurological diseases and infections, this barrier is disrupted, leaving the brain vulnerable to further damage. Worldwide, neurological disorders remain the main cause of disability and the second leading cause of death [185]. Therefore, novel therapies are urgently required. However, given the highly selective nature of the BBB, drug penetration into the CNS has always been a major hurdle in developing treatments for neurological disorders [186]. To overcome this hurdle, several innovative approaches are currently being developed. Some of the novel techniques include, using receptor-mediated transcytosis to deliver nanoparticles [187], cell-penetrating peptide conjugated adeno-associated viruses (AAVs) to deliver novel gene therapies [188], organic cation transporters 1 and 2 (Oct1/Oct2) to deliver novel stroke therapeutics [189], intranasal delivery methods [190], cell therapies [191] and focused ultrasound (FUS) in conjunction with gas-filled microbubble contrast agents [192]. Finally, to make progress, the development of suitably robust and reliable *in vitro* BBB models for high throughput drug screening as well as models that can mimic the complex pathophysiology of BBB dysfunction to elucidate the underlying mechanisms are essential.

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Perspectives

- BBB disruption is implicated not only in neurological infections and diseases but also in peripheral diseases, leading to CNS damage. Yet, our understanding of the complex mechanisms underlying the pathophysiology of BBB dysfunction remains incomplete.
- Deterioration of neuroprotective BBB function plays a major role in the pathogenesis of disease since the BBB dynamically responds to many events associated with inflammation and flow disturbances, which can cause brain damage.
- Novel models that closely simulate the structure and function of the BBB are required to study the complex cellular and molecular pathways that are disrupted during neurological disease. Furthermore, innovative strategies are required to deliver therapeutic drugs across the BBB for CNS disease.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

ACE2, angiotensin-converting enzyme 2; AF, atrial fibrillation; BBB, blood–brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; JEV, Japanese encephalitis virus; MMPs, matrix metalloproteinases; NO, nitric oxide; NVU, neurovascular unit; ROS, reactive oxygen species; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; TEER, transendothelial electrical resistance.

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