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Heterogeneity of the blood-brain barrier

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The brain microvascular network is comprised of capillaries, arterioles and venules, all of which retain – although to a different extent – blood-brain barrier (BBB) properties. Capillaries constitute the largest and tightest microvasculature. In contrast, venules have a looser junctional arrangement, while arterioles have a lower expression of P-gp. Development and maintenance of the BBB depends on the interaction of cerebral endothelial cells with pericytes and astrocytes, which are all heterogeneous in different regions of the central nervous system. At the level of circumventricular organs microvessels are permeable, containing fenestrations and discontinuous tight junctions. In addition, the blood-spinal cord barrier – where the number of pericytes is lower and expression of junctional proteins is reduced – is also more permeable than the BBB. However, much less is known about the cellular, molecular and functional differences among other regions of the brain. This review summarizes our current knowledge on the heterogeneity of the brain microvasculature.

Introduction

The central nervous system (CNS) is separated from the rest of the body by 3 barrier systems (the blood-brain barrier, the blood-cerebrospinal fluid barrier at the choroid plexus and the arachnoid barrier), the most important of which is the blood-brain barrier (BBB). The BBB not only restricts the free transport of different potentially harmful substances to the brain, but plays a critical role in the transport of nutrients to the brain and removal of metabolites.

Anatomically, the BBB is located at the level of the brain microvascular network comprised of capillaries, arterioles and venules. The barrier itself is formed by cerebral endothelial cells (CECs) interconnected by a continuous line of tight junctions (TJs). In addition, an important regulatory role is attributed to pericytes located in the duplication of the basement membrane (for review see refs. [1,2]). Cerebral capillaries are surrounded by specialized structures of astrocytes, called endfeet, which cover more than 90% of capillaries, and together with the deposited parenchymal basement membrane form the glia limitans perivascularis. These three cell types – together with neurons – are the main components of the neurovascular unit (NVU).

The barrier functions of CECs include a paracellular barrier (tight junctions/TJs, apical junctional complex), a transcellular barrier (low level of pinocytosis and transcytosis), an enzymatic barrier (enzymes metabolizing biologically active substances including catecholamines, acetylcholine, peptides) and the presence of efflux transporters (ABC transporters).[3] Transport of water soluble substances which are not able to cross cell membranes is provided by solute-like carrier transporters (SLCs) or receptor-mediated endocytosis. TJs of CECs are composed of transmembrane proteins like occludin, claudins (mainly claudin-5), junctional adhesion molecules, etc.; and plaque proteins including zonula occludens proteins (ZO-1, ZO-2) and associated molecules. TJs are supported by adherens junctions (AJs), which are mainly formed by cadherin-catenin complexes. In human brain microvessels the most abundantly expressed ABC transporters are ABCG2/BCRP, ABCB1/MDR1, ABCC1, ABCC4, ABCC5, ABCA2 and ABCA8, while the main SLC transporters are SLC2A1/GLUT1, SLC7A5/LAT1, SLC16A1/MCT1, SLC1A3/EAAT1 and SLC1A2/EAAT2.[4]

Although this general structure and molecular composition is valid for a large part of the CNS, the functional diversity of the brain is reflected by regional differences in BBB function. These differences may not only be present between anatomically distinct regions, but could appear along the vascular tree among the capillary, arteriolar and venular endothelium as well. Although capillaries constitute the largest microvascular surface in the CNS,[5] the role of arterioles and venules should not be underestimated. Due mainly to technical reasons (difficulty to isolate endothelial cells from different brain regions or vascular compartments), the cerebral endothelium is often regarded as a homogenous population. However, this simplification may lead to an inadequate understanding of brain microvascular function with potentially significant therapeutic consequences as well.

This review summarizes our present knowledge on the heterogeneity in the structure and function of the NVU.

Morphological differences in the vasculature of different CNS regions

The capillary network of distinct brain regions may show differences in topography, and in the morphology and function of the cells of the NVU. The capillary density is higher in

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the gray matter than in the white matter, which is thought to be related to the higher synaptic activity and metabolic demand of neurons. In the rat brain the medium distance among capillaries was shown to be 19.5 μm in the gray matter and 29.8 μm in the white matter, and there was a significant difference in capillary surface areas (179 cm^2/g vs. 107 cm^2/g in the gray vs. the white matter).[6] Comparing 18 gray matter and 5 white matter regions in the rat brain, Borowsky and Collins found that the capillary density was 3-5-times higher in gray matter structures than in the white matter, the highest being in the inferior colliculus (**Table 1**). Regional capillary density showed strong positive correlation with glucose utilization and negative correlation with lactate dehydrogenase (LDH) activity. Cytochrome oxidase activity was lower in the white matter than in the gray matter.[7] Density and branching of capillaries was found to be higher in the gray matter than in the corpus callosum in mice as well.[8] White matter vessels were found to be oriented parallel to the axons.

Even within the gray matter large differences were observed in the vascular density of different regions. In the mouse cortex relatively few capillaries were found immediately below the glia limitans, but capillary density increased in deeper cortical regions to an apparent maximum near 500 μm below the surface of the somatosensory cortex (**Table 1**).[9] The rat hippocampal CA3 region was shown to be rich in microvessels, similar to the parietal or the frontal cortical regions. On the other hand, the CA1 region was shown to have a low vascular density, comparable to the corpus callosum (**Table 1**).[10] The capillary density of the subfornical organ (SFO) (one of the circumventricular organs with increased BBB permeability) and of the inferior colliculus (a metabolically very active part of the brain) was even higher than that of the sensorimotor cortex in rats.[11] In the mouse brain the best vascularized regions were found to be the colliculi, thalamus, parietal cortex and occipital cortex, while the worst vascularized regions were the pons and medulla.[12]

Pathological processes may differently affect vascular densities of different CNS regions. In mouse models both aging and Alzheimer's disease (AD) were shown to contribute to the decrease in vessel densities in the hippocampus, while in the cortex loss of microvascularization was a consequence of the interaction of aging and AD (**Table 1**).[13] It has also been shown that during aging different angiogenesis-related genes were altered in distinct brain regions of the mouse. Although VEGF was not altered, several other factors were found to be differently regulated (e.g. MMP-2 decreased in the cortex, but increased in the white matter of old animals). It was concluded that regional control of multiple angiogenesis-related genes exists in the brain.[8] Although aging induced capillary loss in all brain regions, the severity of this process was higher in the gray matter than in the white matter, while reduction of capillary branch points affected the cortex and the white matter, but not the hippocampus.[8] Physical exercise reversed some aging-associated impairment of angiogenesis-related gene expression in all 3 brain regions studied. On the other hand, it positively impacted brain capillary density and branching in aged mice only in the hippocampus, but not in the cortex or the corpus callosum.[8]

Differences in astrocytes

In respect of the cellular differences in different CNS regions, out of the 3 most important cell types of the BBB/NVU astrocytes were shown to be the most diverse. They differ in their origin, function, density and morphology, possibly adapting to the needs of their environment.[14] The most abundant astrocyte types are protoplasmic astrocytes in the gray matter and fibrous astrocytes in the white matter (**Table 1**).[15] They have different morphologies: protoplasmic astrocytes having several radially extending processes, while fibrous astrocytes having smoother, longer processes. It has been shown that white matter astrocytes have higher levels of intermediate filament proteins (including GFAP, vimentin and nestin) than gray matter astroglial cells (**Table 1**).[14] Moreover, cultured astrocytes derived from the white matter were found to have increased levels of SLC1A3 (GLAST1/EAAT1), SLC1A2 (GLT1/EAAT2) and glutamine synthetase (GS) in comparison with astrocytes originating from the gray matter (**Table 1**).[16]

The cell surface molecule CD44 was found to be predominantly expressed on the surface of white matter astrocytes (**Table 1**).[17] In the gray matter CD44 was found to be associated with the processes of some astrocytes of both protoplasmic and fibrous morphology, which came in association with blood vessels.[18] Interestingly, CD44 can be upregulated during aging [18] and CNS disorders including multiple sclerosis/MS [19] and AD.[18]

In addition, intercellular communication of astrocytes may differ in distinct regions of the CNS. Regional differences exist in the connexin expression of astrocytes, e.g., white matter astrocytes have no or less connexin-30 levels than gray matter astrocytes (**Table 1**).[20] Astrocytes in the neocortex were found to be highly coupled, in contrast to astrocytes in the corpus callosum. As a consequence, astrocytic calcium waves were shown to propagate without gap junctional coupling in the white matter, while in the neocortex calcium wave propagation depended on functional astrocytic gap junctions.[21] In the hippocampus CA1 astrocytes were shown to have a high degree of cell-to-cell coupling, while in CA3 astrocytes' coupling was absent or restricted to smaller cell groups (**Table 1**).[22]

Although the differences in astrocyte morphology and function are well characterized, little is known about their impact on BBB function. The importance of GFAP – which shows regional differences in its expression – in the formation of the BBB is supported by the findings that lack of GFAP impairs the integrity of the BBB [23] and GFAP-deficient astrocytes have a reduced capacity to induce BBB properties.[24] Moreover, it has been suggested that reduced GFAP expression in the endfeet of astrocytes might contribute to the fragility of the germinal matrix vasculature, and therefore might play an important role in the pathogenesis of intraventricular hemorrhages of premature infants.[25]

Since astrocytes are indispensable in the maintenance of the barrier function of CECs, differences in astrocyte functions might influence the BBB as well. Further studies are needed to understand how astrocytes of different type in different CNS

Table 1. Regional differences in the NVU.

	circumventricular organs	spinal cord	white matter (c. callosum)	gray matter (cortex)	hippocampus	cerebellum
capillary density	SFO: high		lower than in gray matter	higher than in white matter, increased in deeper cortical regions, AD+aging: decrease	CA3: high, CA1=white matter, aging or AD: decrease	
astrocytes	GFAP-positive astrocytes: important role in preventing diffusion of blood-derived molecules outside sensory CVOs		most abundant: fibrous, high expression of intermediate filament proteins and CD44, high expression and activity of glutamate transporters	most abundant: protoplasmic, high levels of connexin-30, highly coupled	CA3: low coupling, CA1: highly coupled astrocytes	
pericytes	high NG2 and PDGFRβ expression	lower pericyte coverage, ALS: pericyte loss		high pericyte coverage, AD: pericyte loss	high pericyte coverage, AD: pericyte loss and dissociation	
cerebral endothelial cells	fenestrated capillaries, thinner endothelial cells, vesicles, discontinuous TJs, low expression of TJ proteins, high permeability (higher in the central regions)	reduced amounts of ZO-1, occludin, β-catenin, VE-cadherin and P-gp than in brain endothelial cells, increased permeability as compared with the brain	basement membrane=as in gray matter, CD44-negative endothelial cells, lower P-gp expression in the epileptogenic temporal lobe, increased permeability in MS	basement membrane=as in white matter, medium-sized arterioles: CD44-positive, high P-gp expression and activity	increased permeability in response to histamin, during aging, in pancreatitis or epilepsia compared to cortex, CA3: less permeable in ischemia or during aging than CA1	lower P-gp expression than in the cortex, more permeable (to bilirubin, in response to histamin) than cortical regions

regions influence the BBB in physiological conditions. On the other hand, it is well known that in neurological disorders (ischemia, brain trauma, brain tumors, epilepsy, Parkinson's Disease/PD or AD) reactive astrogliosis occurs during BBB disruption (for review and further information see ref. [26]).

Regional differences in pericytes

Pericytes are also very diverse in their origin and have important regulatory functions on different endothelial functions.[2] Pericyte coverage of brain capillaries was shown to be ~80% in the mouse cortex, caudate nucleus and hippocampus.[27] On the other hand, pericyte coverage was significantly lower (varying between 48 and 68%) in cervical, thoracic, and lumbar spinal cord anterior horn capillaries in comparison to the brain, but not (or to a lesser extent) in the white matter regions of the spinal cord (**Table 1**).[27] Lower pericyte coverage was shown to correlate with the higher permeability of the blood-spinal cord barrier (BSCB) in comparison to the BBB.[27] In amyotrophic lateral sclerosis, a motor neuron disease of the spinal cord, a reduction in the number of pericytes was demonstrated correlating with an increase in the permeability of the BSCB.[28]

In circumventricular organs, pericytes were shown to express high levels of NG2 and PDGFRβ, as seen in the immature vasculature (**Table 1**). Chronic salt loading caused a further increase of pericytic expression of NG2 and PDGFRβ, and increased vascular permeability of FITC in the organum vasculosum laminae terminalis and the SFO.[29]

In the hippocampus (a region critical for learning and memory) age-dependent BBB breakdown was shown to correlate with pericyte injury.[30] In AD a significant loss of pericytes was observed in both the hippocampus and the frontal cortex, correlating with BBB leakage.[31] In mice with AD-like pathology loss of pericytes was observed in both the cortex and the hippocampus, while progressive perivascular dissociation of pericytes was characteristic to the hippocampus, but no to the cortex (**Table 1**).[13]

In conclusion, the extent of pericyte coverage seems to directly influence the barrier function of CECs in different brain regions in physiological and pathological conditions as well.

Differences in cerebral endothelial cells

CECs are the most important cellular components of the BBB; therefore, differences in barrier properties of capillaries in different brain regions are directly determined by the properties of endothelial cells. However, little is known about the heterogeneity of BBB-forming endothelial cells.

The BBB is localized to the level of microvascular endothelial cells, which comprise not only capillaries (vessels <10 μm diameter) but also arterioles and venules (which are 10–100 μm in size). Arterial and venous endothelial cells differently express ephrinB2 and EphB4, several members of Notch signaling, Hedgehog morphogens and VEGF-A [32] – all of which have important role in the regulation of BBB functions. To our best knowledge, however, the regional distribution and function of these proteins in different brain microvascular segments have not been studied so far.

Indeed, CECs are not homogenous all over the microvasculature and this heterogeneity is reflected in their functional diversity. By profiling 87 genes enriched in brain microvessels it was shown that most of the BBB properties reside in both capillaries and venules, but particular functions may specifically associate with different segments. Solute transport seems to be preferentially related to capillaries, while venules showed higher expression of inflammation-associated genes (**Table 2**).[33]

Biochemical and physiological properties related to the barrier functions of endothelial cells seem to be more expressed in capillaries than in larger microvessels. These characteristics include expression of specific transporters (P-gp, Glut-1), receptors (transferrin receptor) and enzymatic activities (e.g., alkaline phosphatase) (reviewed in ref. [34]). Postcapillary venules were shown to have looser arrangement of junctional strands than capillaries (**Table 2**),[35] being therefore the preferred site for leukocyte extravasation. In the thoraco-lumbar spinal cord of mice the intensity of immunostaining for claudin-5 – which forms the backbone of the TJs – was highest in the capillaries and smaller venules, and lowest in the larger venules. During experimental autoimmune encephalitis (EAE), claudin-5 was shown to be selectively lost in venules with permeability increase in both capillaries and venules (**Table 2**).[36] In the human brain P-gp/ABCB1 was found to be localized to the luminal membrane of microvessel endothelial cells, becoming weaker or undetectable

Table 2. Heterogeneity of the BBB in different segments of the brain microvasculature.

	arterioles	capillaries	venules
gene expression		increased expression of genes of the solute transport system	higher expression of inflammation-associated genes
TJs			looser arrangement of junctional strands than in capillaries, EAE: loss of claudin-5
transporters	lower P-gp levels	high and uniform P-gp levels	high and uniform P-gp levels
astrocytic endfeet	thickest astrocytic perivascular sheaths	GFAP-negative astrocytic processes, highest density of contacting processes, thinest astrocytic perivascular sheaths	

on larger vessels.[37] Its expression displayed an increasing gradient from an almost undetectable level in large penetrating arterioles to a high and uniform level in capillaries and venules (**Table 2**).[38] It was suggested that the lower expression of P-gp in arterioles in comparison to capillaries and venules could participate in the preferential arteriolar accumulation of β-amyloid in cerebral amyloid angiopathy.

It has also been shown that differences exist in the glio-vascular interface along the microvasculature. In the rat cortex the surfaces of large to medium-size vessels were densely covered by GFAP-positive astrocytic end-feet; however, astrocytic processes covering capillaries were found to be GFAP-negative (**Table 2**).[39] In addition, arteries were found to have the thickest astrocytic perivascular sheaths, followed by veins, while capillaries had the thinnest astrocytic perivascular sheaths. The highest density of contacting processes was found for capillaries (**Table 2**).[9]

Regarding BBB differences in different brain regions, it is important to understand endothelial heterogeneity in the distinct parts of the CNS. No differences were found between the white and gray matters in the composition of the basement membrane in the cerebral vasculature of human fetuses, premature and mature infants.[40] On the other hand, the adhesion molecule CD44 was found on endothelial cells of medium-sized arterioles in the gray matter but, not in the white matter (**Table 1**).[17]

Using a mouse model expressing human MDR1-luc, both hMDR1 transcription and mouse P-gp protein expression were found to be higher in the cortex than in the cerebellum (**Table 1**).[41] In the removed brain tissues of the epileptogenic temporal lobe higher P-gp expression was found in capillaries of the gray matter than in the white matter. Seizure recurrence coincided with higher P-gp expression, especially in the white matter, of the resected lobe.[42]

Barrier properties of CECs, however, principally depend on the TJs. Differences in the permeability of the BBB are mainly a consequence of the differences in the TJs, which are responsible for the first defense line (i.e. the paracellular barrier) of CECs. Regional differences in TJs and BBB permeability are discussed in the next chapter.

Fuctional differences: Permeability properties of the BBB in different brain regions. Role of tight junctions

The barrier function of the BBB is reflected by its relative impermeability to blood-borne solutes. Due to the presence of a complex network of TJ strands, the intact BBB prevents the diffusion of hydrophilic tracers into the CNS parenchyma. Size-selective opening or permeability differences can be monitored using fluorescent tracers (sodium fluorescein – 376 Da, fluorescein isothiocyanate/FITC – 389 Da, Lucifer yellow – 457 Da or fluorescently labeled dextrans – 3–70 kDa) or radiolabeled compounds (e.g., 3H-mannitol – 180 Da, 3H-sucrose – 342 Da, 3H/^{14}C-inulin – ~5 kDa). Flux of tracers can be assessed both *in vivo* and *in vitro*. Ion permeability of TJs can be estimated *in vitro* by measuring the transendothelial electrical resistance (TEER). Both TEER and permeability measurements reflect not only the paracellular pathway, but a composite of the para- and the transcellular routes.[43] The transcellular route is mainly reflected by the flux of Evans blue-labeled albumin (66 kDa) or horseradish peroxidase (44 kDa).

The most striking functional differences in BBB permeability are associated to circumventricular organs (CVOs). Here the BBB is leaky and the capillaries are fenestrated, allowing a relatively free exchange of substances between the blood and CNS. CVOs are important elements of the neuroendocrine system and include the area postrema (AP), the subfornical organ (SFO) and the organum vasculosum laminae terminalis (OVLT) (these regions are capable of sensing the concentration of different peptides), the pineal gland, the posterior pituitary, the intermediate lobe of the pituitary gland, the median eminence and the subcommissural organ (which are secretory organs). Morphologically here the capillaries are fenestrated with discontinuous TJs, thinner endothelial cells which contain more vesicles than capillaries of other brain regions (**Table 1**).[44] Although the exchange of circulating substances is relatively free in the CVOs, they do not provide direct passage of blood-borne substances to the rest of the brain due to the presence of diffusion barriers. For example, ZO-1- and GFAP-positive columnar cells were described between the AP and nucleus tractus solitarius.[45] Both the outer basement membrane and also astrocytes and tanycytes are considered alternative barriers to prevent diffusion of blood-derived molecules outside the sensory CVOs.[46]

However, not even the endothelia of CVO microvessels constitute a homogenous population. In the median eminence and the SFO many capillaries lack detectable levels of ZO-1; low level of ZO-1 can be detected in the AP, but in the subcommissural organ the ZO-1 staining is similar to barrier forming capillaries.[47] In the SFO the blood-to-tissue flux of ^{14}C-α-aminoisobutyric acid was shown to be 100–400 times faster than in the gray or the white matter [11] and the water permeability was shown to be one of the highest in the brain.[48] The AP region could be clearly localized in living humans by SF administration,[49] indicating the permeability of the AP to the dye.

In addition, sensory CVOs of mouse capillaries were found to be permeable to FITC and to 3 kDa dextran; however, the 10 kDa dextran remained in the perivascular space between the inner and outer basement membranes. Interestingly, not even the lower molecular weight markers were able to diffuse beyond the dense network of GFAP-positive astrocytes and tanycytes, which have a higher density at distal subdivisions of the sensory CVOs as compared with central subdivisions (capillary plexus in the OVLT, the ventromedial core in the SFO and the central zone in the AP). It is important to note that the capillary permeability was higher in the central regions of secretory CVOs (**Table 1**). In line with these findings, TJ proteins like occludin, claudin-5 and ZO-1 were undetectable at the central subdivisions, whereas a low level of staining was detectable in distal subdivisions.[46]

Although the rest of the CNS is protected by an intact BBB under physiological conditions, its permeability is not uniform. The most important differences are related to the spinal cord. The blood-spinal cord barrier (BSCB) has a similar cellular and molecular structure as the BBB; however, specific differences have been described. Literature data indicate that in general the BSCB has an increased permeability to 3H-mannitol and ^{14}C-inulin in comparison to the BBB, the lumbar cord appearing to be the most permeable (**Table 1**).[50] Cultured rat endothelial cells isolated from the spinal cord reached lower TEER values than endothelial cells isolated from rat brains; however, both showed similarly low permeability to Lucifer yellow.[51] In the murine spinal cord – predominantly in the anterior horn – the number and capillary coverage of pericytes was found to be reduced as compared with the brain, leading to decreased expression of occludin and ZO-1, but not of claudin-5 in spinal cord capillaries. As a consequence, increased permeability to 350 Da-150 kDa tracers was observed in the cervical, thoracic and lumbar spinal cord regions when compared to the brain.[27] In accordance, murine spinal cord microvascular endothelial cells in culture were shown to express reduced amounts of ZO-1, occludin, β-catenin, VE-cadherin and P-gp than brain endothelial cells (**Table 1**).[52] The differences between the 2 barrier systems, i.e., the BBB and the BSCB, might be responsible for some of the differences between the pathologies of the brain and the spinal cord.[53]

Permeability differences have been described among different regions of the BBB-protected brain as well, mainly in non-physiological conditions. Multiple sclerosis patients, for instance, were shown to have increased BBB permeability in the total white matter, but not in gray matter regions.[30] In 2-day old piglets the brainstem and the cerebellum were shown to be more permeable (at least to bilirubin) than cortical regions.[54] In response to the histamine antagonist DPPE, an increased permeability for sodium fluorescein was seen in the hippocampus, striatum and cerebellum, but not in the parietal cortex of rats, while Evans blue-labeled albumin extravasated in all brain regions.[55] The rat parietal cortex was also found to be more resistant to acute pancreatitis-induced BBB permeability increase than the hippocampus, striatum and medulla (**Table 1**).[56] In ischemic conditions extravasation of albumin was shown to be more pronounced in the hippocampal CA1 region than in the highly vascularised CA3 region of the rat brain (**Table 1**).[10] After status epilepticus induced in rats, BBB leakage was observed only in limbic brain regions (in the entorhinal cortex, amygdala and the piriform cortex, and to a lower extent in the hippocampus and thalamus), while cortical brain regions such as the motor cortex were not affected. BBB impairment was still present 6 weeks after staus epilepticus in the hippocampus, entorhinal cortex, amygdala and piriform cortex.[57]

Aging and hormonal status may also differently influence the BBB of different brain regions. Increased IgG leakage was observed in the hippocampus and thalamus, but not the hypothalamus of reproductive senescent female rats.[58] In the aging human brain a progressive leakage through the BBB was observed in the hippocampus (the CA1 and DG, but not the CA3 region) (**Table 1**) and the caudate nucleus, but other brain regions (frontal cortex, temporal cortex, thalamus, striatum, subcortical white matter fibers, corpus callosum and internal capsule) were not affected.[30] The process affected persons with no cognitive impairment, but was accelerated in cognitively impaired individuals.

Taken together, pathological processes of specific brain regions are usually associated with BBB impairment of the region affected. However, interventional approaches may also differently affect the BBB of diverse CNS regions. Systemically-injected reduced graphene oxide, for example, was found to induce a transient opening of the BBB mainly in the thalamus and hippocampus of rats.[59]

Conclusions

The largest part of the CNS is protected by a tight BBB composed of CECs interconnected by continuous TJs. At the level of circumventricular organs – especially in their central regions – capillaries are more permeable, containing fenestrations and discontinuous TJs. However, other CNS regions are also heterogenous in the tightness of the barrier. In special, the blood-spinal cord barrier (BSCB) was shown to be more permeable than the BBB. This is in line with the reduced expression of TJ proteins and the lower number of pericytes covering the outer surface of capillaries. However, much less is known about the permeability differences among other regions of the brain in physiological conditions. Considering the regional restriction of several brain pathologies and the diversity of astrocytes, it is to be expected that the NVU is not uniform throughout the brain. Further studies are needed to understand differences in brain endothelial properties in the white matter, cortex, hippocampus, cerebellum, etc. Regional NVU differences are summarized in **Table 1**.

Moreover, heterogeneity of CECs appears among capillary, arteriolar and venular segments as well, which are all parts of the BBB. Barrier properties seem to be more expressed in capillaries than in larger microvessels (**Table 2**).

Disclosure of Potential Conflicts of Interest

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