Healthy aging and the blood-brain barrier

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Abstract

The blood-brain barrier (BBB) protects the central nervous system (CNS) from unregulated exposure to the blood and its contents. The BBB also controls the blood-to-brain and brain-to-blood permeation of many substances, resulting in nourishment of the CNS, its homeostatic regulation and communication between the CNS and peripheral tissues. The cells forming the BBB communicate with cells of the brain and in the periphery. This highly regulated interface changes with healthy aging. Here, we review those changes, starting with morphology and disruption. Transporter changes include those for amyloid beta peptide, glucose and drugs. Brain fluid dynamics, pericyte health and basement membrane and glycocalyx compositions are all altered with healthy aging. Carrying the *ApoE4* allele leads to an acceleration of most of the BBB's age-related changes. We discuss how alterations in the BBB that occur with healthy aging reflect adaptation to the postreproductive phase of life and may affect vulnerability to age-associated diseases.

The blood-brain barrier (BBB) is an old concept, originating during the last part of the nineteenth century. Observations that dyes and biologically active substances did not stain the brain or affect behavior when injected systemically, but could when injected directly into the central nervous system (CNS), led the pioneers to conclude that there must be a selective barrier between the blood and the CNS[1,2]. Currently, the BBB is often divided into four barriers: the vascular BBB (vBBB), residing in vertebrates at the capillary bed and the adjacent arterioles and venules, and whose fundamental unit is the brain endothelial cell (BEC); the blood–cerebrospinal fluid (CSF) barrier, residing at the choroid plexus, and whose fundamental unit is the ependymal cell; the tanycytic barrier that separates

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circumventricular organs (small areas of the brain lacking a vBBB) from their adjacent areas of the barriered brain, and whose fundamental unit is the tanycyte; and the meningeal barrier, residing primarily within the arachnoid mater, and whose fundamental unit is an epithelial cell. Additionally, there are specialized extensions or regions of the BBB, such as the blood–retinal barrier and the otic barrier. All barriers are subject to changes with aging; the changes that occur at the vBBB are the best studied. Here, we use the term vBBB when referring specifically to the vascular BBB and the term BBB when a more general concept is needed.

The BBB prevents the unregulated leakage of blood-borne materials into the CNS[3]. All the BBBs prevent leakage between their barrier cells by their possession of tight junctions, which are complexes of proteins that bind the adjacent barrier cells together so tightly as to impede the passage of electrons as efficiently as the cell membrane (Fig. 1). BECs have also lost nearly all of the macropinocytosis and the fenestrae that contribute to the leakiness of other peripheral capillary beds[4,5]. Thus, an ultrafiltrate, and its accompanying unregulated leakage of blood-borne molecules, is not produced by the vBBB.

Without the production of an ultrafiltrate by its capillary bed, the CNS is without its usual source of nutrition or the fluid that in the periphery requires a classic version of the lymphatic system for drainage. Thus, the CNS must find other ways to import nutrients and export toxins. The BBB largely fills these functions by a variety of mechanisms, most notably the use of influx and efflux transporters. The BBB also regulates the influx and efflux of informational molecules, such as peptides and regulatory proteins, and the bidirectional trafficking of immune cells and exosomes. The cells comprising the BBB respond to and secrete a variety of substances at both its peripheral- (luminal; basolateral) and CNS-facing (abluminal; apical) surfaces. Thus, in addition to serving as a barrier, the BBB functions as a blood-brain interface[6].

The BECs forming the vBBB do not act in isolation but in crosstalk with other cells forming the neurovascular unit (NVU)[7]. Pericytes and astrocytes are the best studied of these cells, but other cells of the CNS and even those of the periphery (for example, circulating immune cells) influence barrier functions. Pericytes in particular have emerged as important at the two ends of the life cycle: vBBB formation during the fetal period and vBBB preservation during aging[8–11]. Many of the molecules participating in this crosstalk are immune-related (for example, cytokines and chemokines), with the BBB first separating and then rejoining the CNS and immune system[6]. By secreting and responding to circulating substances, the BBB acts much like an endocrine tissue[12]. Models that appreciate the role of the BBB in the neuroimmune axes or as an endocrine tissue underscore its complex and multiple functions, many of which change with healthy aging. See Box 1 for a glossary of technical terms.

The BBB and models of aging

All the BBBs are subject to changes with aging, but those occurring at the vBBB are the best documented and will be the focus of this Review. The cells of the NVU have been studied to varying degrees during healthy aging. However, much of that literature does not include studies of BBB function. For example, the literature on the aging astrocyte is substantial, but

very little of that literature relates to the aging vBBB. Here, we focus on only the literature on healthy aging and do not review the much larger literature on age-associated diseases.

One's concept of aging influences one's definition of 'healthy aging'. Most theories of aging, such as the hallmarks of aging[13] and pillars of aging[14], would define aging as the time-dependent accumulation of cellular damage. The contributions to cellular damage are interrelated and include oxidative stress, epigenetic changes, genomic instability, telomere attrition, dysregulation of cell signaling and inflammatory responses, and senescence. Cellular senescence includes a senescence-associated secretory phenotype and biomarkers such as beta galactosidase and p16[Ink4A]. Recent transcriptomic studies have observed age-associated increases in the number of BECs that exhibit high levels of senescence-related gene expression[15]. One regulator of senescence that mediates vBBB dysfunction is sirtuin-1; the loss of sirtuin-1 with aging is associated with vBBB dysfunction[16].

That a brain can be both aged and healthy is supported by the idea of adaptive senectitude[17]. BBB functions change throughout the life cycle in response to the changing needs of a maturing brain. For example, the transport of amino acids by the vBBB differs between neonates and adults[18]. As such, one would expect that a healthily aging brain would be accompanied by changes in BBB functions. This view is consistent with the concept of the BBB as a physiological interface and does not rely on disease models to define age-related changes. Nevertheless, the picture of the healthily aging BBB that emerges from the literature is based on a normative view (that is, what is typically encountered) and may not reflect what is truly healthy.

Morphological changes in the vBBB with aging and the challenges of copathologies

Morphological changes of the vBBB that occur with aging have been recognized for over 50 years, with many of the early works utilizing electron microscopy to view ultrastructural changes of the brain capillary endothelial cells and associated cellular and acellular components of the NVU. The results of studies that have assessed morphological changes are summarized in Table 1. Additionally, aged rats exhibited increased damage by free radicals, but no change in brain microvessel composition[19]. Many studies do not include a middle-aged group; therefore, it is unclear if changes occur in a stepwise or gradual fashion with aging.

Other morphological changes that have been assessed for the aging vBBB are capillary density and diameter[20,21]. The findings of studies on these changes are mixed, which may reflect aspects of study design, such as small sample sizes, the age of the aged group (which should be defined by the median survival time of the species), the brain regions examined and whether comorbidities were assessed. This latter point is a critical consideration; a challenge of studying healthy human aging is that some level of pathology is usually present even in the absence of apparent clinical disease. For example, a comprehensive neuropathological assessment of over 300 brains from cognitively normal donors revealed that an absence of neuropathology in seemingly healthy individuals may be the exception rather than the rule[22]. On average, about 4% of cognitively normal subjects showed no

neuropathological changes, whereas nearly 60% had evidence of Alzheimer's disease (AD) pathology and almost 40% had mixed pathology that included AD and microvascular brain injury or Lewy bodies. Only about 1% of the cohort had microvascular damage in the absence of neurodegenerative-disease-associated pathologies. Notably, the cases assessed in this study were limited to the upper four quintiles on a cognitive-screening test that was performed, on average, within one year prior to death to avoid selection of participants with prodromal dementias[22].

Ongoing advances in neuropathological diagnoses and subsequent classification will further complicate the study of healthy brain aging[23]. In summary, this area is challenged by methodological issues and has not reached a clear consensus regarding morphological changes in the healthy aging vBBB.

vBBB disruption in aging

Whether BBB disruption occurs in healthy aging has been debated for decades. As reviewed here, disruption refers to the unregulated leakage of plasma solutes across the vBBB and not to other types of BBB dysfunction, such as alterations in transporters, diapedesis, enzymatic activity or receptor function.

As discussed in the morphology section, there is no consistent ultrastructural evidence of vBBB disruption, either from changes in tight-junction proteins (TJPs) or increased numbers of pinocytic vesicles, with aging. Older studies using sucrose as a tracer in rats have also suggested an absence of vBBB disruption with age[24]. The older literature included CSF/blood ratios and immunohistochemical studies; in these areas, as previously reviewed[25,26], findings regarding disruption of the aging vBBB were mixed. Immunohistochemical analysis usually relied on demonstration of cuffing around blood vessels by substances typically excluded by the BBB, such as albumin, fibrinogen, Evans blue, plasminogen or IgG. Their presence is taken as evidence of vessel leakage. However, there is some degree of cuffing in young, healthy brains, and it is not clear that vascular cuffing increases with healthy aging[27]. IgG is not an acceptable candidate for this type of analysis as it mostly originates from secretion by immune cells within the CNS and it has a vascular efflux system that may participate in its binding to the abluminal surface of endothelial cells. Evans blue also has several problems[28,29]. As established by a recent study involving a cohort of 20,000 participants, an age-dependent increase in CSF/blood albumin ratios occurs, especially in males[30]. This increase lessened when only healthy donors were analyzed. However, CSF reabsorption slows with age, which also increases CSF/blood ratios of albumin. The extent to which the age-associated rise in CSF/serum albumin ratios is caused by a leakage of the BBB or the decrease in CSF reabsorption remains unresolved[31].

More recent studies in aging rodents have also been controversial, with some showing evidence of vBBB leakage and/or decreased expression of TJPs[16,32,33], whereas others have observed an absence of vBBB leakage with aging[34]. Recently, it has been shown using novel methods for labeling the plasma proteome that plasma protein transport, which occurred abundantly in young mice, was reduced in aged mice. Aging was accompanied by a decrease in the number of clathrin-coated vesicles for receptor-mediated transcytosis and an increase

in caveolae that mediated non-specific protein leakage[35]. Macropinocytosis, the absence of which is central to vBBB integrity, is independent of both clathrin- and caveolin-mediated transcytosis[36]. Therefore, we suggest that this work does not point to a return to leakage caused by macropinocytosis but to other transcytotic mechanisms.

Imaging currently offers the best approach in humans to determine the degree to which BBB disruption occurs with healthy aging. Studies using dynamic-contrast-enhanced magnetic resonance imaging (DCE-MRI) have found disruption of the vBBB in the hippocampus[37] and in gray and white matter with healthy aging[38]. The disruption correlates with some measures of the cognitive decline that are accepted to accompany healthy aging, with the strongest correlation between leakage in white matter and delayed recall[39]. These studies strongly support vBBB disruption in healthy aging and an association with some types of cognitive decline. Although any disruption in the BBB, especially one that would be present for decades, is likely detrimental to brain health, these studies must be interpreted in context. In the study of Montagne et al.[37], for example, leakage to gadolinium is predicted to increase between the ages of 20 and 100 by about 80%. This contrasts with about a sevenfold increase in leakage in contrast-enhancing lesions in multiple sclerosis[40]. The increase in tracer leakage is highly variable with age and is well within the range of normal values; for example, in a study by Verheggen et al.[38], the single individual over the age of 90 years had white matter that was less leaky than that of the two individuals who were younger than 50. That this leakage is small and varies between people explains why it has been so hard to detect BBB disruption by immunohistochemistry. In conclusion, imaging supports a small increase in leakage in the healthy aging vBBB that correlates with some measures of cognitive impairment. The increase in leakage is small and variable, which likely accounts for why the histological findings have been ambiguous.

Altered transport systems at the vBBB

Saturable systems at the BBB serve diverse functions and can be categorized in a variety of ways. Functions include providing the CNS a homeostatic environment, nutrition and communication with peripheral tissues[18]. Transporters can be constructed as vesicles, channels, or pores that are either energy-dependent or energy-independent, and transit bidirectionally or unidirectionally across the BBB. As discussed below, transporters can also be used by drugs to enter the brain, and two such transporters, large neutral amino acid transporter (LAT-1) and p-glycoprotein (P-gp), are altered with healthy aging. Other vBBB transporters that are decreased with healthy aging include those for the interleukin-1 family, choline, triiodothyronine, tumor necrosis factor-alpha, glucose, choline, and Tyr-MIF-1 and the enkephalins[41–46]. Kinetics analysis shows an uncompetitive (decreases in both Michaelis–Menten constant (K_m) and velocity (V_{max})) pattern of inhibition for some of these transporters, suggesting loss of, or interference with, a transporter cofactor rather than the presence of a competitive inhibitor or loss of transporter number.

Are the changes with aging in BBB transporters an adaptation to healthy aging, a failure of BBB functioning or a response to brain disease? Changes in BBB transporters between neonates and adults reflect the changing needs of the maturing brain[18]. Similarly, alterations in BBB functions could reflect changing demands of an otherwise healthy brain or an

adaptive response to the needs of a diseased brain. Alternatively, a mismatch between the services of the BBB and the needs of the brain would lead to CNS dysfunction. For example, inhibition of the brain-to-blood transporter low-density lipoprotein receptor-related protein-1 (LRP-1), as occurs with normal aging, leads to decreased efflux of amyloid beta (Aβ) peptide and its subsequent accumulation, resulting in cognitive impairment[47].

Whether the changes in BBB transport rates are in response to, or the cause of, CNS dysfunction has been debated most vigorously for glucose. Glucose transport across the vBBB is near its maximum, and almost all of the glucose transported into brain is used. Glucose transport and glucose metabolism are tightly correlated to each other and to cerebral blood flow. All three decrease with age, but cerebral blood flow becomes uncoupled from glucose transport utilization temporally and regionally[42,45,48]. However, the decrease in glucose transport is obviated when corrected for brain atrophy[49]. The question remains: is glucose transport across the vBBB in response to a lower demand by aging brain tissue, or does a decrease in glucose transport across the vBBB drive a starving brain into dysfunction?

In summary, changes in BBB transport systems clearly occur with healthy aging, although only a few transport systems have been investigated. Why most show uncompetitive changes in kinetics is unclear, but suggests a cofactor effect. The decrease in glucose transport illustrates the dilemma of determining whether a change is in response to, or a cause of, changes in the CNS.

The BBB and altered passage of drugs in the aging brain

Almost 90% of the US population 65 years old and older report using at least one prescription medication, and about 35% report concurrent use of five or more prescription medications, underscoring the trend of increased medication use among older adults[50]. The pharmacokinetics and pharmacodynamics of many drugs are altered in advanced age[51], including CNS-active drugs[52]. Most drugs entering the brain use the mechanism of transmembrane diffusion; the degree of penetration is determined mainly by lipid solubility, hydrogen bonding and molecular weight, but it is also influenced by binding to serum proteins and peripheral pharmacokinetics.

Hydrophilic drugs diffuse across the BBB much less readily than do lipophilic drugs, and the expression of specialized efflux transporters such as P-gp impede the passage of a number of otherwise membrane-permeant drugs. Evidence suggests that P-gp function at the vBBB decreases in humans beginning in middle age[53–55] and continuing into old age, with expression decrements synergizing with neuropathological burden[56]. P-gp activity is also decreased in vivo by factors like inflammation that accompany normal aging as well as many age-related diseases[57,58]. Older populations are more sensitive to intended or adverse effects of CNS-active drugs, such as antipsychotics, benzodiazepines and opiates, many of which are P-gp substrates or inhibitors[52,59,60]. Therefore, it is plausible that P-gp deficiencies with aging could contribute to changes in drug disposition in the brain. Since P-gp is an efflux transporter for Aβ[61,62], xenobiotic P-gp substrates or inhibitors could

contribute to Aβ accumulation in the brain under conditions where the capacity of this efflux system decreases, such as aging.

Other drugs, such as L-DOPA and gabapentin, which are too hydrophilic to freely diffuse across membranes, can cross the vBBB because they are substrates for BBB transporters, such as LAT-1 (ref. [63]). Thus developing a better understanding of how age-associated changes at the vBBB influence CNS drug delivery is important for developing safer, more effective therapies for the geriatric population.

Aging and changes in the movements of brain fluids

The brain naturally creates waste products that must be removed to maintain homeostasis. Without the production of a capillary ultrafiltrate, the brain does not possess a classic lymphatic system. For solutes not readily cleared across the vBBB in the brain-to-blood direction, the major mechanism of brain clearance has long been associated with exchange into the CSF compartment. CSF is produced primarily by the choroid plexus of the lateral, third and fourth ventricles, moving outward through the ventricular system by bulk flow and exiting via the foramina of Magendie and Luschka to enter and fill the subarachnoid space surrounding the brain and spinal cord (Fig. 2). Solutes exchanging between the interstitial and CSF compartments are then cleared as the CSF is reabsorbed into the blood stream via arachnoid villi or drainage along cranial nerves to the cervical lymphatics[64–66].

Exchange of fluids and their solutes between the CSF and interstitial compartments is dominated by two forces: diffusion, driven mainly by Brownian motion, and convection (more appropriately referred to as advection). Diffusion is characterized by solute movement being symmetric (that is, equal in all directions from the point of origin), from a higher to a lower concentration, and with low-molecular-weight solutes diffusing faster than high-molecular-weight solutes (Fig. 2). These characteristics of diffusion are not altered by aging. Convection/advection is characterized by solutes following the movement of their solvent (bulk flow), which creates asymmetric gradients and a movement of solutes that is independent of molecular weight. Solutes administered into the interstitial space migrate towards the ventricles more rapidly than ventricular CSF solutes migrate into the brain parenchyma, illustrating that convection occurs in the brain interstitial space[67]. Proposed mechanisms of convection within the brain interstitial fluid and/or the CSF are the production of metabolic free water, CSF secretion and reabsorption, vascular pulsations and water secreted by aquaporin-4 (AQP4) located on astrocytic endfeet (glymphatic system)[68–71]. Anatomic heterogeneities of the brain that are thought to magnify asymmetric solute distribution are the Virchow–Robin spaces (fluid-filled spaces surrounding penetrating vessels that support the movement of fluid between CSF and brain tissue[72–74]), a separation of brain parenchyma by astrocytic endfeet surrounding the vBBB into a perivascular region and a brain interstitial-fluid region and tortuosity of the brain interstitial-fluid channels that surround brain cells.

Changes with healthy aging are observed for all of the proposed mechanisms of convection. The rate of CSF turnover is decreased with healthy aging[75,76]. The glymphatic system is impaired in aged rodents[77] because of a loss of perivascular AQP4 localization. Perivascular

movement is more active during slow-wave sleep[78] and so could be disturbed with the fragmentation of sleep, which often occurs with healthy aging. Reduced arterial compliance occurs in otherwise healthy human aging[79–81], and this affects both the vascular pulsations in capillaries[80] and glymphatic activity[71]. Age-associated decreases in the metabolic rate of brain cells or a reduction in the number of brain cells reduces the production of metabolic free water. The disruption of the BBB that occurs in healthy aging would be expected to modify convection in the aging brain. Most age-related changes in convection, except for BBB disruption, reduce convection.

Some of the consequences of altered convection of brain fluids are understood. Decreased CSF turnover is one reason that CSF/serum ratios of albumin increase with healthy aging[75,76]. Reduced convection decreases the clearance of substances with higher molecular weights more so than that of lower-molecular-weight substances[69]. Extrapolation of data presented by Ray et al. suggests that convection accounts for more movement than does diffusion for solutes with molecular weights greater than about 200 Da[69]. Thus, the clearance from the aging brain of low-molecular-weight solutes would be less impacted than those in the size range of peptides and larger compounds[70,82].

Changes in cell types of the NVU with aging and implications for vBBB health

Although all cell types in the brain are in direct communication with BECs, the astrocytes and pericytes are the most important in the induction of BECs to form a vBBB and are the most important in regulating BBB structure and function. While there is a great deal of literature on the impact of aging on astrocytes in general, the information on how these changes relate to or affect the BBB is sparse. Therefore, the direct impact of aged astrocytes on the vBBB is an area that warrants further investigation. The effect of aging pericytes on BEC function is better studied and suggests a critical role for pericyte function in BBB aging.

With aging, astrocytes undergo hypertrophy, and the number of astrocytes expressing neuroinflammatory genes increase in number, taking on a more 'reactive' morphological phenotype, while proliferation and total astrocyte number decrease[83]. In addition, aged astrocytes have increased oxidative metabolism and altered glutamate regulation[84]. The reactive phenotype of astrocytes and the impaired antioxidant capacity affects their ability to respond to stressors or insults. Therefore, during healthy aging, this phenotype may not emerge until an injury or insult to the CNS arises. It should be noted that astrocyte reactivity does not affect the ability of the endfeet to surround BECs[85]. Additionally, astrocytes are known to be the primary secretors of sonic hedgehog (Shh), a protein known to act on BECs to regulate vBBB permeability[86]. Shh signaling is impaired in reactive astrocytes[87], which increase in prevalence with aging and could be a contributor to vBBB dysfunction with age.

Despite historical conflicting data, recent methodological advances suggest that pericyte loss occurs with age[9–11]. Additionally, pericytes can lose contact with the BECs[88], potentially through age-related alterations in platelet-derived growth factor receptor β (PDGFRβ) signaling[9,10]. A 20% loss in pericytes is sufficient to cause vascular damage in mice, without

neuronal damage[9]. Indeed, pericytes are critical to vBBB integrity, especially under conditions of stress[89], and loss of pericytes leads to severe alterations in blood flow and circulatory failure[90]. The soluble form of PDGFRβ shed from pericytes directly correlates with vBBB breakdown in humans, and CSF levels of this protein predict changes in cognitive status[10]. Therefore, the age-dependent loss of pericytes may lead to vBBB dysfunction.

Specific mechanisms regarding the age-related loss of pericytes, especially due to oxidative DNA damage and repair, are poorly understood compared with those regarding other BBB cell types[84] and deserve further exploration. Further mechanisms for pericyte loss, including oxidative stress, are expanded on in Box 2.

Pericytes are also critical to regulation of transport systems, both of the BEC and of the pericyte itself. Sweeney et al. recently reviewed the transport systems present in pericytes[91]. How these transport systems change with age, with the exception of the transporter for Aβ, is largely unknown. A recent transcriptional study has shown that the gene *ARHGAP42* declines with age in vBBB samples collected from both humans and mice[92]. This protein is enriched in pericytes[93] and is known to regulate blood pressure[94], underscoring a role in vascular maintenance. Using an inducible pericyte knockout mouse model, investigators were able to show pericyte loss concomitant with a dysfunctional vBBB led to neurodegeneration within days and then to behavioral changes within 2 weeks[90].

In summary, age-related changes in BECs and pericytes, and probably in astrocytes, alter NVU–BEC communications, resulting in age-related alterations in BBB functions. Changes in BBB functions, in turn, may predispose to, or increase vulnerability to, age-related diseases. Mechanisms to prevent pericyte loss and astrocyte reactivity are described in Box 2, and studies still needing to be performed to investigate NVU–BEC communication are described in Box 3.

Altered extracellular matrix and the abluminal basement membrane

The basement membrane (BM) of the BBB is a 40- to 100-nm-thick composite of structural extracellular matrix (ECM) that lies next to the abluminal membrane of the brain endothelium. The BM is synthesized predominantly by BECs, pericytes and astrocytes at the vBBB, and is designed to undergo minimal turnover during the vascular lifespan. Resistance to breakdown is facilitated by a backbone of the heterotrimer laminin and the sheet-like collagen IV (col IV). The deposition of laminin and col IV are interconnected and further stabilized by cellular fibronectin and heparan sulfate proteoglycans (HSPGs), such as perlecan, agrin and nidogen[95]. The individual contribution of these components to BM function has been delineated by knockout and mutation studies, which have been recently reviewed by Xu et al.[96]. The BM of the brain microvasculature is typically described as having endothelial and parenchymal compartments that are separated by pericytes. This distinction reflects the increasing appreciation of pericytes as regulators of cell–ECM interactions in the BBB, although the composition of the BM is identical in regions of endothelial and parenchymal BM having a reduction in pericytes[96–98].

Studies of changes in the BM during human aging are confounded by the 'stiffening' of the vasculature that occurs with normal aging. The resulting increases in systolic blood pressure and widened pulse pressures produce pulsatile downstream effects that stress the microvasculature[99,100]. In this context, early observations noted general widening of the BM of tissue beds with normal human aging[101–103]. Studies in animal models, which do not typically have age-related increases in systolic blood pressure and widened pulse pressure, also noted overall thickening of the cerebral microvascular BM[104].

The thickening of the BM with aging reflects variations in composition that are specific to the BM molecules and the brain location in both human and animal models[103,105–108]. Moreover, the impact of these alterations on age-related changes in brain microvascular function is yet to be determined. Studies of brain regions have shown significant reduction in col IV in the cortex, and a reduction in laminin and nidogen 2 in the cortex and striatum in aged C57BL/6 mice relative to young controls[104,105]. In contrast, the brains of aged humans (mean age of 74) demonstrated increased col IV accumulation in the BM of putamen microvessels compared with those of controls (mean age of 39)[107]. Examination of related organs, such as the human retina, confirm an overall increase in BM thickness with age, which was attributed to col IV and agrin, with a concomitant decrease in laminin content[109].

Increases in perlecan in small-vessel BM have been noted in BALB/c mice aged 16 months relative to in that of 8-month-old mice[104]. Measures of fibronectin are complicated by the range of sizes found in the BM. Early reports noted a substantial decrease of fibronectin content in rat brain BM with aging[110]. Later measures of fibronectin showed no changes in the large 250-kDa monomer, but at the same time, there were increases in the 60-kDa monomer, in the brain cortex microvasculature of aged mice relative to that of young BALB/c mice[104]. Subsequent regional analyses found an increase in fibronectin content throughout the aged brain of C57BL/6 mice[104,105]. Measures in aged human occipital cortex showed an increase in nidogen 2, which could reflect a region-specific finding[106].

Detailed descriptions of alterations in brain BM composition with aging are further complicated by a recent ultrastructural study[111] that found a twofold increase in BM thickness, which was as high as fivefold in some portions of the microvasculature, due to deposition of lipid droplets and aggregates in aged C57BL/6 mice relative to young controls. Accumulation was on the glial side of the BM, suggesting that astrocytes contributed to the defect in lipid metabolism. Whether the deposition reflects age-related alterations in lipid synthesis, turnover or both is unclear. These changes are also likely to be brain-region-specific and underscore the potential for the BM of the vBBB to serve as a repository for accumulation of components that are not classically studied in the context of ECM.

In summary, alterations in the brain BM with normal aging affect all components and are specific to each brain region. These changes are likely to have effects on local microvascular function that impact the normal brain, as well as the progression of concurrent neurodegenerative and injury processes.

The aged endothelial glycocalyx

The endothelial glycocalyx layer (EGL) is considered the first line of 'defense' between the blood and the brain[112,113]. This thin gel-like layer located on the luminal side of the vBBB is comprised primarily of proteoglycans (PGs), including membrane-bound syndecans and glypicans[114–116], and glycosaminoglycans (GAGs), including heparan and chondroitin sulfates, and hyaluronan[115,117]. The abundance and composition of each EGL component can regulate BEC function[118]. In peripheral blood vessels, the EGL was first described by Luft in 1966 as an endocapillary interface that may regulate vascular function[119]. More recently it was determined that the EGL senses circulating factors and maintains vascular homeostasis. More specifically, the EGL functions to regulate blood flow[120] and immune cell interactions[121], as well as to respond to shear-stress[122].

Our current understanding of the EGL comes from the early work performed by Danielli in 1940 on peripheral blood vessels in frogs[123], and subsequent work on rodent peripheral blood vessels by Duling and colleagues[124,125]. In reference to the brain vasculature, few studies have investigated the EGL[126–128], and only recently has the neurovascular EGL been visualized with intravital imaging[112,129].

Recent advancements in our understanding of brain EGL function may enhance vBBB delivery strategies to treat CNS disease as well as highlight new avenues for developing CNS therapeutics.

The EGL is altered by age-related diseases such as stroke and diabetes[126,130,131], and should be considered a viable therapeutic target for the aging population. BBB dysfunction is associated with age-related cognitive decline in humans[37], but little is known about how the neurovascular EGL specifically contributes to this impairment. Recent work has demonstrated age-related changes to the EGL in peripheral blood vessels. Machin et al. discovered a thinner EGL in peripheral blood vessels of aged mice and humans compared with younger controls[132]. In addition, humans with age-related diseases show significant changes in their peripheral EGL structure and function[126,131]. It is unknown whether the brain vasculature also exhibits these EGL changes observed with age and age-related diseases. Recent ultrastructural evidence suggests that the brain EGL is vastly different than that observed in blood vessels of the liver and lung[113]. Age-related changes to the neurovascular EGL may also be unique, and future research should explore these effects so that novel therapeutics for age-related CNS diseases can be developed.

Genetic impact on BBB integrity and transport systems

There are genetic links to changes at the BBB with healthy aging. The majority of genes in the human vBBB are downregulated with age and are primarily related to DNA binding[92]. The impact of apolipoprotein E (ApoE) and its various isoforms (E2, E3, E4) on the vBBB has been the most extensively studied (see Box 4). Early work showed that a deficiency in apolipoprotein E (ApoE) was associated with an age-related disruption of the BBB[133–135]. People with ApoE4 make up approximately 25% of the population, and the *ApoE4* allele is linked to a shortened lifespan, especially in women[136]. ApoE4 can affect BBB integrity

during normal cognitive aging[10,137] and is known to play a role in both vBBB structure (that is, by regulating TJPs, BM production and inflammatory pathways) and function (that is, transporter expression and activity)[138] (Fig. 3).

There are multiple changes in the structure of the vascular BBB due to ApoE4. First, tight junctions are impacted with reduced phosphorylation of occludin, leading to a reduction of vBBB integrity[139]. However, total levels of the TJPs, occludin and claudin-5, were not altered in aged mice due to ApoE4 (ref. [140]). Second, ApoE4 alters proinflammatory signaling that affects not only vBBB permeability but also cerebral blood flow[141,142]. Cerebral vessel density in aged ApoE4 mice is decreased, indicative of vascular atrophy[140]. However, vascular space does not seem to be affected by ApoE genotype[140,143]. Third, even though aging results in a thickening of the BM[111], aged ApoE4 mice have a thinner BM compared with that of E2 or E3 mice[140]. The thinner BM in the ApoE4 BBB could be due to ApoE4 expression by pericytes and a decrease in col IV levels[144]. Lastly, the interaction of LRP-1 with ApoE, which has previously been shown to aid in vBBB integrity, is reduced with ApoE4 (ref. [141]).

Another protein expressed in BECs that is altered by ApoE4 is the receptor for advanced glycation end-products (RAGE). RAGE is a multiligand receptor, and interactions with its receptors often result in proinflammatory gene activation. RAGE protein levels are increased in brain capillaries from ApoE4 aged mice compared with those in ApoE2 mice[140]. While most of these changes occur in young mice, they can be accentuated with age[140]. Some of the detrimental effects of ApoE4 on the structure of the vBBB during aging are summarized in Fig. 3.

In summary, ApoE has a major role in the regulation of BBB structure and function. ApoE4 in comparison to other forms of ApoE is associated with age-related deterioration in BBB structure and functions. Further examination of the molecular mechanisms by which ApoE affects the vBBB and why ApoE4 is associated with decline in the aging BBB is warranted.

Conclusions

The BBB is a highly complex interface. As part of the NVU, it influences, communicates with and otherwise responds to other components of the CNS and to circulating cells, exosomes and hormones. It also secretes and transports a host of substances, establishing communication links with and between the periphery and CNS. The BBB is vital to CNS nutrition and homeostasis, and undergoes myriad alterations during normal aging. Few morphological changes are well documented to occur at the BBB with healthy aging. Imaging with DCE-MRI supports a small increase in leakiness at the vBBB with healthy aging that correlates with some types of age-related cognitive decline. Transport systems are clearly altered, including those for glucose and the efflux of Aβ and xenobiotics. Those systems responsible for the circulation of brain interstitial fluid and CSF, including CSF bulk flow and the glymphatics, are decreased with healthy aging and likely by age-associated conditions, such as sleep disturbances and systolic hypertension. Of all the cells with which BECs associate, the pericyte is the one that is in the most intimate contact and is arguably the most influential on vBBB function. The pericyte is very vulnerable to oxidative stress,

and pericyte levels decrease with age. Animals with decreased pericytes have altered BBB functions, including disruption. The roles of the BM and glycocalyx in vBBB protection and function are just beginning to be explored but are known to be altered with aging. The E4 isoform of ApoE is associated with age-related BBB dysfunctions, including changes in tight-junction regulation, altered transport systems, decreased insulin binding to BECs and pericyte loss. In summary, the BBB undergoes many changes with healthy aging that likely are adaptive, but may also be in response to or affect susceptibility to age-associated diseases.

Acknowledgements

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Box 1 | Glossary

Adaptive senectitude: some biological changes occurring in old age, including those that are pathological at younger ages, are adaptive for survival in the postreproductive phase of life.

Basement membrane: the extracellular matrix on the abluminal side of BECs.

BBB: the processes and structures that prevent the unregulated exchange of plasma and CNS fluids.

Convection: the movement of a solute attributed to the movement of its solvent. Notably, in most other fields, this is the definition of advection, whereas convection encompasses both diffusion and advection.

Diffusion: the net movement of a substance from a region of higher concentration to one of lower concentration. As applied here, it is driven by Brownian motion within its solute and is symmetric, and the diffusion rate is more rapid for smaller molecules.

Efflux transporters: transporters that move their ligands in the brain-to-blood direction.

Neurovascular unit: for the purposes of this Review, those cells, especially those located within the CNS, that form or influence the structure and functions of the vBBB.

Glycocalyx: glycolipid/glycoprotein matrix located on the luminal surface of BECs.

Glymphatic system: a conception of fluid movement within the brain that emphasizes the role of free water produced by astrocytic AQP4 water channels as a driving force in convection and the importance of fluid movements in the clearance of waste products from the brain.

Influx transporters: transporters that move their ligands in the blood-to-brain direction.

Metabolic (free) water: water produced by a cell resulting from glucose metabolism.

Senescence: originally defined as the loss of a cell's ability to divide, it increasingly describes cells with a specific proinflammatory secretome (senescence-associated secretory phenotype) with expression of biomarkers such as beta galactosidase and p16$^{\text{Ink4A}}$.

Transcellular diffusion: a mechanism of crossing the cell membranes that comprise the BBB that depends in large part on the physicochemical properties of the compound.

vBBB: the BBB formed by the capillary bed of the brain and its adjoining arterioles and venules.

Box 2 | Treatments for preventing pericyte loss and astrocyte reactivity

Since pericyte loss and increased astrocytic reactivity are associated with BBB disruption in healthy aging, preventing pericyte loss and astrocytic activation could have great therapeutic potential for the healthy aging BBB. In addition, administration of beneficial factors secreted by pericytes could also aid in BBB maintenance and function.

Pericyte implantation has been used to improve transport functions at the BBB[153]. An intracerebroventricular injection of pericytes derived from a mesenchymal stem cell line was given into the right hemisphere and vehicle in the contralateral side of aged mice. Two weeks later, the cells were still alive and preserved properties of pericytes by facilitating microvessel formation and increasing microcirculation.

Growth factors are broadly produced by pericytes and offer therapeutic properties. Clinical trials have shown that exogenous administration of PDGF-BB to people with Parkinson's disease was well-tolerated[154]. PDGF signaling plays a critical role in not only blood-vessel formation, but also blood-vessel maintenance. Another growth factor produced by pericytes, glial cell-line-derived neurotrophic factor (GDNF), upregulates claudin-5 expression in endothelial cells and increases BBB tightness[155]. Intracerebroventricular infusion of pericyte-derived pleiotrophin (PTN) prevented neuronal loss, independent of restoring blood flow[90]. PTN is a secreted growth factor enriched in pericytes compared with other CNS cell types, and is not present in the periphery. Therefore, CNS delivery of these critical pericyte secreted factors, PDGF-BB, GDNF or PTN, potentially through intranasal administration, might have therapeutic potential for repairing the BBB in aging.

Topiramate treatment can prevent the loss of pericytes[156], suggesting that this mitochondrial carbonic anhydrase inhibitor can protect the NVU by reducing the oxidative stress resulting from glucose metabolism, thus potentially delaying age-related CNS diseases. In vitro studies have shown the antioxidant ascorbic acid can prevent pericyte apoptosis induced by high glucose[157]. These findings suggest an important role in restoring the antioxidant capacity in pericytes to prevent loss related to aging.

Interventions that extend lifespan, such as long-term exercise, caloric restriction and rapamycin, can prevent pericyte loss and/or alter astrocyte morphology in aging mice[158–160]. Behavior and synaptic function are improved with exercise. Pericyte number and vascular coverage are also increased in the cortex and the hippocampus, similar to the levels of young mice. In addition, exercise has been shown to increase GDNF expression[161], which, as mentioned above, can help maintain BBB integrity. Exercise has been shown to decrease astrocyte reactivity in middle-aged female mice, but in young mice and other mouse models of neurodegeneration exercise it increases astrocyte reactivity, which has been shown to be protective in some disease states[159,162,163]. The role of astrocytic reactivity in aging remains to be determined in addition to whether caloric restriction and rapamycin treatment can improve pericyte number in aging. Therefore, treatments already known to extend lifespan have the potential to aid in

preventing age-related pericyte loss, improving astrocyte reactivity and ultimately restoring BBB function.

Box 3 | Key research questions for a healthy aging BBB

Cells that help support BECs at the NVU include pericytes and astrocytes. The function of these cells declines with age, which impairs their ability to maintain a healthy BBB. The presence of these cells is critical for BBB homeostasis, and loss of either cell type results in BBB disruption. Reasons for pericyte loss and changes in astrocyte reactivity as well as the direct implications on the BBB are important considerations. We have generated a list of the most prominent gaps in the literature regarding the impact of aging on cells supporting BBB function and how answering these questions will advance this field.

1. Determine why pericyte loss and astrocyte reactivity occurs with age. Pericyte loss during aging leads to many changes at the BBB and within the CNS. However, the reason for such changes still remains to be determined. Is pericyte loss and increased astrocyte reactivity part of an evolutionarily influenced program? What mechanisms underlie pericytes loss and astrocyte hyperactivity in healthy aging? To what degree are changes in pericytes and astrocytes induced by BECs? By other cells of the NVU?

2. Determine the rate of loss of pericytes and astrocyte reactivity during aging. While it is known that these events occur with aging, it is unclear when in the aging process changes begin and if these changes occur linearly or exponentially. Insults and stressors can increase the rate of aging, but at least in animal models, pericyte loss and astrocyte reactivity can occur independently of stressors. Determining what makes these cells susceptible to change could extend the health of the BBB.

3. Determine the downstream impact of pericyte loss and astrocyte reactivity on BBB transport systems. The impact of aging, pericyte loss and astrocyte activation has been studied for only a few of the BBB transport systems. The question arises as to which changes in the healthy aging BBB are inherent to the aging BEC, and which are in response to age-related changes in supporting cells.

4. Determine how aging affects the BBB regionally and zonally. It is now recognized the BBB is not uniform throughout the brain. It can vary inter- and intraregionally. Different zones of the BBB include various types of arterioles, capillaries and venules[164]. Importantly, the regulation of the BBB by supporting cells, including astrocytes, varies in each of these zones. Lastly, the development of two-photon imaging now allows for regional and zonal visualization of the BBB.

Box 4 | Potential impact of ApoE4 on BBB function in aging

While some work has been performed on the impact of ApoE isoforms on BBB transport, such as that of Aβ and glucose, the full extent of the ApoE isoform on transport at the aging BBB has not been evaluated[165]. ApoE4 can impact BBB function by altering the binding of substrates to BECs or the transport (influx or efflux) of substances across the BBB. We recently found that young mice expressing ApoE4 have greater insulin binding to BECs than do ApoE3 mice, although transport of insulin across the BBB did not differ[143]. It is known that, in neurons, ApoE4 interacts with the insulin receptor in an age-dependent manner, limiting signaling and trafficking[166]. Whether these results extend to BECs, and thus the BBB, is unknown. Lipoprotein receptors (LDLR and LRP-1) have been shown to aid in Aβ transport. Increased membrane shedding of LDLR and LRP-1 occurs with expression of ApoE4, resulting in decreased levels of these functional transporters at the cell membrane[150].

There is a genotype effect on the amount of ApoE secreted from cells, which could be a reason for a beneficial effect of ApoE2 and ApoE3, as they are secreted more abundantly than ApoE4 (ref. [167]). Investigation of the cell-specific contributions of ApoE4 on BBB functions has only recently begun. ApoE is primarily produced by astrocytes, with about 25–30% of ApoE coming from pericytes and a negligible amount from BECs[144]. ApoE4 expression in astrocytes has been linked to a disruption of BBB integrity[139,141]. Recently it has been suggested that the impact of ApoE4 on BBB integrity is mediated through pericytes[144,168,169]. ApoE4 carriers have a greater loss of pericytes than do ApoE3 carriers. This loss of pericytes correlates with BBB disruption[10]. Since it is known that pericyte loss occurs with age, it is possible that older ApoE4 carriers have an even greater amount of pericyte loss. The expression of the endothelial ApoE isoform had no effect on barrier tightness, suggesting endothelial ApoE does not affect endothelial structure. Overall, the literature suggests there is a tight link between the ApoE genotype and BBB integrity and function. How these effects on the BBB explicitly change with age remains to be determined.
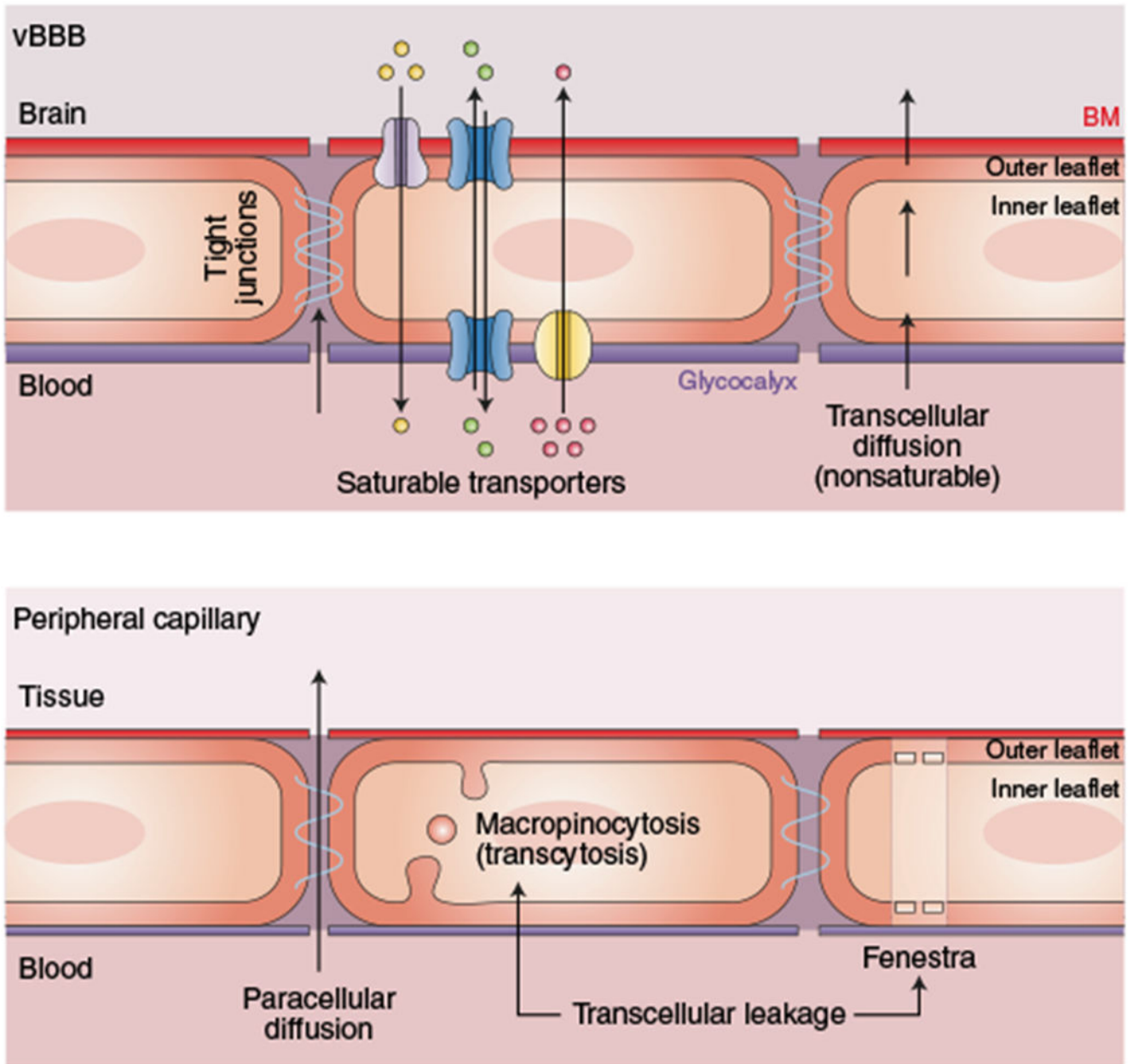
Fig. 1 |. Properties of the peripheral endothelial cell versus the vBBB or brain endothelial cell.
The upper portion of the figure shows the major characteristics of the vBBB: absence of macropinocytosis and fenestrae, tight junctions limiting paracellular diffusion, polarized cell membrane with different proteins in the luminal versus abluminal membrane leaflets, unidirectional and bidirectional saturable and nonsaturable (transcellular diffusion) mechanisms of BBB penetration, a glycocalyx and a BM. The lower portion of the figure shows the morphological basis for leakage of peripheral capillaries: paracellular diffusion, macropinocytosis and the presence of window-like fenestrae. Some peripheral capillaries possess tight junctions but are leakier than the BBB is.
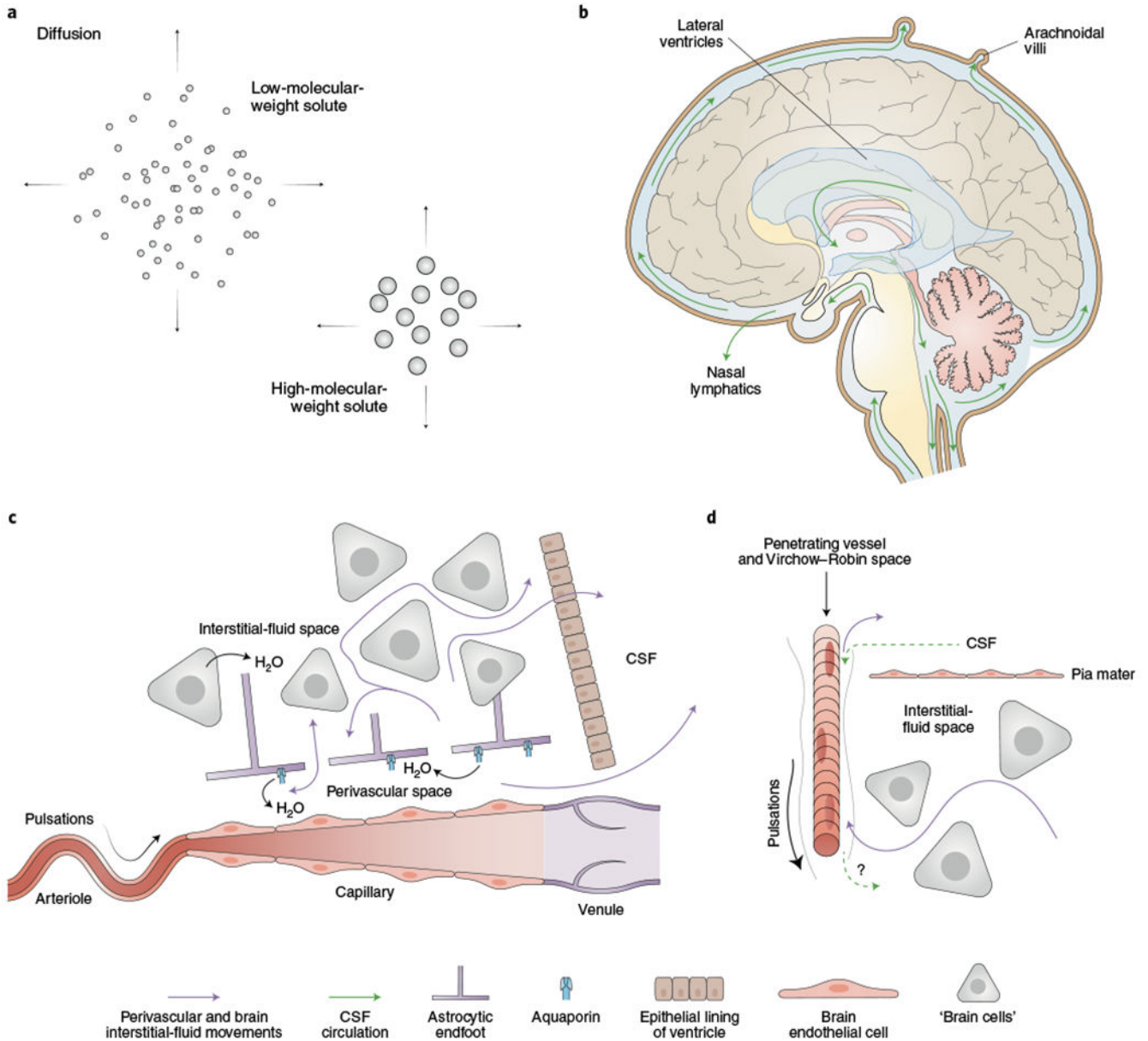
Fig. 2 |. Movement of brain fluids via diffusion and convection.
Diffusion is unaltered with normal aging, whereas many aspects of convection are. **a**, Diffusion, mainly driven by Brownian motion, is characterized by dispersion that is symmetrical and faster for substances with lower molecular weights. **b–d**, Convection is driven by several forces that differ among the regions. **b**, CSF is produced by the choroid plexus located in the ventricular system, is reabsorbed at the arachnoid villi and drains to the nasal lymphatics. **c**, In the perivascular space, fluid is mixed or driven towards the venule end of the capillary bed and into the brain interstitial-fluid space by arteriole pulsations and production of free water by astrocytic AQP4 channels; production of free water increases oncotic pressure. Interstitial fluid moves through tortuous channels between the various cells that form brain parenchyma. Metabolic free water increases oncotic pressure in the

interstitial-fluid space, driving fluid towards CSF compartments. **d**, CSF and interstitial fluid can enter spaces around blood vessels that penetrate into brain (Virchow-Robin spaces). Interstitial fluid entering a Virchow–Robin space can mix with CSF, and pulsations aid in movement and mixing. CSF entering this space may be able to penetrate deep into brain parenchyma (see question mark and dashed arrow).
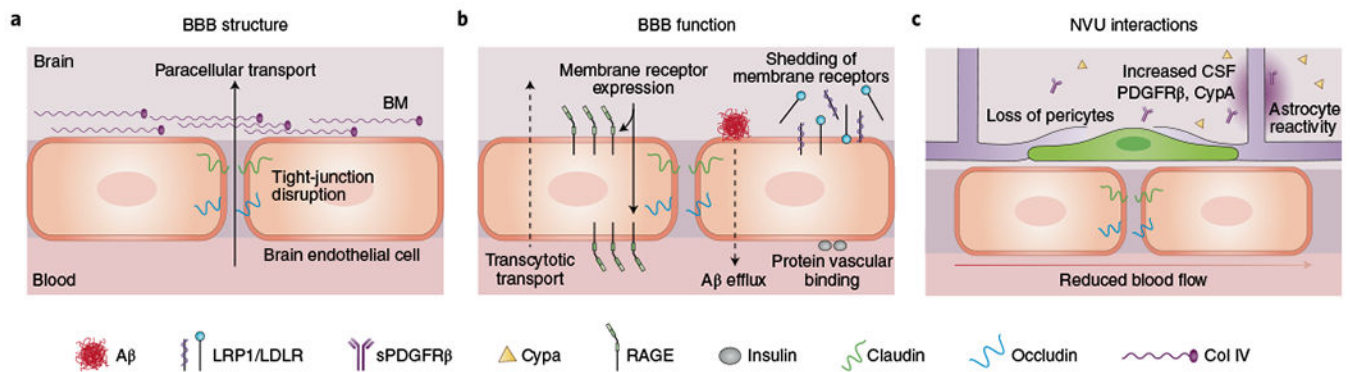
Fig. 3 |. Impact of ApoE4 on the vBBB.

ApoE4 is associated with an acceleration of many of the age-associated changes that occur at vBBB, both in humans and rodent models. **a–c**, ApoE4 can lead to structural (**a**), functional (**b**) and NVU interaction (**c**) changes. **a**, Structural changes at the vBBB include a disruption of the vBBB to various vascular substrates with an increase in paracellular transport[10,137–139,141,147,148], possibly associated with altered tight junction protein regulation (shown in **b**), such as changes in claudin and occludin[139] In addition, there are changes in BM production, with decreased levels of col IV leading to a thinner BM[140,144,149] **b**, Functional changes at the vBBB include transporter dysfunctions such as changes in LRP-1 transporter expression[150] and activity[140,141] due to shedding of this membrane receptor; increased membrane receptor expression of RAGE[140]; reduced amyloid β efflux[151]; and reduced transport of various vascular substrates, including DHA[152], glucose[140] and diazepam[140]. There are also alterations in the ability of vascular proteins to bind to brain endothelial cells due to ApoE4, such as decreased binding of insulin[143]. **c**, Changes in the NVU include pericyte loss[10] and astrocyte inflammation[141], which could also contribute to vBBB disruption and other BBB dysfunctions. The loss of pericytes is correlated with an increase in CSF levels of a pericyte receptor, PDGFRβ and cyclophilin A (CypA)[10,141]. There are also decreases in cerebral blood flow[141,142] and in vascular density[140]. However, there is no change in vascular space due to ApoE4 (refs. [140,143]). These data suggest clear evidence for the detrimental effects of ApoE4 on the vBBB with aging.

Table 1 |

Structural changes in the aging vBBB

Structural change evaluated	Species/tissue source	Age range	Method	Parameters	Change with age	Ref.
BBB leakiness/ coverage	Human surgical resections	10–79 years	TEM, regression analysis	Vesicular density	No	11
				TJP length	No	
				TJP wide gaps	No	
				TJP close gaps	No	
	C57BL6/J mouse	3–4 months versus 18 months	TEM, statistical comparison of means	TJP gaps	No	145
				vacuoles	No	
	Male Wistar rats	2–3 months versus 14–16 months	TEM, statistical comparison of means	TJP average length	No	146
				TJP number	Yes (trend), decrease	
				TJP total length per capillary	Yes (trend), decrease	
Endothelial organelles	Human surgical resections	10–79 years	TEM, regression analysis	Cytoplasmic area, gray matter	No	11
				Cytoplasmic area, white matter	Yes, increased	
				Mitochondria number	No	
				Mitochondria area	No	
				Mitochondrial density	No	
	Male Wistar rats	2–3 months versus 14–16 months	TEM, statistical comparison of means	Capillary thickness	Yes, increased	145
NVU composition	Human surgical resections	10–79 years	TEM, regression analysis	BM thickness	No	11
				Pericyte area, gray matter	No	
				Pericyte area, white matter	Yes, decreased	
	C57BL6/J mouse brains	3–4 months versus 18 months	TEM, statistical comparison of means	Pericyte coverage	No	145
				Astrocyte endfeet area	No	
				BM thickness	No	
	C57BL6/J mouse brains	6 months versus 24 months	TEM, statistical comparison of means; no statistics	BM thickness	Yes, increased	111
				Lipid-droplet accumulation between BM and astrocyte endfeet	Yes, increased	
	Male Wistar rats	2–3 months versus 14–16 months	TEM, statistical comparison of means	BM thickness	Yes, increased	146

Structural change evaluated	Species/tissue source	Age range	Method	Parameters	Change with age	Ref.
				Astrocyte endfeet coverage	Yes, increased	
				Pericyte numbers	No	