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Tension at the gate: sensing mechanical forces at the blood–brain barrier in health and disease

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Abstract

Microvascular brain endothelial cells tightly limit the entry of blood components and peripheral cells into the brain by forming the blood–brain barrier (BBB). The BBB is regulated by a cascade of mechanical and chemical signals including shear stress and elasticity of the adjacent endothelial basement membrane (BM). During physiological aging, but especially in neurological diseases including multiple sclerosis (MS), stroke, small vessel disease, and Alzheimer's disease (AD), the BBB is exposed to inflammation, rigidity changes of the BM, and disturbed cerebral blood flow (CBF). These altered forces lead to increased vascular permeability, reduced endothelial reactivity to vasoactive mediators, and promote leukocyte transmigration. Whereas the molecular players involved in leukocyte infiltration have been described in detail, the importance of mechanical signalling throughout this process has only recently been recognized. Here, we review relevant features of mechanical forces acting on the BBB under healthy and pathological conditions, as well as the endothelial mechanosensory elements detecting and responding to altered forces. We demonstrate the underlying complexity by focussing on the family of transient receptor potential (TRP) ion channels. A better understanding of these processes will provide insights into the pathogenesis of several neurological disorders and new potential leads for treatment.

Keywords Blood–brain barrier, Mechanical forces, Wall shear stress, Basement membrane stiffness, Transendothelial migration, TRP, Multiple sclerosis, Alzheimer's disease, Stroke, Small vessel disease

Background

Exchanges of circulating molecules and cells between the bloodstream and central nervous system (CNS) are regulated by the neurovascular unit, a multi-layered structure consisting of perivascular astrocytes, microglia, pericytes, and specialized brain microvascular endothelial cells (BMECs) comprising the blood–brain barrier (BBB) [1–3] (Fig. 1, central part). The BBB is the innermost, monocellular layer of the brain microvasculature forming a tight physical barrier that is characterized by a unique network of adherens junctions (AJs) and tight junctions (TJs) [3, 4], which respond to internal and external mechanical forces of the local microenvironment [5, 6] (Fig. 1B). AJs consist of transmembrane protein complexes, such as vascular endothelial (VE-) cadherin [1, 4], which connect to the intracellular actin cytoskeleton via catenins [4, 7]. TJs are located at the basolateral side of BMECs and include claudin-5, occludin, junctional adhesion molecules (JAMs), and tri-cellular junction proteins

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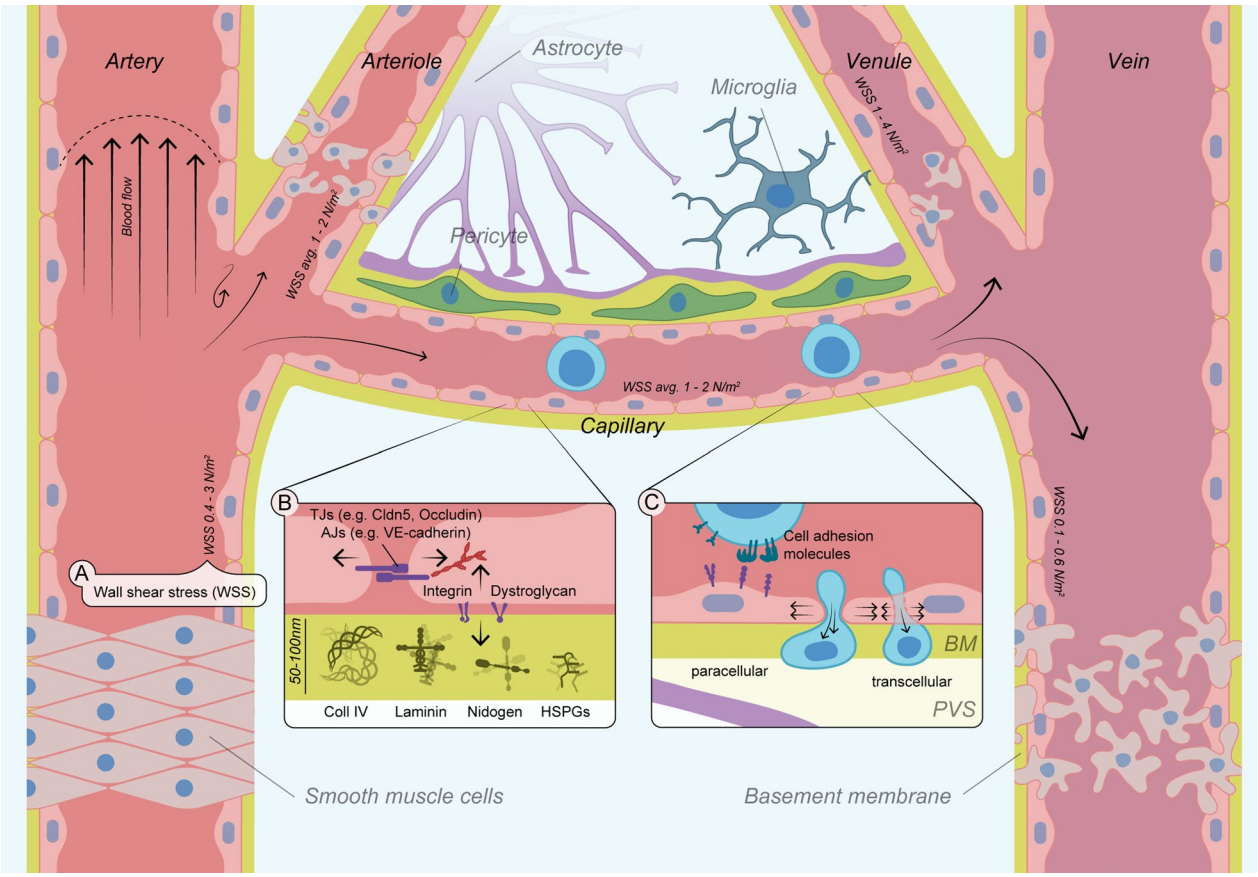

Fig. 1 A trio of mechanical forces acting on the brain endothelium. Forces and their directionality are presented by black arrows. **A** Wall shear stress (WSS) acts on the luminal side of the BMECs in the direction of the cerebral blood flow (CBF). **B** Abluminally, BMECs are anchored to the basement membrane (BM) and use integrin-mediated mechanotransduction to respond to the underlying stiffness. Basolateral junction complexes include tight junctions (TJs) and adherens junctions (AJ) creating inter- and intracellular tension. (**C**) Mostly under pathological conditions: Immune cell migration across the BBB is a multi-step cascade with mechanical forces acting on both cellular players, the immune cell, and BMECs. Figure is based on Vanlandewijk et al. [37]. *HSPGs* Heparan sulfate proteoglycans, *Coll IV* Collagen IV, *PVS* perivascular space, *BM* basement membrane

such as tricellulin [8–10]. Together, they form a robust paracellular connection and generate intercellular tension, giving rise to the exceptionally low permeability of the BBB [1, 3]. Finally, integrin-based focal adhesions anchor BMECs to the underlying basement membrane (BM), a network of extracellular matrix (ECM) proteins, and an important source of mechanical force (Fig. 1B) [11]. To maintain their integrity, BMECs sense and adapt to changes in forces including cerebral blood flow (CBF), BM elasticity, and cell–cell interactions such as during transendothelial migration (TEM) of peripheral immune cells across the BBB (Fig. 1A–C) [1, 4, 8].

Pathological changes of the BBB are found in many cerebral disorders. In multiple sclerosis (MS), a chronic neuroinflammatory disease, BBB impairment, and immune cell infiltration are disease hallmarks and tightly connected to the formation of demyelinated plaques [12, 13]. Alzheimer's disease (AD) is characterized by aggregation of amyloid beta (Aβ) and accumulation of hyperphosphorylated tau forming neurofibrillary tangles [14]. These are further accentuated by vascular changes including BBB injury, reduced CBF, and enlarged perivascular spaces [15]. Impaired Aβ clearance is a common feature of AD and cerebral amyloid angiopathy (CAA), whereas in the latter Aβ accumulates particularly around the vasculature, further impairing vascular reactivity and possibly permitting haemorrhages [16]. Reduced vascular function through vessel stiffening and chronic hypertension is commonly found in aging cerebral vasculature and is known to increase the risk for stroke (haemorrhagic and ischemic) and sporadic small vessel disease [17, 18]. Overall, vascular stiffening and disturbed CBF likely result in reduced vascular reactivity, enhanced perivascular aggregate formation, and eventually focal hypoxia, contributing to the (vascular) pathology observed in numerous age-related neurodegenerative disorders [19–28].

Many detrimental vascular changes in cerebral disorders follow and include distinct mechanical components such as alterations of BM composition and wall shear stress (WSS) acting on the microvasculature (Fig. 1A, B). WSS or often referred to as simply shear stress defines the arising force within a moving fluid and between the fluid and vessel wall [29]. Additionally, neuroinflammation facilitates the migration of peripheral immune cells across the BBB, which adds cell–cell interaction forces to the mechanical trio [30, 31] (Fig. 1C). TEM of immune cells into the brain is a hallmark of MS, but also gains increasing attention in AD patients, stroke, and healthy elderly [32, 33]. This migration cascade governs many biomechanical and biochemical interactions from start to finish. Whereas biochemical signalling has been described and reviewed in detail [1, 3, 8, 34], the importance of mechanical stress has only recently been realised.

It becomes apparent that changes in mechanical forces largely influence vascular function and dysfunction and might be early drivers of neuroinflammatory and neurodegenerative diseases. In this review, we briefly summarize our current knowledge of the two main mechanical stressors, WSS and BM stiffness, acting on the BBB and their pathological changes. Then, we highlight the forces measured during TEM of immune cells across the BBB and BBB-specific migration behaviour. Finally, we focus on mechanosensory/sensitive elements of BMECs and spotlight members of the transient receptor potential (TRP) ion channel family to showcase the intricacies of mechanical signalling [35, 36].

Mechanical forces at the BBB

Cerebral vessels are subjected to a range of mechanical stressors (Table 1), which play a key role in BBB maintenance, activation, and function [38, 39]. Changes in mechanical stimuli are sensed by specialized mechanosensors and translated into biochemical signalling cascades [40], culminating in cytoskeletal reorganization, gene regulation, and epigenetic chromatin modification [41]. Disturbances in mechanical forces including CBF and BM stiffness are often concomitant and recognized as early signs of numerous cerebral disorders [5, 39]. Below, the force ranges and impact on the cerebral vasculature are discussed.

External, non-cellular forces

Wall shear stress

Wall shear stress (WSS) or endothelial WSS is calculated from the blood viscosity (highly dependent on concentration and mechanical properties of red blood cells) and axial flow velocity gradient at the vessel wall [66]. WSS, arising from laminar or disturbed CBF at the luminal side of BMECs, plays a critical role in maintaining the structure and function of the BBB [38]. Under healthy conditions, the average capillary WSS in the brain ranges from 1 to 2 N/m^2 [46, 47] (Table 1, Fig. 1A). ECs experience the highest WSS within capillaries and declining hyperbolically to 0.28 N/m^2 at larger diameter post-capillary venules [48, 67]. This WSS was shown to be required for the differentiation of vascular ECs into a BMEC phenotype [68] using a dynamic in vitro BBB model. In vitro application of capillary-like WSS also inhibited cell proliferation, probably due to induction of the cyclin-dependent kinase inhibitor p21, causing cell cycle arrest [5, 69]. Recent advances in BBB models, which incorporate external

Table 1 Mechanical stressors and forces acting at the vasculature. Summary of non-cellular and cellular mechanical forces at the endothelium measured in N/m^2 (equals 10 dyne/cm^2 or 1 Pa)

Stressor	Area/cell type	Force range	References
External non-cellular forces			
Wall shear stress	Arteries	0.4–3 N/m^2	[39, 42–45]
	Arterioles & capillaries	Avg. 1–2 N/m^2	[46–50]
	Post-capillary venules & veins	< 0.1–4 N/m^2	[39, 48, 49, 51]
BM stiffness	Arteries & microvessels	~ 50 K to 150 K N/m^2 or higher	[39, 52–55]
	Venules/venous tissue (outside brain)	~ 3 K-50 K N/m^2	[54, 56]
Cell–cell interaction forces			
Selectin catch bonds	Leukocytes & ECs	10–30 pN/bond, maybe up to 60 pN	[57–60]
Integrin catch bonds	Leukocytes & ECs	10–30 pN/bond	[61, 62]
Crawling	T cells	up to 0.6 nN/cell	[63]
Diapedesis	Leukocytes	up to 60 nN/cell	[58]
Cell–cell junctions e.g. VE-cadherin and PECAM-1 binding	ECs	10–100 pN/bond (depending on number and type of interacting proteins)	[64, 65]

mechanical forces such as shear stress and ECM stiffness, have been reviewed elsewhere [70–74].

CBF dynamics strongly alter with age, occlusion and inflammation, whereas both high and low WSS are associated with brain pathology [75]. Aberrant high WSS (>4 N/m^2) occurs in hypertension and causes vascular dysfunction through loss of TJ proteins and activation of inflammatory signalling at the BBB [38, 76–78]. Hypertension and cerebrovascular injury can manifest in small vessel disease, which often presents an intermediate state leading to ~ 30% of ischemic stroke and ~ 80% of haemorrhagic intracerebral bleedings [79–82]. In small vessel disease, the stiffened/damaged arteries are unfit to absorb the heightened pulsatility, causing damage to the proximate microvasculature of the brain [83, 84]. In vitro studies confirm that pulsatile WSS on BMECs causes the loss of TJ proteins, reduced P-glycoprotein expression and delocalization of zona occludens-1 (ZO-1) from the cell borders to the cytoplasm and nucleus [77]. Disease severity of small vessel disease is also associated with global and local CBF alterations and recent work has pinpointed distinct region-specific CBF patterns in patients, which were linked to the occurrence of periventricular white matter hyperintensities and total disease burden [85].

During ischemic stroke, blood flow is blocked to a part of the cerebral vascular network, instantly severely lowering CBF in this region, which can be restored upon timely treatment (reperfusion). As a result, BMECs first experience hypoxia and oxidative stress which partially increases their permeability for blood components, fluids, and peripheral cells, causing post-ischemic inflammation [86]. Edema formation follows ischemia-induced sodium and water uptake through shear stress-regulated ion transporter [87]. A number of signalling pathways (including Wnt and Notch pathways) underlying ischemic/reperfusion injury across organs have been recently reviewed [88]. Very low levels of CBF and WSS also prompt increased BMEC proliferation and enhanced BBB permeability through cell layer remodelling [38, 69, 89]. Following reperfusion, BMECs secondly experience enhanced WSS which promotes endothelial-to mesenchymal transition [90]. This (partial) shift to mesenchymal cell properties further enhances BBB permeability [91], which may however reverse at a later timepoint of reperfusion [91]. A recent study, using two-photon imaging to follow vascular leakage post ischemic stroke in mice, showed BBB leakage as early as 30 min after the occlusion, steadily increasing in the hours after (120 min) [92]. Disruption of BBB function from ischemia/reperfusion has been reported as a predictor of poor disease outcome and haemorrhagic transformation in ischemic stroke patients [93].

Hypertension-induced vascular injury correlates with age and increases the risk for AD, yet the CBF is frequently reduced globally and locally in AD patients, which has been suggested as an early marker for AD [20, 94–96]. Similarly, MS patients show reduced CBF in white matter regions, specifically periventricular areas, concomitant with increased BBB permeability [97, 98]. Reduced CBF in MS may be the result of impaired flow-mediated endothelial dilation, both of which are correlated with disease severity and progression [99]. Whereas numerous studies hint towards changes in CBF in age-related and neuroinflammatory diseases [100, 101], the underlying causes and consequences for BMECs and BBB function remain elusive. Especially data on human brain vasculature is missing to fully comprehend different forms of WSS and its effects on the BBB during aging and neuroinflammatory disorders [51].

Basement membrane changes

Vascular stiffness is a complex property governed chiefly by vascular reactivity, BM properties, and blood pressure [102]. Here we focus on the BM and its changes in mechanical properties during pathology. The brain endothelial BM separates BMECs from neighbouring pericytes and supports BBB maintenance [103]. The BM is composed of extracellular matrix (ECM) proteins including laminins, nidogens, heparan sulphate proteoglycans, and collagen IV (Fig. 1B). They form a 50–100 nm thick three-dimensional network that interacts with BMECs to hold them in place [104, 105]. BM stiffness is influenced by the composition of ECM proteins, their concentration and elasticity, crosslinking between the proteins and the created intrinsic tension [106]. Degradation through proteases (e.g. matrix metalloproteases), remodelling and (limited) synthesis of ECM proteins can change the degree of membrane stiffness [106].

In vitro studies show that ECs respond to the rigidity of the neighbouring BM by enhanced expression of ECM receptors, including integrins. These cell–matrix contacts are crucial for BMEC identity and BBB function [107, 108]. Consequently, global knockout models for components of the ECM or pharmacological blockage of integrins often demonstrate cerebral bleeds and pre- or postnatal lethality [109, 110]. Growing cells on polyacrylamide hydrogels of varying stiffness showed that cells on soft hydrogels developed a lower density of actin fibres and more rounded nuclei than those grown on stiffer substrates [39, 111]. BM stiffness further cues endothelial progenitor cell differentiation along the arterial-venous axis, as higher stiffness induces arterial EC marker expression via the Ras-Mek pathway [112]. Interestingly, when exposed to physiological WSS, ECs on soft hydrogels displayed tighter cell junctions, more profound

elongation, and fewer (RhoA-mediated) cytoskeletal changes, revealing a direct relation between ECM stiffness and WSS [111]. These results show that ECs can directly modulate internal tension in response to external forces by altering the assembly and dynamics of actin fibres and junctional proteins [40, 113].

BM thickening has been linked to a narrowing of the vessel lumen during inflammation, aging and AD [114–118]. Over time, the BM composition shifts towards thicker collagen fibres with enhanced crosslinking, while hyaluronan levels decrease by degradation and elastin elasticity function is reduced [119, 120]. Consequent narrowing of the vessel lumen diameter restricts local CBF, causing lack of sufficient oxygen and nutrient supply for the brain [121, 122]. Prolonged reduced CBF can be further amplified by decreased endothelial-dependent dilation and overactive contraction, e.g. by pericytes evoked through reactive oxygen species or Aβ signalling in ischemic stroke and AD [123, 124]. Moreover, the increase in BM rigidity affects mechanosensitive processes such as angiogenesis [125, 126]. Functional angiogenesis requires a delicate balance between matrix remodelling/degradation and mechanical support of the BM for BMEC adhesion and migration. Age-related changes in BM composition and elasticity result in a lack of vessel support and might be linked to microvessel rarefaction and regression following impaired angiogenesis [127]. Lastly, the endothelial BM is a reservoir for growth factors and signalling crucial for vessel formation. Changing ECM protein composition thus also leads to a dysregulation in growth factor and other ligand availability [127].

In AD pathogenesis, changes in levels of collagen IV and the proteoglycan perlecan occur early on (Braak stage > 2 & ≤ 4) and correlate with parenchymal Aβ plaque deposition in the frontal and temporal cortex, suggesting BM changes in parallel with disease progression [128]. In AD patients with CAA, CAA severity correlates with $A\beta_{1-42}$ and collagen IV abundance in vessels of the frontal cortex [129]. It remains to be investigated if changes in the endothelial BM facilitate vascular Aβ deposition or merely occur concomitant with disease development. In MS, non-invasive pulse wave velocity measurements, a readout for arterial stiffness, are associated with disease duration and severity. Another study showed that increased arterial stiffness correlated with a reduced cognitive processing speed, as assessed via the Symbol Digit Modalities Test [130, 131]. Similar measurements in stroke patients connected arterial stiffness with enlarged perivascular spaces and cerebral microbleeds and revealed vessel stiffness as a potentially more important risk factor than blood pressure for cerebral small vessel disease [132, 133]. Lastly, a meta-analysis demonstrated a negative association between arterial stiffness and cognitive function, together highlighting the importance of vascular fitness in disease pathogenesis and global memory function [134, 135].

Due to their dense network, BMs also create a rate-limiting step for immune cells to enter the perivascular space and the parenchyma, prolonging the migration duration by 3–fourfold [136–138]. Pathological changes in BM composition can facilitate the infiltration of immune cells and cue cell differentiation as previously reviewed elsewhere [139]. Matrikines, peptides released during ECM remodelling, might also affect BMEC phenotype and inflammatory state as suggested for the peripheral endothelium [140, 141]. Ex vivo measurements of BMs have shown a large range of stiffness (Young's elastic module) from 500 to 4,000,000 N/m^2 depending on the tissue, but similar to WSS, measuring the stiffness of BMs in vivo remains an ongoing challenge (Table 1) [142].

Cell–cell interaction forces during transendothelial migration

Immune cell migration across the BBB under homeostasis is limited to a small subset of activated immune cells [143]. Dysfunction of the BBB due to inflammatory and age-related pathology greatly enhances TEM. Immune cell entry further potentiates inflammatory processes within the CNS, fuelling disease progression and cognitive decline [144–146]. In addition to chemical cues, leukocytes prepare for migration by following a stiffness gradient of the underlying substrate, a process termed durotaxis [147, 148]. In the following sections, we highlight the forces acting between BMECs and immune cells during the multi-step process of TEM. Studies on the mechanical forces between BMECs and immune cells have predominantly focused on T cells, which are key players in neuropathological events. However, many of these mechanisms are likely universal to immune cell populations entering the CNS.

First steps of engagement of leukocytes with BMECs

In order to exit the bloodstream and enter the brain parenchyma, immune cells must come into contact with the vessel wall [149]. Thus, migration primarily takes place at inflamed postcapillary venules where WSS is greatly reduced, increasing the chance of immune cells interacting with the brain endothelium [150]. This is further facilitated by erythrocytes, which are thought to push immune cells to the edges of the blood vessel [149] (Fig. 2).

The first contacts of the leukocyte with the endothelial cells (ECs) are generally made between the family of molecules known as selectins (L-selectin on leukocytes, P- and E-selectin on ECs) and/or α_4-integrins (such as

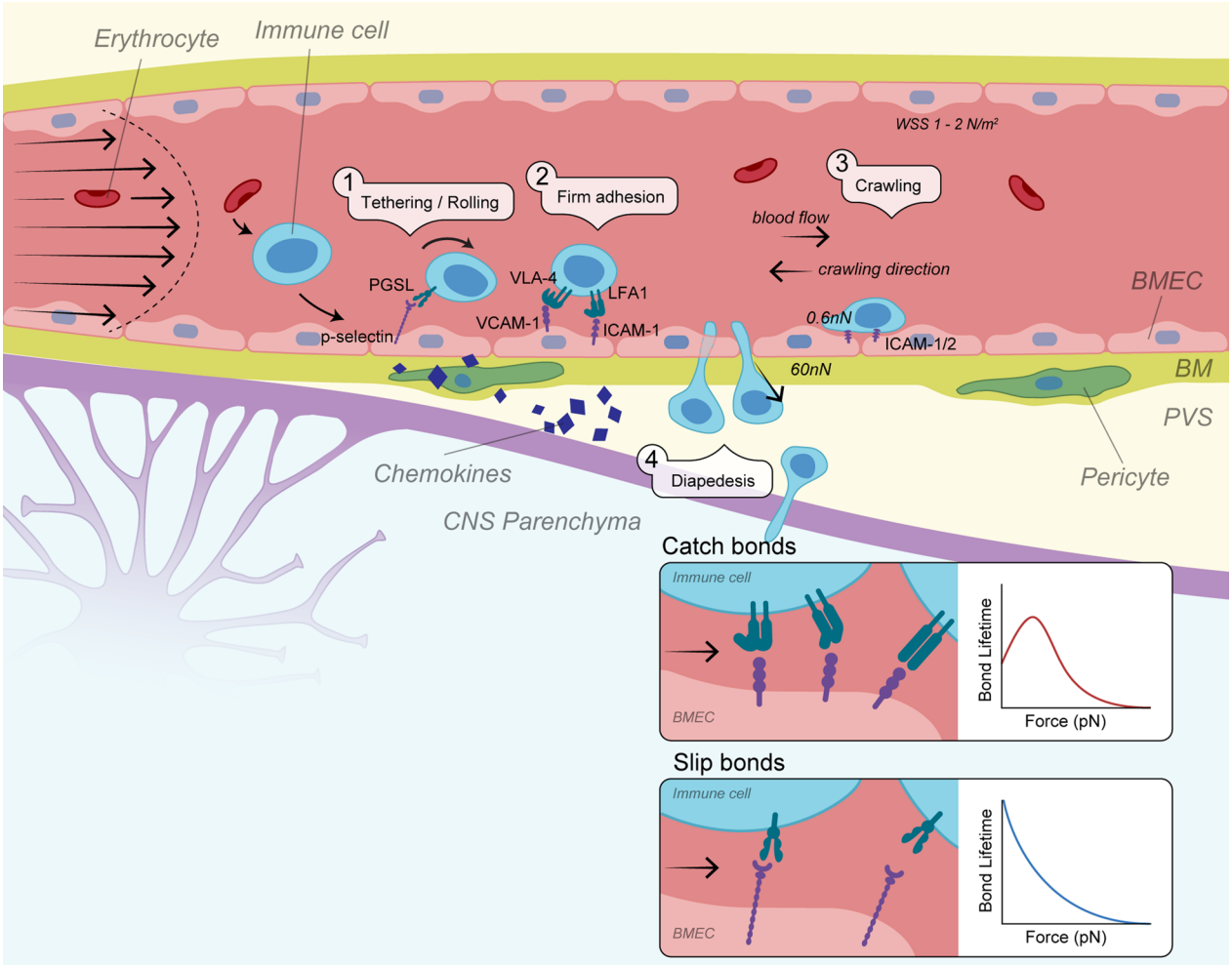

Fig. 2 TEM cascade of migratory T cells across the brain endothelium. Upon engagement of catch bonds between selectins/α_4-integrins and their ligands, T cells start rolling and eventually firmly adhere to the ECs. Cells then crawl against the direction of CBF and probe BMECs for sites permissive for diapedesis. Diapedesis eventually takes place either paracellularly through bi-/tricellular endothelial junctions or transcellularly by inducing the formation of pores within ECs. *WSS* Wall shear stress, *PGSL-1* P-selectin glycoprotein ligand-1, *VLA-4* very-late antigen 4, *VCAM-1* vascular cell adhesion molecule-1, *LFA1* lymphocyte function-associated antigen 1, *ICAM-1* Intercellular adhesion molecule 1, *PVS* perivascular space, *WSS* Wall shear stress, *BM* basement membrane

very-late antigen 4 (VLA-4) and lymphocyte function-associated antigen 1 (LFA-1) on leukocytes), and their respective ligands [151–153]. During inflammatory insults such as in MS, selectin and integrin expression increases in both BMECs and leukocytes [154]. In fact, inhibiting T cell adhesion by blocking the interaction of α_4-integrins with brain endothelial vascular cell adhesion molecule-1 (VCAM-1) through natalizumab is currently one of the most effective treatments for relapsing–remitting MS [8, 155, 156]. The initial tethering is followed by rolling of the immune cell along the vascular wall with reduced speed. Interestingly, rolling does not seem to be required for successful T cell migration [58, 152, 157]. A direct capture mechanism of T cells, mediated by α_4-integrins/VCAM-1, has been shown predominantly under non-inflammatory conditions and might be specific for migration into the CNS [158–161].

During rolling, immune cells experience a tangential force caused by WSS, as well as a rotational torque due to their rolling motion, resulting in shear forces dragging on newly formed adhesion complexes [162]. Surprisingly, these forces were found to stabilize adhesive interactions rather than opposing them. This discovery led to the definition of 'catch bonds'—a bond whose lifetime increases with rising tension, as opposed to 'slip bonds' whose interaction is destabilized by force [58, 163–165] (Fig. 2, insert). Catch bond formation is essential for the interaction of selectins and integrins whose lifetime is maximal

in a force range of 10–30 pN [57, 61, 62]. Application of lateral force in form of WSS is thought to accelerate the conformational transition of integrins into an extended high-affinity state and thus facilitate immune cell arrest in an outside-in manner [166–168].

Rolling at reduced velocity enables immune cells to probe the endothelial surface for chemokines [58]. These chemokines bind to chemokine receptors on the immune cell surface, delivering a G-protein coupled receptor (GPCR)-mediated inside-out signal to α- and β-integrins. GPCR signalling increases the affinity of integrins for their endothelial ligands via activation of the cytoskeletal adaptors talin and kindlin in the immune cell [169]. Interaction pairs include LFA-1 (T cell) and intercellular adhesion molecule-1/2 (ICAM-1/2) (ECs), as well as VLA-4 (T cell) and VCAM-1 (EC) [152, 153, 167, 170]. This is further facilitated by integrin clustering into focal adhesions and ultimately results in integrin-mediated immune cell arrest, polarization, and firm adhesion on the endothelium [58]. Catch bonds again play a crucial role in this process as integrin activation by chemokines alone is insufficient to stimulate adhesiveness [62, 171]. In summary, the adhesion of immune cells to the post-capillary bed is facilitated by both WSS and intercellular forces, together stabilizing the interaction and allowing for cell migration to occur.

T cell crawling on BMECs is directed against the flow

While adhering to the vascular wall, lymphocytes polarize with respect to the blood flow by adopting an elongated morphology with a wide and flat F-actin-rich lamellipodium at the leading edge and a tail-like projected uropod at the trailing edge [160, 172]. Crawling is an active movement facilitated by Rho GTPases [8, 58], involves the formation of additional adhesive contacts, and occurs at a highly reduced velocity of a few µm per minute [173, 174]. Forward propulsion along the endothelium is driven by actin polymerization and myosin II contractility (retrograde flow), which together push the lamellipodium forward, parallel to the surface [58, 63]. Thereby, the actin cytoskeleton generates internal forces of up to 0.6 nN [63], which are transferred to integrins via cytoskeletal adaptors such as talin, increasing the ability to form catch bonds [172]. This actin-talin-integrin linkage, however, is not absolute, leading to varying degrees of slippage [175]. This has been termed a 'molecular clutch' and is exploited by the cell to dynamically adjust migration speed and direction in response to changes in substrate adhesiveness and matrix stiffness [175, 176]. Enhanced stiffness, as found in inflammation and aging [148], has been linked to more persistent directional crawling [177] and increased efficiency of transmigration [178].

T cell crawling along the cerebral post-capillary venules is unique in two aspects. Firstly, crawling was observed in vivo to be directed preferentially against the direction of blood flow [8, 138, 152, 179]. This has been linked to the presence of shear forces and the adhesion molecules ICAM-1 and ICAM-2 [161, 172]. T cells can also crawl downstream by engagement of VLA-4 and VCAM-1, but prefer upstream migration under shear rates above 400 s^{-1} [172]. Interestingly, in the absence of VLA-4 or VCAM-1, T cells still adhere and crawl against the flow, but do not maintain directionality after the flow is terminated [161]. VLA-4 thus appears to be required for migratory memory, the capacity to remember directionality, under WSS [180].

Secondly, T cell crawling at the brain post-capillary venules takes place over significantly longer distances, frequently exceeding 150 µm, which is likely due to the unique high barrier integrity rarely allowing for diapedesis even under inflammatory conditions [160]. In contrast to rapid rolling and tethering, crawling takes on average several minutes and rarely up to hours [138, 160, 161]. On primary mouse BMECs, 95% of T cells crawled or transmigrated within half an hour, whereas 5% arrested and remained immobile [160]. Similarly, intravital imaging of autoreactive T cells showed 80–85% of cells crawled and extravasated from the leptomeningeal vessels within 30 min [138]. The purpose and mechanism of preferential crawling against the flow at the BBB still remain elusive, and it has yet to be determined whether this phenomenon is immune cells specific and how frequently this event occurs in other tissues [179].

Diapedesis by the path of least resistance

Immune cell migration across BMECs occurs either paracellularly through endothelial junctions or transcellularly by inducing the formation of pore-like structures in the ECs themselves [152]. In search for permissive sites, leukocytes sense endothelial substrate stiffness, mainly defined by their F-actin density [148, 181]. Several factors determine which route will be preferred. High ICAM-1 levels, increased caveolin-1 expression, and chemokine presentation have all been linked to transcellular migration [182–186]. On the other hand, with high stress fibre density and lower levels of inflammation with intermediate ICAM-1 expression might favour paracellular diapedesis [182, 187]. Although increased stiffness appears to facilitate crawling, diapedesis of T cells locally takes place at the path of least mechanical resistance [182, 188]. Transcellular migration thus primarily occurs at the cell periphery, where F-actin density is highly heterogeneous [58, 182, 189]. As multiple forces and chemical signals act in concert, plus in vivo studies are sparse, it is challenging

to predict which migration route is preferred in neuroinflammatory conditions like MS, AD, or during aging.

Finally, diapedesis takes place within seconds to minutes [190], during which both leukocytes and ECs undergo drastic cytoskeletal remodelling. Leukocytes adopt an elongated shape and drastically reduce stiffness by inducing actin disassembly and breaking down vimentin intermediate filaments and microtubules [189]. Once the leading edge of the leukocyte has breached the endothelial monolayer, the elongated leukocyte nucleus squeezes through the endothelium with lateral forces in the order of 60 nN to extrude through the narrow space [58]. This is sensed by the endothelial cytoskeleton, which responds with the disassembly of filaments to withstand these forces and allows for transmigration to succeed [189]. Leukocyte squeezing also triggers the formation of an F-actin ring surrounding the migrating immune cell. This ring limits pore size and prevents plasma leakage, allowing the endothelium to preserve its low permeability to macromolecules [189, 191]. Upon successful diapedesis, BMECs respond to the sudden loss of tension and utilize ventral lamellipodia to close the gap [189]. During transmigration, ECs and leukocytes exchange surface proteins [192, 193], which might draw additional immune cells to these sites, creating a hotspot for transmigration [194]. However, it is unclear whether the endothelium returns to its previous state eventually or retains long-lasting imprints at these sites.

The unique structural properties of the BBB would suggest that mechanical force transmission and response are distinct from those observed in other tissues, and might help explain molecular mechanisms directing transcellular or paracellular diapedesis. However, no studies have yet reported mechanical properties of diapedesis across BMECs.

Mechanosensory elements of endothelial cells

Considering the wide variety of mechanical stresses at the BBB, BMECs express a variety of mechanosensory complexes, which convert physical stress into biochemical signals (Fig. 3). Here we focus on cell–cell junction complexes and mechanosignalling through receptors and ion channels of BMECs, highlight their relevance in immune cell migration across the BBB, and changes during inflammation and aging. Importantly, we try to distinguish mechanosensing elements, which were shown to respond to mechanical forces directly, from mechanosensitive players, which may be activated indirectly by force transmission and biochemical signals downstream of primary mechanosensing components or actin fibres.

Cell–cell junctions

Cell–cell junctions play a crucial role in endothelial barrier function and have thus adapted multiple ways to respond to mechanical forces (Fig. 3) [4, 195]. Formation of stable AJs requires intracellular coupling of VE-cadherin to actomyosin through α-catenin [196, 197]. Both external forces on VE-cadherin and internal forces generated by actomyosin pull α-catenin into an extended conformation, leading to the recruitment of vinculin and other binding partners [4, 196, 197]. Vinculin further stabilizes the unfolded conformation of α-catenin, leading to enhanced cell–cell adhesion and F-actin polymerization [40, 195, 196]. Increasing force strengthens the binding (catch-bond) between the cytoskeleton and junctional partners vinculin, talin, and α-catenin upon formation of dimers/multimers (order of 10 pN), while e.g. a bond between monomeric α-catenin and F-actin disrupts under increasing tension (slip-bond) [198–201]. The actin cytoskeleton of ECs also directly responds to tension via polymerization and interaction with actin filament-associated proteins, leading to stress fibre formation, increased stiffness, junctional changes and activation of focal adhesions [40, 202].

Extracellular detachment forces between VE-cadherin proteins (Ca^{2+}-dependent) range from 15 to 150 pN depending on their cumulative interactions and on the applied stretch, which extends their lifetime (catch-bonds) [65, 203]. Cadherins are also thought to be mechanosensitive themselves, acting in concert with PECAM-1 and VEGF receptors to initiate further downstream signalling including PI3K/Akt and integrin/RhoA [6, 40, 64, 76, 204, 205]. Mechanosensing by PECAM-1 regulates force-dependent cellular stiffening [204] and PECAM-1 is critically involved in the regulation of BBB junctional integrity [206], WSS sensation [207], integrin activation, cytoskeletal rearrangement [195, 208] and TEM [209]. Loss of PECAM-1 increases vascular permeability of the BBB, but prevents paracellular transmigration, suggesting that intact (to a certain degree) cell junctions and PECAM-1 mechanosignalling are required for paracellular diapedesis [8, 185, 206]. On the other hand, in pathologies with disturbed WSS, such as atherosclerosis and ischemic stroke, PECAM-1 is increased and defined as a main contributor to atherosclerotic lesions, neutrophil invasion, and post-ischemic neuroinflammation [210, 211].

BM stiffness and WSS regulate the force on focal adhesions and TJs by traction force, hence mechanosensory properties have also been shown for TJ proteins. [6, 212]. Under physiological laminal WSS, mechanosensing via occludin promotes vascular integrity by recruiting further occludin and ZO-1, whereas disturbed flow causes loss of occludin and barrier

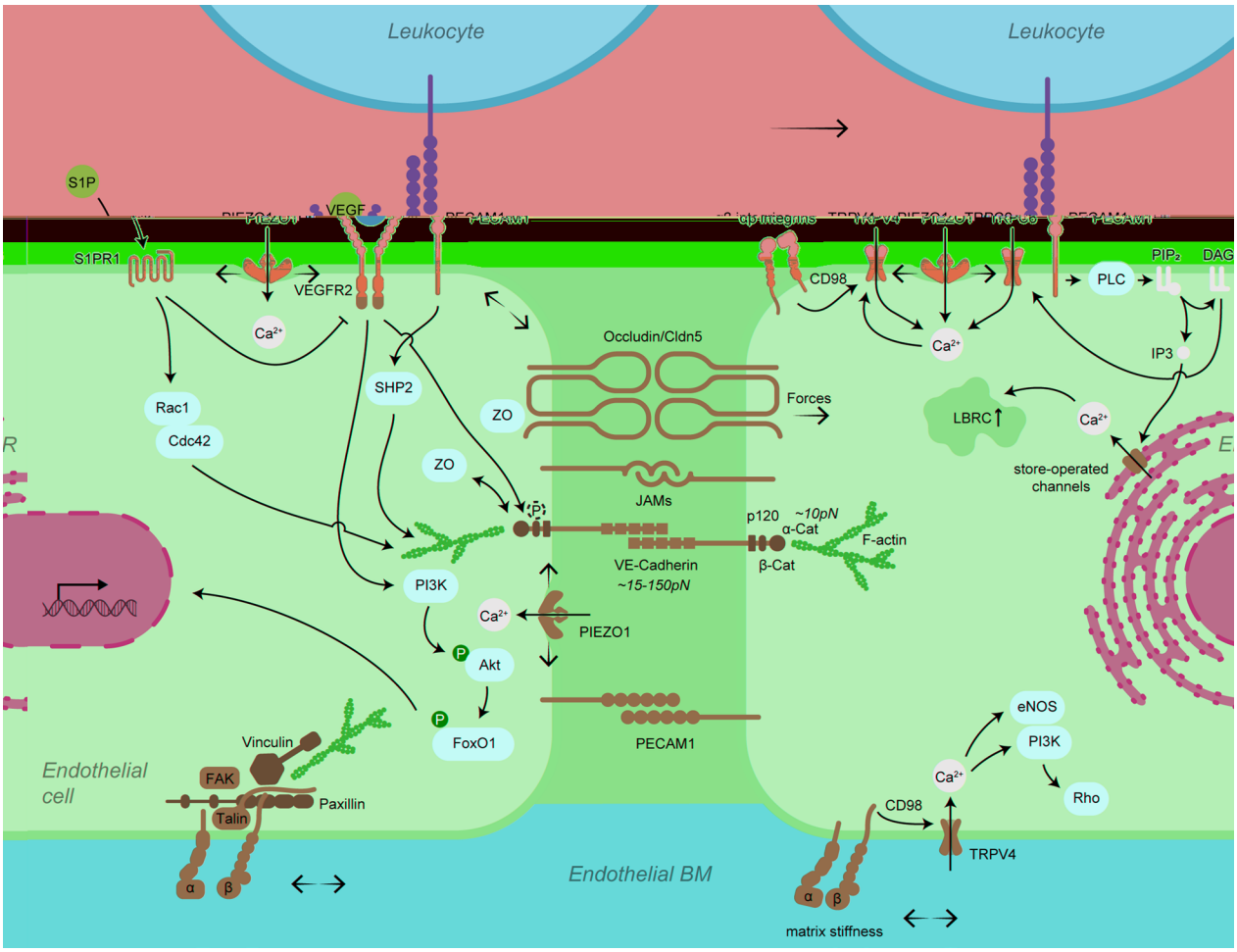

Fig. 3 Biomechanical signalling via mechanosensing/sensitive elements at the brain endothelium. Intercellular tension is sensed by junctional molecules such as PECAM-1, VE-cadherin, and catenins. Interactions between PECAM-1, VE-cadherin, Piezo1, S1PR1, and VEGFR2 regulate barrier stability. Integrin signalling transmits ECM stiffness and stretch to the actin cytoskeleton and ion channels. Transient receptor potential (TRP) ion channels may be activated by direct mechanical force transfer from primary mechanosensing components (integrins) or via intermediate proteins (CD98) or cytoskeletal adaptors. Alternatively, they may be activated via biochemical signalling downstream of primary mechanosensing elements, involving synthesis and modification of intracellular messengers (DAG and Ca^{2+}). *WSS* wall shear stress, *S1P/S1PR1* sphingosine-1-phosphate receptor 1, *VEGFR2* vascular endothelial growth factor receptor 2, *Rac1* Rac family small GTPase 1, Cdc42 cell division cycle 42, *JAMs* junctional adhesion molecules, *PECAM-1* platelet and endothelial cell adhesion molecule 1, *α/β* cat catenins, *ZO* zonula occludens, *PLC* phospholipase C, *DAG* diacylglycerol, *PIP2* Phosphatidylinositol 4,5-bisphosphate, *Ca^{2+}* calcium, *ER* endoplasmic reticulum

dysfunction [213]. In addition, the TJ adaptor protein ZO-1 also regulates tension acting on VE-cadherin in response to WSS, similar to the function of α-catenin at AJs [205, 212, 214]. Together these components enable the dynamic response of junctional proteins to mechanical forces, which are required for endothelial barrier formation and maintenance [196, 215], and cell orientation in the direction of flow [195]. In neurological pathologies, both mechanical forces as well as organization and expression of cell–cell junctions change drastically. However, it remains challenging to create a causative relationship between these two components, as other factors, such as cytokine signalling, majorly affect junctional integrity [216].

Mechanosensitive receptors and ion channels

Both ECs and migrating immune cells display a wide range of receptors and ion channels responding to mechanical stress, although mechanisms of their localization and activation are often not fully understood [217]. Mechanosensitive proteins can be activated by direct stress applied to the lipid bilayer, opening e.g. the ion channel through membrane tension, or via additional linked components outside the membrane [218, 219].

Activated proteins often undergo large conformational changes, such as unfolding of domains in talin, which are reversed upon force dissipation [220, 221]. Of note, translation of mechanical signals into cellular alterations occurs on different time scales e.g. with integrin activation (seconds to minutes) and longer duration for cytoskeletal rearrangements (minutes to hours) [220].

Tyrosine kinase receptors on ECs, including vascular endothelial growth factor receptor 2 (VEGFR2) and Tie-2, become phosphorylated under mechanical stretch, which triggers ligand-independent signalling [222, 223]. Importantly, VEGFR2 has multiple phosphorylation sites, which can regulate vascular permeability through VE-cadherin phosphorylation, but also proliferation and migration [224]. As a result, mechanical cues can regulate angiogenesis via VEGFR2-signalling [225]. ECM stiffness regulates the angiogenic potential via yes-associated protein (YAP)-Dll4-Notch1-VEGFR signalling, and promotes pro-angiogenic factors on softer vs. stiffer substrates [226]. YAP/transcriptional coactivator with PDZ-binding motif (TAZ) activation also induces an inflammatory endothelial phenotype under disturbed blood flow, hence inhibition of this pathway has been effective against atherosclerotic lesion development [227, 228]. Exploration of these tightly connected mechanosensitive pathways for therapeutic approaches could reinstate functional angiogenesis in cerebral vessels with stiffer BM and support areas of disturbed or reduced WSS such as in elderly, AD and ischemia [229].

Mechanosensing GPCRs change conformation under mechanical stress [230]. For example, endothelial GPR68 is activated by high WSS in small diameter arteries, triggering flow-mediated dilation [231, 232]. Interestingly, GPR68 is also activated by acidosis, a lowered blood pH, which can occur through prolonged lack of oxygen and CO_2 accumulation [233]. However, CO_2-induced CBF increase was not changed in mice lacking GPR68, thus the role of GPR68 for H+sensing at cerebral arteries could be less prominent [234, 235]. Sphingosine-1-phosphate receptor 1 (S1PR1) is another mechanosensitive GPCR expressed in BMECs but also widely throughout other tissues [236]. Vascular S1PR1 can be activated by WSS and circulating S1P resulting in vasodilation and maintenance of endothelial barrier stability [237]. Of note, S1PR modulators, such as fingolimod (S1PR1,3,4,5 modulator) and siponimod (S1PR1,5), are approved as disease-modifying treatment in MS, and tested in clinical trials for ischemic and hemorrhagic stroke [238, 239]. Fingolimod also reduces VCAM-1 expression and stabilizes claudin-5 levels on BMECs [240]. In line with SP1R1s mechanosensitive functions in vasodilation and vascular network maintenance, prolonged treatment with fingolimod resulted in hypertension in mice [241] and patients with chronic inflammatory demyelinating polyradiculoneuropathy [242]. In conclusion, mechanosensitive GPCRs present a promising target group for multiple CNS diseases, however, their widespread expression and functional spectrum may limit therapeutic potential.

Mechanosensitive ion channels are primarily cation permeable and regulate ion flux to initiate Ca^{2+} signalling, alter the membrane potential, and regulate Mg^{2+} homeostasis [36, 40, 243]. Due to their rapid action and the fast propagation of Ca^{2+} waves, ion channel activity is usually among the first steps in mechanical signalling [40, 244]. This Ca^{2+} signalling is critical for maintaining the low permeability of the BBB [245], whereas dysregulation of intracellular Ca^{2+} due to high mechanical stress or pathological conditions can compromise BBB integrity [245–248]. For example, Ca^{2+} influx through the nonselective cation channel Piezo1 is involved in flow-induced cell alignment, vasodilation of cerebral capillaries and angiogenesis [40, 249–251]. Overstimulation of Piezo1 due to disturbed flow conditions in atherosclerosis and hypertension, however, causes the breakdown of AJs, actin remodelling and inflammation [78, 252–255]. The underlying mechanism is a direct connection between Piezo1 and VE-cadherin as well as PECAM-1 regulating junction integrity [256].

Mechanosensitive ion channels also include the diverse family of transient receptor potential (TRP) ion channels, which mediate large parts of the mechanical stress response experienced by BMECs. They fulfil a multitude of functions in immune cell migration and barrier function and are involved in both progression and recovery of several neurological and vascular-related pathologies [35, 36, 257]. The following section will highlight the functional diversity of this family of ion channels.

Mechanosensitive TRP channels

TRP channels are widely expressed throughout the CNS and are involved in cellular response to a variety of external cues, including mechanical signals [258] (Table 2). Based on sequence homology, they are grouped into six subfamilies termed canonical (TRPC1-7), vanilloid (TRPV1-6), ankyrin (TRPA1), polycystic (TRPP1-3), melastatin (TRPM1-8) and mucolipin (TRPML1-3) [36]. TRP channels can directly or indirectly trigger Ca^{2+} signalling [40, 245] and can also be Ca^{2+}-regulated themselves via Ca^{2+}-calmodulin or direct Ca^{2+} binding [40]. Although TRP channels are commonly involved in mechanosignalling cascades, likely none of them are mechanosensing themselves [259]. Mechanisms for direct mechanical activation, such as deformation due to membrane stretch, have been proposed, but lack sufficient evidence [260]. TRP channels are thus most likely activated by mechanical transduction via interaction with mechanosensing

Table 2 Mechanosensitive TRP channels of vascular ECs and leukocytes

Channel	Cell type	Upstream components	Reference(s)
TRPA1	BMECs, T cells, B cells, macrophages	$[Ca^{2+}]_i$	[262–264]
TRPC1	cBAECs, mBMECs, T cells, B cells, macrophages, neutrophils	PLC, DAG, $[Ca^{2+}]_i$	[246, 265–268]
TRPC3[a]	cBAECs, mBMECs, T cells, B cells, macrophages, neutrophils	PLC, IP_3	[246, 265, 266, 269–272]
TRPC5[a]	cBAECs, mBMECs, macrophages	PLC, PIP_2	[246, 266, 273, 274]
TRPC6	cBAECs, macrophages, neutrophils	DAG, $G\alpha_q$, PECAM-1	[266, 275–278]
TRPM4[a]	BMECs, T cells, macrophages	IP_3, Ca^{2+}-calmodulin	[279–282]
TRPM7[a]	HUVECs, T cells, macrophages		[265, 283]
TRPP1	HUVECs		[284]
TRPP2	BMECs	glycocalyx, IP_3	[247, 261]
TRPV1[a]	BMECs, T cells, macrophages, neutrophils		[285–288]
TRPV2	BMECs, T cells, B cells, macrophages, neutrophils	phosphatidylinositol-3 kinase, Akt	[285, 286, 288–290]
TRPV4	BMECs, T cells, macrophages, neutrophils	β_1-integrins, CD98, Piezo1, PLC, PIP_2, Ca^{2+}-calmodulin, arachidonic acid metabolites	[78, 285, 286, 291–295]
TRPV5[a]	T cells, granulocytes		[296]
TRPV6[a]	Jurkat T cells, granulocytes		[297]

Expression of mechanosensitive TRP channels in human primary vascular ECs (BMECs where data available) and leukocytes, and associated suggested upstream signalling components; BMECs–(human) brain microvascular endothelial cells; $[Ca^{2+}]_i$–intracellular Ca^{2+}; cBAECs–cultured brain artery endothelial cells; DAG–diacylglycerol; HUVECs–human umbilical cord endothelial cells; mBMECs–mouse brain microvessel endothelial cells; PIP_2–phosphatidylinositol-4,5-biphosphate; PLC-phospholipase C

[a] Mechanosensitive properties derived from cell types other than ECs or leukocytes

proteins, such as β_1-integrins and the glycocalyx [40, 261]. Alternatively, force transmission may activate TRP channels via intermediary membrane proteins and the cytoskeleton [40]. Table 2 provides an overview of known mechanosensitive TRP channels and associated upstream components. For several TRP subunits mechanosensitive properties have not been shown specifically for vascular ECs, but are likely based on findings in other cell types (indicated by superscript **a** in Table 2).

TRPV4 is a key mediator of mechanical signalling

TRPV4 is a non-selective cation channel triggering downstream signalling via influx of extracellular Ca^{2+} [298] and is involved in mechanosignalling of various cell types, including BMECs [286], T cells [285], neutrophils [294] and macrophages [295]. TRPV4 has been proposed to react to diverse mechanical forces such as WSS [78, 299–302], circumferential stretch [303, 304], stiffness [305, 306], osmotic swelling [298, 307], shrinkage and surface expansion [298].

Endothelial TRPV4 is an important regulator for flow-induced vasodilation [299, 302], cell volume [243, 293], cytoskeletal remodelling and barrier function [248, 308, 309]. TRPV4 activation can occur within milliseconds in focal adhesions of ECs by force transfer from mechanosensing β_1-integrins via CD98 to TRPV4 [292, 310]. TRPV4 activation by β_1-integrins might explain why high ECM stiffness, such as during inflammation or aging, causes endothelial dysfunction in a pathway that involves TRPV4-mediated Ca^{2+} signalling [305]. TRPV4 is also involved in flow-induced vasodilation and cytoskeletal remodelling via Piezo1. Upon mechanical stimulation, Piezo1 causes a local increase in intracellular Ca^{2+} and activates TRPV4 via Ca^{2+}-calmodulin binding to its C-terminal calmodulin binding site [78, 298, 311]. TRPV4 then triggers a more prolonged Ca^{2+} signal, which leads to Piezo1-mediated disassembly of AJs under disturbed blood flow [78]. TRPV4 inhibition in a mouse model of haemorrhagic stroke reduced BBB dysfunction, likely due to reduced Piezo1 signalling [309, 312]. These findings suggest a regulatory role of TRPV4 in vascular permeability, which contributes to BBB disruption under altered mechanical conditions [308, 309]. This role in BBB function and fast activation via β_1-integrins render TRPV4 a potential contributor to immune cell crawling and diapedesis [248]. It remains to be determined at what stage of the migration cascade TRPV4-mediated signalling occurs.

TRPC channels form mechanosensitive homomeric and heteromeric channels

Channels of the TRPC subfamily are involved in stretch and shear stress sensing of ECs and migrating immune cells [313, 314]. Although the expression of TRPCs in primary human BMECs has not explicitly been shown, their frequent expression in other human endothelial

tissue [315] would suggest that they are also common at the BBB. This is further supported by findings of TRPC expression in cultured brain artery ECs (cBAECs) [266] and mouse brain microvessel ECs (mBMECs) [246, 316]. All TRPC channel complexes in humans can be activated by PLC-mediated hydrolysis of PIP_2 [278, 317]. This can directly lead to TRPC channel opening by binding of diacylglycerol (DAG) or removal of PIP_2-dependent inhibition [317–319]. Alternatively, IP_3 may travel through the cytoplasm, bind to its receptor and trigger the release of Ca^{2+} from intracellular stores, which in turn activates TRPC channels by Ca^{2+} or Ca^{2+}-calmodulin binding [35, 260, 317, 320]. Activation by mechanical stress might thus involve mechanosensing GPCRs activating $G_{q/11}$ proteins, which in turn stimulate PLC and lead to TRPC channel opening further downstream [313].

Whereas TRPC1 is essential for cell migration and polarization [203, 243, 313], TRPC6 plays an important regulatory role in barrier-forming tissue [40, 209]. Endothelial TRPC6 channels mediate the intracellular Ca^{2+} signalling to allow leukocyte diapedesis [189, 209]. Weber et al. [209] showed that TRPC6-deficient ECs prevent paracellular diapedesis, whereas selective activation of TRPC6 by a DAG analogue allows for transmigration even when PECAM-1 is blocked. These results suggest that TRPC6 is activated downstream of PECAM-1-mediated mechanosensing and is required for the cytoskeletal adaptations in ECs enabling leukocytes passing through. The same study also showed that TRPC6 channels accumulate around PECAM-1 and promote translocation of intracellular stores of PECAM-1 and other adhesion molecules to the site of diapedesis [209]. TRPC6-induced Ca^{2+} signalling is likely also responsible for the formation of the F-actin ring surrounding migrating immune cells [189]. Under pro-inflammatory and disturbed flow conditions, TRPC6 expression and activation are elevated independently of diapedesis, leading to EC contraction and increased vascular permeability [321, 322].

TRPC channels function both as homomeric complexes and in heteromeric combinations, which differ in terms of activation characteristics, sensitivity and cation selectivity [40, 314, 323]. For example, vasorelaxation by ECs in response to high WSS is in part realized by heteromeric complexes of TRPC1 and TRPV4 [40, 314, 324]. The formation of heteromers might explain the multiple and often overlapping functionalities of TRP subunits [40, 314] and may be a major reason why most TRP knockouts in animal models do not show a detrimental phenotype [309]. How the formation of these complexes is regulated and how their functionalities differ from their homomeric counterparts requires further investigation.

TRPP, TRPM and TRPA1 channels regulate vascular tone, permeability and function

TRPP1 and TRPP2, also known as Polycytin-1 and −2, are nonselective cation channels involved in flow and pressure sensing of vascular ECs [323, 325]. Both subunits accumulate at primary cilia where they regulate vascular tone in response to WSS by inducing nitric oxide production [284, 325, 326]. Upon mechanical injury of BMECs, TRPP2 is involved in stress fibre formation and cytoskeletal remodelling leading to BBB dysfunction [245, 247]. Interestingly, TRPP2 may only be mechanosensitive in combination with other TRP subunits, including TRPP1, TRPC1 and TRPV4, once again highlighting the relevance of heteromeric TRP complexes [323, 325]. Accordingly, pressure sensing by ECs was shown to be dependent on relative expression of TRPP1 and TRPP2 [325], and heteromeric complexes of TRPV4 and TRPP2 are much more sensitive to laminar WSS than homomeric TRPV4 channels [327]. Changing composition of heteromeric TRP channels might thus present a useful way by which cells regulate their sensitivity to mechanical stress.

TRPM channels are widely expressed in the brain and are involved in many neurological disorders [35]. TRPM4 was shown to be activated by membrane stretch in vascular smooth muscle cells [280], but its role in BMECs still remains elusive. In vascular ECs, TRPM4 associates with Sulfonylurea receptor 1, which is upregulated in BMECs after ischemic stroke [35]. This causes continuous Na^+ influx and can lead to cell death, disintegration of capillaries and secondary bleeding [35, 328]. TRPM7 is another potentially mechanosensitive channel, which is activated by shear stress in fibroblasts [329], vascular smooth muscle cells [330] and mesenchymal stem cells [331]. Knockdown of TRPM7 in human umbilical cord ECs (HUVECs) prevents adhesion of ECs and promotes their growth and proliferation, suggesting a regulatory role in cell differentiation [283].

TRPA1 is involved in vasodilation of BMECs [262, 332] and is also expressed in T cells, where it promotes immune functions in response to mechanical stress and is implicated with several inflammatory conditions [263, 264]. TRPA1 can be activated by Ca^{2+} influx [35] and may even be mechanosensing itself. Although a recent study showed that TRPA1 in reconstituted proteoliposomes is insensitive to membrane stretch [259], another study revealed that single-channel currents of TRPA1 in artificial lipid bilayers respond to mechanical stretch in a pressure-dependent way [333]. This discrepancy might be explained by its redox state, as mechanosensing of TRPA1 was abolished under reducing conditions [333]. Clearly, TRP channel activation and function are not yet fully explained. Further studies should focus on the

effects of redox and other environmental conditions on TRP subunits, as these might help to better explain their activation, function and changes under pathological conditions.

Discussion and future perspectives

Mechanical stressors are crucial regulators of BBB function. Physiological WSS and BM stiffness support the formation of AJs and TJs and suppress inflammatory signalling, whereas alterations under pathological conditions lead to enhanced vascular permeability, endothelial dysfunction, reduced vascular reactivity and facilitate leukocyte infiltration. In this review, we took a holistic view of the various aspects of mechanical forces and mechanosensing at BMECs in different neuropathologies, which open new future intervention avenues.

Increasing evidence suggests that mechanisms of immune cell infiltration are not conserved across the body and depend on the tissue and immune cell type involved. Such cell-type-specific events offer great opportunities for therapeutic interventions. In MS, for example, Th1, Th17 and $CD8^+$ T cells are thought to promote inflammation in the CNS, whereas Th2 cells are associated with a protective effect [334, 335]. Th2 cells show lower ability to form tethers and slings and thus egress to a much lesser extent under high WSS [336, 337]. T cell subtype-dependent behaviour has also been observed for crawling [338] and diapedesis [185], but the underlying molecular mechanisms governing these differences remain mostly undescribed [189]. Closer examination of the molecular players driving cell-type-specific events throughout migration could reveal novel therapeutic targets for the treatment of MS and other neuroinflammatory or age-related conditions.

TEM is controlled by a range of mechanosensory elements on both migrating leukocytes and the endothelium. It is not always clear whether certain mechanosensory proteins adapt to pathological conditions to maintain vascular integrity, or whether these conditions throw mechanosignalling events off-balance and thereby contribute to pathological hallmarks. For example, PECAM-1 mediates vasodilation to alleviate high shear stress and might be involved in restoring the BBB after disruption in MS [206, 339], but also contributes to immune cell infiltration and inflammation in atherosclerosis, stroke and MS [211, 339, 340]. Moreover, disturbed flow and inflammation frequently affect expression and localization of endothelial proteins, further altering their response [341]. Decreased surface expression of VE-cadherin during inflammation increases permeability and reduces the ability of ECs to respond to WSS [342]. To arrive at a better understanding of pathological effects on the mechanosensory response, future research will require a more detailed exploration of mechanosensing and -sensitive proteins during neuroinflammation and aging.

Mechanosensitive TRP channels fulfil a wide range of functions and thus offer a plethora of potential drug interventions. Tremendous effort has been put into the development of specific TRP channel agonists and antagonists as drug targets for a range of diseases, including MS, Alzheimer's disease and stroke [343]. A recent study from our lab explores specifically the potential of TRPV4 inhibition to reduce leukocyte migration across the BBB in MS, however without integrating WSS or BM stiffness [248]. Although numerous potential compounds have entered clinical trials, several of them were eventually withdrawn due to lacking effectiveness and a variety of adverse effects [343, 344]. To effectively use TRP channels for drug discovery, a better understanding of channel expression and activity during pathology is required.

In conclusion, biomechanical signalling is an essential component of BBB function, yet many questions remain open on how these signals change during neuropathology. Continuing research in this field will provide new insights into the cumulative effects of mechanical alterations in WSS, BM stiffness and cell–cell interactions during immune cell migration. Ideally, such understanding would lead to the development of novel drug candidates selectively altering mechanosensory properties of immune cell subsets while leaving other functionalities unaffected, thus counteracting the problem of multifunctionality.

Abbreviations

Aβ	Amyloid beta
ACA	Anterior cerebral artery
AD	Alzheimer's disease
AJ	Adherens junction
BAEC	Brain artery endothelial cells
BBB	Blood–brain barrier
BM	Basement membrane
BMEC	Brain microvascular endothelial cell
CAA	Cerebral amyloid angiopathy
CBF	Cerebral blood flow
CCA	Common carotid artery
Cdc42	Cell division cycle 42
CNS	Central nervous system
CSF	Cerebrospinal fluid
DAG	Diacylglycerol
EC	Endothelial cell
ECM	Extracellular matrix
WSS	Wall shear stress
GPCR	G-protein coupled receptor
HSPGs	Heparan sulfate proteoglycans
HUVEC	Human umbilical cord endothelial cells
ICA	Internal carotid artery
ICAM-1	Intercellular adhesion molecule 1
IJV	Internal jugular vein
IP_3	Inositol 1,4,5-trisphosphate
JAM	Junctional adhesion molecules
LBRC	Lateral border recycling compartment
LFA1	Lymphocyte function-associated antigen 1
mBMEC	Mouse brain microvessel endothelial cells

MRI	Magnetic resonance imaging
MS	Multiple sclerosis
NFkB	Nuclear factor kappa B subunit
PECAM-1	Platelet and endothelial cell adhesion molecule 1
PIP_2	Phosphatidylinositol-4,5-biphosphate
PLC	Phospholipase C
Rac1	Rac family small GTPase 1
ROS	Reactive oxygen species
S1P/S1PR1	Sphingosine-1-phosphate receptor 1
TAZ	Transcriptional coactivator with PDZ-binding motif
TEM	Transendothelial migration
TJ	Tight junction
TRP	Transient receptor potential
VCAM-1	Vascular cell adhesion molecule-1
VEGFR2	Vascular endothelial growth factor receptor 2
VLA-4	Very-late antigen 4
YAP	Yes-associated protein
ZO	Zonula occludens

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CEH and DH designed and wrote the manuscript. AK created the figures and revised the manuscript. HEdV contributed to the design of the study, obtained research grants, and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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