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**CRITICAL REVIEW****Neurovascular unit dysfunction as a mechanism of seizures and epilepsy during aging**Erwin A. van Vliet<sup>1,2</sup>  | Nicola Marchi<sup>3</sup> 

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**Abstract**

The term neurovascular unit (NVU) describes the structural and functional liaison between specialized brain endothelium, glial and mural cells, and neurons. Within the NVU, the blood-brain barrier (BBB) is the microvascular structure regulating neuronal physiology and immune cross-talk, and its properties adapt to brain aging. Here, we analyze a research framework where NVU dysfunction, caused by acute insults or disease progression in the aging brain, represents a converging mechanism underlying late-onset seizures or epilepsy and neurological or neurodegenerative sequelae. Furthermore, seizure activity may accelerate brain aging by sustaining regional NVU dysfunction, and a cerebrovascular pathology may link seizures to comorbidities. Next, we focus on NVU diagnostic approaches that could be tailored to seizure conditions in the elderly. We also examine the impending disease-modifying strategies based on the restoration of the NVU and, more in general, the homeostatic control of anti- and pro-inflammatory players. We conclude with an outlook on current pre-clinical knowledge gaps and clinical challenges pertinent to seizure onset and conditions in an aging population.

**KEYWORDS**

aging, Alzheimer's disease, blood-brain barrier, cognitive decline, inflammation, late-onset epilepsy, neurodegeneration, seizures, stroke, traumatic brain injury

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## 1 | INTRODUCTION

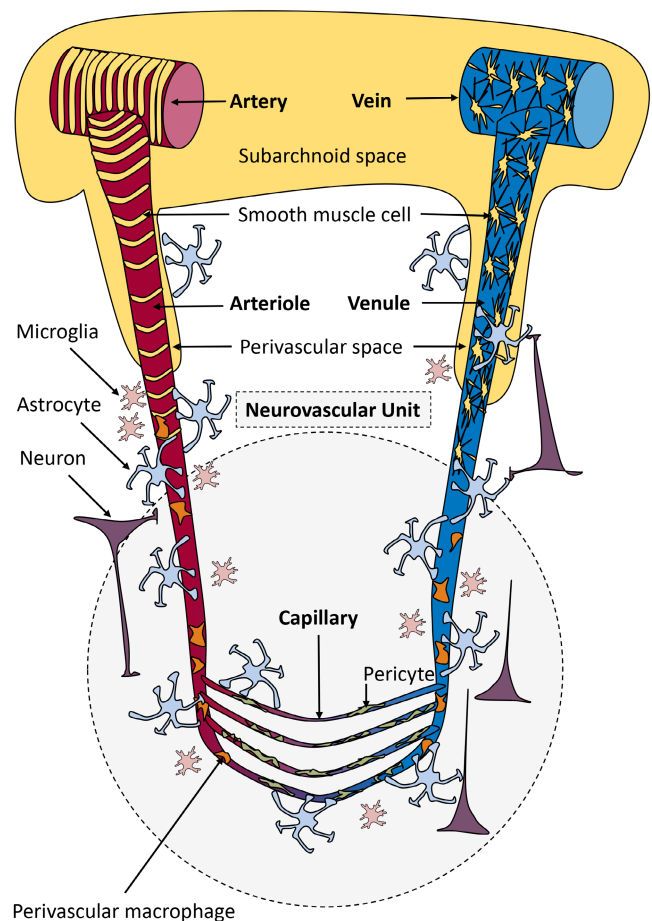
Older adults account for 12% and 20% of the United States and Europe population, respectively.<sup>1,2</sup> By 2050, one in six individuals will be 65 years of age or older.<sup>3,4</sup> These numbers are important considering that the incidence of seizures and epilepsy increases from 50 years of age, and it becomes the highest in subjects older than 65 because of epileptogenic brain insults and diseases.<sup>3</sup> This clinical fact, and its limited understanding, stirs discussion about pathological mechanisms and trajectories, risk of cognitive impairment, and the adequacy of available antiseizure medications for treating older patients.<sup>3</sup> From this context, we examined whether neurovascular unit (NVU) dysfunction during aging<sup>5-8</sup> could play a role in the onset of seizures and epilepsy. We searched for relevant literature using PubMed and Google Scholar databases. We focused on, but did not limit to, the past decade. We used combinations of two or more keywords<sup>9</sup> (eg, seizures, aging, late-onset epilepsy, neurovascular unit, blood-brain barrier (BBB), inflammation, glial cells, stroke, traumatic brain injury, neurodegeneration, tau-hyperphosphorylation, amyloid beta, Alzheimer's disease, and vascular dementia) to retrieve and select pertinent knowledge, including perspective views, hypotheses, and knowledge gaps. We first summarize the structural and cellular elements of the healthy NVU. Next, we focus on clinical and experimental examples supporting the involvement of NVU dysfunction, within a pro-inflammatory environment and not limited to BBB permeability, in the occurrence of seizures and epilepsy during aging. Finally, we discuss diagnostic and therapeutic opportunities that could be further studied and potentially applied to an aging population, proposing a contextualization to seizure conditions.

## 2 | THE NEUROVASCULAR UNIT: AN OVERVIEW OF CELLULAR COMPONENTS AND FUNCTIONS

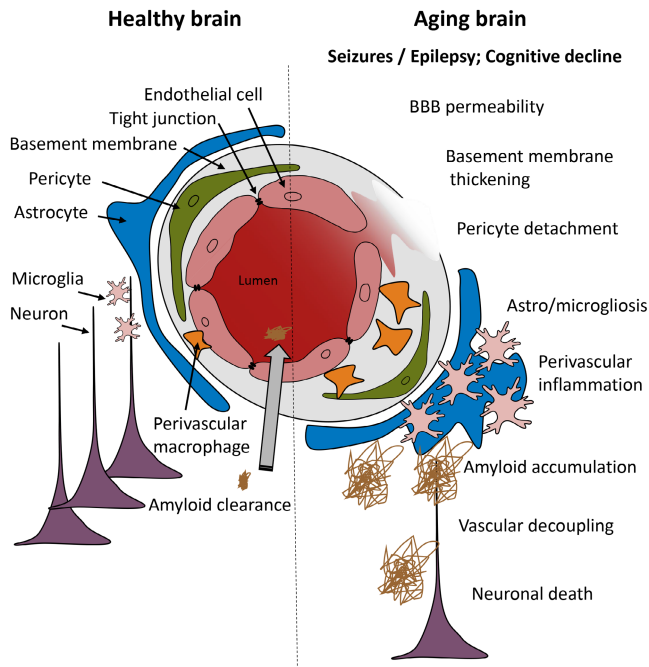
The NVU is unique to the brain and is a complex functional and multi-cellular anatomic structure. The NVU includes vascular cells (brain microvessel endothelial cells [BMECs]) with mural cells (pericytes and smooth muscle cells) that are in close contact with BMECs, glial cells (astrocytes, microglia, and oligodendrocytes), perivascular macrophages, and neurons (Figures 1 and 2). Within the NVU, the BBB includes a vast system of capillaries and postcapillary venules regulating the passage of molecules and cells in and out of the brain. The BBB is characterized by a highly selective endothelial-pericyte-astrocyte layering, separating the peripheral blood from the brain

### Key points

1. Neurovascular unit (NVU) dysfunction is a converging disease-associated element contributing to seizures during aging.
2. Acute brain insults and disease progression promote or intersect with seizures during aging.
3. Blood-brain barrier dysfunction and brain inflammation could be hallmarks of age-related seizures and neurodegenerative diseases.
4. Refining NVU diagnostics and therapies is essential to control seizures and epilepsy in the aging population.



**FIGURE 1** Schematic overview of the cerebrovasculature. The brain receives blood supply from the carotid and vertebral arteries, which regulate blood flow via the contraction of smooth muscle cells. Arteries leave the subarachnoid space to penetrate the brain parenchyma, branching into arterioles and capillaries. At this site, the endothelium is surrounded by a multi-cellular assembly (see Figure 2), forming the neurovascular unit. Blood is collected in venules and distributed via veins to exit the brain



**FIGURE 2** The neurovascular unit in the healthy and aging brain: intersections with seizure conditions. Left: in the healthy brain, endothelial cells are connected by tight junctions and form the blood-brain barrier (BBB). These endothelial cells are surrounded by pericytes (and perivascular macrophages) embedded in the basement membrane. Astrocytic endfeet cover the basement membrane and enclose the blood vessel, and neighboring microglia provide immune surveillance. Changes in local neuronal activity lead to subsequent changes in cerebral blood flow, a process that is called neurovascular coupling. Furthermore, several transporters are active, for example, to clear amyloid from the brain. Right: detrimental processes during aging can intersect with seizure pathophysiology. These changes include increased BBB permeability, basement membrane thickening, pericyte detachment, astrocytosis and microgliosis, perivascular inflammation, amyloid and tau accumulation, and neuronal death, which may converge to seizure, epilepsy and/or cognitive decline

parenchyma (Figure 2; see Ref. 10). The BBB is a gateway, and the first cell type encountered when entering the brain from the blood is the BMEC. These cells are connected via tight and adherens junctions, restricting the paracellular diffusion of ions (eg,  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$ ), macromolecules, and polar solutes, but allowing oxygen/ $\text{CO}_2$  via diffusion (see Ref. 10 and 11). Tight junction proteins have a size-selective permeability to uncharged molecules of up to 4 nm, suggesting that larger molecules can passively traverse only when the permeability of the BBB increases or via ad hoc transport or metabolic mechanisms.<sup>12–14</sup> BMECs produce a variety of molecules, including inflammatory mediators, growth factors, and extracellular matrix proteins, that modulate diverse molecular pathways and allow adaptations during physiological and pathological stimuli (see<sup>15,16</sup>). BMECS are

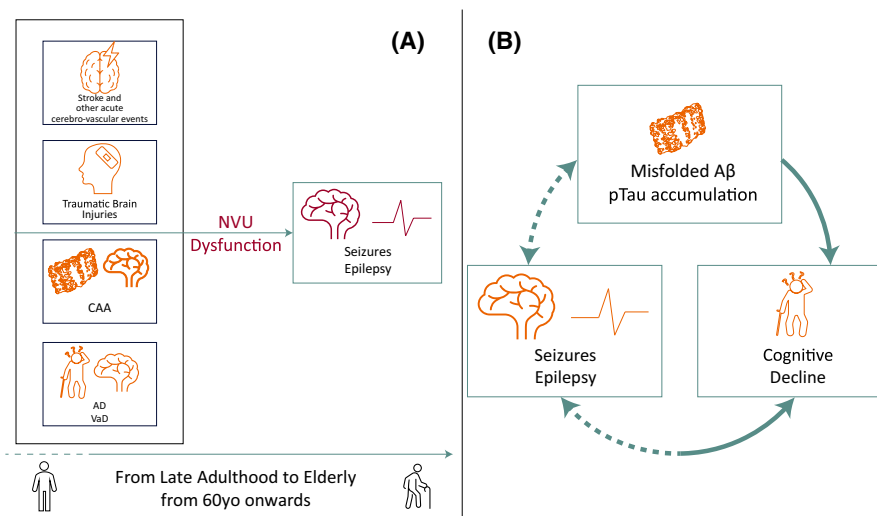
encased by a continuous basement membrane, consisting of an inner layer produced by BMECs and pericytes and a parenchymal layer shaped by astrocytes (Figure 2).<sup>17,18</sup> The basement membrane consists of laminin, collagen IV, nidogen, and heparan sulfate proteoglycans that support mutual interactions between BMECs, pericytes, and astrocytes.<sup>15,18,19</sup> The basement membrane is a physical barrier with an ideal location for signaling processes.<sup>20</sup> Pericytes are mural cells located within the basement membrane, where they interact with BMECs and astrocytic end-feet to participate in structural and homeostatic BBB functions (Figure 2; and<sup>21,22</sup>). Pericytes communicate directly with BMECs through gap junctions and adjacent pericytes via peg-and-socket contacts.<sup>22</sup> They are essential for the normal development and function of the brain arterioles and capillaries, including tightness or permeability properties and cerebral blood flow regulation.<sup>22,23</sup> Pericytes interplay with immune cells<sup>24–25</sup>; they may have macrophage-like properties<sup>26</sup> and rapidly relay inflammatory signals from the periphery.<sup>27</sup> Astrocytes ensheath with their endfeet perivascular pericytes, the basal lamina, and neuronal processes (Figures 1 and 2). They are centrally positioned between neurons and BMECs. They are essential for neurovascular coupling, the dynamic control of local blood flow in response to increased metabolic needs due to neuronal activity.<sup>28–30</sup> They express ion channels, transporters, and receptors, enabling their critical roles in modulating synaptic activity via potassium buffering, pH regulation, neurotransmitter uptake, gliotransmitter release, and maintenance of neuronal homeostasis.<sup>30</sup> Astrocytes produce inflammatory mediators and extracellular matrix proteins, with either BBB-promoting or BBB-disrupting effects, depending on the signals received from neurons, microglia, and endothelial cells (see<sup>15,16,30</sup>). In addition, they are essential for perivascular clearance via the glymphatic system.<sup>31</sup> Microglia and oligodendrocytes neighbor the BBB. Microglia are the primary brain immune effector cells that become activated and undergo a morphological and functional transformation during brain injuries and diseases (see Ref. 32). Oligodendrocytes are responsible for forming myelin sheets that provide support and insulation to axons in the central nervous system.<sup>32</sup> Finally, perivascular macrophages lie on, or close to, the outer (abluminal) surface of blood vessels.<sup>33</sup> Like pericytes, they can regulate blood vessel permeability and immune functions by producing inflammatory mediators and performing phagocytosis.<sup>32,33</sup> These fundamental descriptions (comprehensively reviewed in Ref. 20 and 34–36) illustrate the cellular complexity of the BBB interface within the NVU, underpinning direct liaisons between regional cerebrovascular functions and permeability, inflammation, and neuronal activity regulations. Failure of these precise cellular and homeostatic interactions during

pathological conditions favors abnormal neuronal firing or seizures.

### 3 | NEUROVASCULAR DYSFUNCTION IS A RISK FACTOR FOR SEIZURES AND EPILEPSY DURING AGING: CLINICAL CLUES.

Particularly in the aging population, brain insults or diseases can promote seizures and late-onset epilepsy (LOE).<sup>37</sup> A significant subset of LOE remains of uncertain origin,<sup>38</sup> and clinical evidence indicates that cerebrovascular dysfunction could represent an underlying and converging pathological substrate.<sup>6,39</sup> This is coherent with the fact that an increase in BBB permeability favors seizure activity or abnormal neuronal transmission by homeostatic and inflammatory dysregulations (see Ref. 6, 24, and 40–44). Acute (eg, stroke and traumatic brain injury) and long-term (eg, neurodegenerative disorders, chronic traumatic encephalopathy) pathologies can be epileptogenic and are associated with an increased risk for seizures and epilepsy in the elderly (Figure 3).<sup>3,45</sup> These acute and chronic conditions can present with NVU dysfunction intertwined with brain inflammation. Here, we focus on cerebrovascular diseases, traumatic brain injury, and neurodegenerative disorders.

Cerebrovascular diseases (subarachnoid hemorrhage, intracerebral hemorrhage, occlusion and stenosis of cerebral arteries, transient cerebral ischemia<sup>46</sup>) are known risk factors for LOE.<sup>47</sup> Post-stroke epilepsy (PSE) accounts for up to 50% of LOE cases.<sup>45,48</sup> The risk for seizures and PSE increases with age, estimated to be six times higher in patients >60 years than younger subjects.<sup>49</sup> Adult subjects with hypertension presented a 2-fold risk of developing epilepsy. Hypertension was proposed as a potentially modifiable vascular risk factor.<sup>45</sup> Patients with hemorrhagic stroke or presenting hemorrhagic transformation are at higher risk of seizures<sup>50</sup> (eg, within 7 days from the stroke episode<sup>51</sup>). The presentation of early seizures post-stroke significantly increases the incidence of delayed PSE. Seizures are most common when the stroke occurs at the middle cerebral artery branch, associated with disturbances in the microcirculation.<sup>50–52</sup> At the cellular level, an early post-stroke seizure may result from the sudden increase of BBB permeability, leading to a loss of parenchymal ionic and neurotransmitter homeostasis. It can be associated with local pro-inflammatory elements.<sup>52</sup> Proteomic studies performed on mechanically retrieved thrombi unveiled the presence of pro-inflammatory cytokines, adhesion molecules, and T cells, supporting the existence of a local inflammation that can negatively impact the brain endothelium.<sup>53–55</sup> Cerebrovascular permeability and inflammation are proposed mechanisms for PSE and seizures.<sup>34,53</sup>



**FIGURE 3** Neurovascular dysfunction as a converging risk factor for late-onset seizures or epilepsy and cognitive decline. (A) An acute insult (e.g., stroke, traumatic brain injury, generalized status epilepticus) or a progressive disease (e.g., cerebral amyloid angiopathy, CAA; vascular dementia, VaD; Alzheimer's disease, AD) to the aging brain can precipitate seizures by a mechanism encompassing neurovascular unit (NVU) dysfunction, including increased blood-brain barrier (BBB) permeability, within an inflammatory environment. (B) Summary of bidirectional associations (to be demonstrated: dotted lines; likely or demonstrated: solid lines) and the vicious cycle between seizures, neurodegenerative pathways, and cognitive decline over time. Seizure activity may accelerate brain aging or promote markers of neurodegeneration, and neurodegenerative pathologies present with abnormal neuronal discharges or seizures

### 3.1 | Traumatic brain injury

Due to reduced autonomy and increased motor disabilities, older individuals are at high risk of falls<sup>56</sup> and traumatic brain injury (TBI). The latter is a leading cause of subdural and intracranial hemorrhage, diffuse cerebral edema, and skull fracture, all risk factors of post-traumatic epilepsy (PTE) in the aging population.<sup>47,57</sup> Although the risk for a single early seizure following TBI is higher at a young age (<5 years), PTE can develop in older individuals.<sup>57</sup> A computerized tomography (CT)-scan visible cortical insult is associated with a greater risk of developing PTE.<sup>58</sup> Experimentally and clinically, BBB dysfunction was indicated as one early event post-TBI, potentially underpinning the development of neurological defects or seizures over time.<sup>59,60</sup> Evidence shows a topographic association between cerebrovascular hypoxia, rapidly occurring post-TBI, and long-term changes of gamma waves.<sup>59</sup> Repeated TBI during early ages (eg, contact sports) or lifespan (eg, military, repeated accidents) can lead to chronic traumatic encephalopathy (CTE), a condition characterized by the accumulation of hyperphosphorylated tau (p-tau),<sup>61</sup> with extensions to Alzheimer's disease (AD). Clinically, CTE presents with impairments in cognition, behavior, mood, and in some cases, chronic headache and motor and cerebellar dysfunction.<sup>61</sup> Patients with CTE are at higher risk of developing epilepsy, with a suspected pathophysiological implication of repeated BBB damage consequent to TBI.<sup>62,63</sup>

### 3.2 | Neurodegenerative disorders

AD and vascular dementia (VaD) present with cerebrovascular alterations.<sup>64–67</sup> AD displays structural or permeability modifications of the BBB,<sup>68</sup> associated with accumulation or inadequate clearance of misfolded A $\beta$ .<sup>64</sup> Neurovascular dysfunction can precede the clinical diagnosis of AD.<sup>64,66,69</sup> VaD is the result of widespread microangiopathy-related cerebral dysfunction (eg, vascular leukopathy), and it is associated with cardiovascular risk factors (hypertension, smoking, dyslipidemia, overweight).<sup>70</sup> Cerebral amyloid angiopathy (CAA), with misfolded amyloid beta at the pial and cortical vessels,<sup>68</sup> represents a risk factor for AD and hemorrhagic stroke, with an extension to seizure onset.<sup>71</sup>

Bidirectional associations between seizures, AD, and VaD are suspected.<sup>72</sup> Poorly controlled seizures are a risk factor for neurodegenerative diseases. In AD, seizures can occur during early, or pre-symptomatic disease stages.<sup>73</sup> The relative risk of seizures for patients with AD, VaD, or no dementia is 5.6/1000, 7.5/1000, and 0.8/1000

individuals each year, respectively.<sup>74–77</sup> Furthermore, abnormal tau phosphorylation exists in temporal lobe epilepsy (TLE) brain specimens, with subjects presenting comorbidities evocative of neurodegenerative conditions.<sup>75, 76</sup> Nevertheless, the prevalence of p-tau and amyloid plaques in human TLE remains debated.<sup>78</sup> These studies provide pathological elements of comparison and overlap between clinical TLE, AD-like patterns, and chronic encephalopathy.<sup>73,79,80</sup>

BBB dysfunction, specifically in the hippocampus, is associated with mild cognitive impairment.<sup>81</sup> A recent 2-year follow-up study performed in patients with cerebral small vessel disease, showed white and cortical gray matter BBB permeability associated with cognitive and executive function decline.<sup>82</sup> Hippocampal capillary dysfunction exists in individuals with early cognitive defects, irrespective of A $\beta$  and tau-related biomarkers, suggesting BBB dysfunction as an initial biomarker of disease.<sup>83</sup> In addition, a transient EEG slowing of the cortical network occurs in AD patients, correlated with cognitive impairment.<sup>84</sup> Collectively, this clinical evidence delineates a context where acute insults and disease progression converge into NVU dysfunction as a possible unifying mechanism contributing to abnormal neuronal activities (Figure 3).

## 4 | NVU DYSFUNCTION IN THE AGING AND EPILEPTOGENIC BRAIN: EXPERIMENTAL EVIDENCE.

Experimentally, aging and seizures present overlapping NVU cellular changes or adaptations (Figure 2). With age, density and coverage of BMECs and pericytes decrease,<sup>85</sup> resulting in BBB dysfunction, particularly in brain regions vulnerable to age-related deteriorations, such as the hippocampus.<sup>81,86</sup> Furthermore, the expression of transporters located at the luminal and abluminal endothelial sides decreases,<sup>7</sup> leading to reduced uptake of nutrients into the brain and accumulation of waste products. In addition, thickening of the basement membrane ensues with aging, resulting in stiffening of the vasculature, underlined by increasing systolic blood pressure.<sup>7</sup> Although the number and size of astrocytes increase during aging,<sup>87</sup> a decline in the number of oligodendrocytes and heterogeneity of microglia can occur,<sup>88,89</sup> altogether leading to changes in brain clearance via the glymphatic system, reduced myelination, and altered immune surveillance. An inadequate response of the aging cerebrovasculature to specific vasodilators has been shown, such as reduced NO-mediated dilatation, oxidative stress, and vascular inflammation.<sup>8</sup> These events will decrease neurovascular coupling efficiency, further aggravated by myelin loss.<sup>90</sup>

BBB dysfunction during aging triggers the hyperactivation of transforming growth factor  $\beta$  (TGF $\beta$ ) signaling in astrocytes and causes neural dysfunction and age-related pathology in rodents.<sup>6</sup> Infusion of serum albumin into the young rodent brain (mimicking BBB leakiness) induced astrocytic TGF $\beta$  signaling and an aged brain phenotype including aberrant electrocorticographic activity, vulnerability to seizures, and cognitive impairment.<sup>6</sup> Infusion of albumin into the cerebral ventricles of young rats led to a transient slowing of the cortical network. This event was also observed in cortical brain regions with BBB dysfunction in patients with AD and epilepsy and in three rodent models.<sup>84</sup>

Alterations in the composition of the extracellular matrix (ECM) are evident during aging and epileptogenesis, triggered by matrix metalloproteinases, endopeptidases that are critical for tissue formation, neuronal network remodeling, and BBB permeability.<sup>91</sup> Higher matrix metalloproteinase (MMP) expression and activity are associated with BBB permeability<sup>92</sup> via degradation of the basal lamina and tight junction proteins and promote the infiltration of neutrophils into the brain parenchyma,<sup>93</sup> which can contribute to epileptogenesis. This is corroborated by studies performed in MMP knockout mice in which BBB dysfunction, brain inflammation, and epileptogenesis could be attenuated or decreased after an initial insult.<sup>94,95</sup>

Pericytes are a cellular entry point and offer an opportunity to study new BBB restorative pharmacological strategies in central nervous system (CNS) diseases.<sup>34,96,97</sup> In general, the perivascular deconstruction, redistribution, and reactivity of pericytes, in coordination with glial cells, negatively impacts BBB permeability and contributes to brain inflammation.<sup>97,98</sup> Deficiency of pericytes during aging leads to brain hypoperfusion, resulting in secondary neurodegenerative changes.<sup>99</sup> Pericyte (NG2DsRed) detachment from the capillary occurs after experimental status epilepticus in the hippocampus and cortices.<sup>24,34,44,100,101</sup> In this condition, pericytes lose their bump-on-a-long appearance and perivascular processes, becoming hypertrophic over time.<sup>40,44,102</sup> The latter is qualitatively similar to the pericyte pathology reported after experimental traumatic brain injury or neurodegenerative disease during aging.<sup>97,103</sup> In a model of TLE, pericytes detach from the capillary during seizure progression.<sup>24</sup> Loss of pericyte coverage is followed by the formation of new cells at the capillary level after status epilepticus, disclosing complex dynamics and equilibriums of damage and repair processes.<sup>100</sup> At the functional level, a redistribution of mural cells modifies vasoreactivity and blood flow when endothelin-1 or glutamate was applied in vivo after status epilepticus.<sup>100</sup> Increased BBB permeability is preceded by capillary constriction with indications of pericyte damage mediating loss of capillary integrity,

with involvement of pericyte mitochondrial depolarization.<sup>104</sup> In addition to their critical structural functions, pericytes have immunological features, and they can participate in peripheral or brain inflammation (see<sup>98, 105</sup>). Pericytes can express inflammatory mediators and present antigens.<sup>98</sup> For example, during experimental TLE progression, pericytes, microglia, and astrocytes become reactive and form a perivascular multi-cellular assembly.<sup>24,34,106</sup> The activated microglial cells accumulate at the outer BBB wall, potentially eliminating the damaged pericytes.<sup>24</sup> This regional multi-cellular cluster corresponds to areas with increased BBB permeability. Similar events occur in experimental autoimmune encephalopathy<sup>107,108</sup> and AD,<sup>109,110</sup> where the perivascular accumulation of microglial cells topographically overlays with the entry of blood fibrinogen or albumin into the brain parenchyma.

Along with the perivascular multi-cellular assembly developing during seizure conditions, increased platelet-derived growth factor receptor beta (PDGFR $\beta$ ) expression and ectopic collagen III and IV perivascular deposits suggest the existence of pro-fibrotic mechanisms.<sup>44,111</sup> A perivascular multi-cellular activation in response to seizures could promote a localized scarring process, negatively affecting vascular tone<sup>100</sup> and neurovascular coupling.<sup>104</sup> Pro-inflammatory cytokines, such as IL-1 $\beta$ , can promote pericyte damage and microglia-pericyte assemblies,<sup>24</sup> along with BBB permeability changes.<sup>112-118</sup>

A pericyte-endothelial pathology also occurs in experimental and human neurodegenerative disorders, including AD.<sup>83,97</sup> Elegant studies revealed how the BBB directly contributes to, or even precedes, the onset or the progression of cognitive decline in AD patients with the apolipoprotein E (*APOE*)  $\epsilon$ 4 variant.<sup>69,119</sup> Furthermore, soluble PDGFR $\beta$ , a pericyte damage biomarker, was elevated in the cerebrospinal fluid of AD *APOE*  $\epsilon$ 4 carriers, predictive of subsequent cognitive decline. Experimentally, *APOE*  $\epsilon$ 4 accelerates BBB dysfunction, reduces cerebral blood flow over time, and promotes behavioral defects. This evidence illustrates a pathological neurovascular intersection between aging, AD, and epilepsy, supporting the hypothesis that regional BBB breakdown represents a mechanism, or a risk factor, for developing or accelerating neurodegenerative pathways.<sup>72,75,76</sup>

Aging is associated with gliosis, with complex transcriptome adaptations over time (see Ref. 120 and 121). A higher number of GFAP<sup>+</sup> astrocytes and Iba-1<sup>+</sup> microglia in the cortex of aging mice and increased receptor for advanced glycation end products were also reported.<sup>122</sup> These events are involved in the inflammatory response, brain entry of thrombin and albumin in hippocampal parenchyma, and indicate BBB permeability.<sup>123</sup> These changes were also observed in the cortex of APP/PS1 mice (an AD model), followed by the formation of A $\beta$  plaques.

In the hippocampus, the appearance of A $\beta$  plaques preceded these alterations.<sup>123</sup> Furthermore, at the peak of inflammation in APP/PS1 mice, microglial cells are activated, increasing BBB permeability.<sup>123</sup> Similarly, astrogliosis and microgliosis occur in models of epilepsy,<sup>30</sup> promoting epileptogenesis via disturbance of energy metabolism and gliotransmission, BBB permeability, dysregulation of blood flow, and the release of pro-inflammatory molecules.<sup>30</sup> Glial cells play an essential role in the elimination of A $\beta$ .<sup>124</sup> Impaired amyloid-beta efflux and clearance, diminished cerebrospinal fluid (CSF) production, decreased enzymatic and metabolic activities, augmented influx of peripheral A $\beta$  through the BBB, and overexpression of amyloid precursor protein contribute to cognitive decline.<sup>99,123</sup>

The role of perivascular macrophages during aging and epileptogenesis is less studied. Of interest, the number of hippocampal CD163-positive perivascular macrophages positively correlates with BBB permeability in rats with recurrent spontaneous seizures.<sup>125</sup> In addition, the expression of CD68-positive monocytes/macrophages, chemokine (C-C motif) ligand 2, and CD163-positive perivascular macrophages correlated with the number of spontaneous seizures,<sup>125</sup> suggesting that these factors may contribute to epileptogenesis. Collectively, these data illustrate the multi-facet involvement of NVU cells during the aging processes, with specific overlaps and contextualization to epilepsies. This emerging knowledge requires systematic studies to define the cellular mechanisms and pharmacological targets tailored to seizure conditions during aging.

## 5 | OVERVIEW OF MODALITIES AND BIOMARKERS TO ASSESS NVU DYSFUNCTION: RELEVANCE TO SEIZURES AND AGING.

NVU dysfunction is etiological to brain diseases, and it enables the diagnosis of neurological disorders.<sup>126</sup> Over the last decades, new imaging and molecular biomarkers have emerged to track NVU dysfunction during age-related pathological conditions; now there is an opportunity to clinically and experimentally apply imaging and blood biomarkers to late-onset seizures and epilepsies. Depending on the required spatial and temporal resolution, experimental imaging tools vary from two-photon microscopy, laser speckle contrast imaging, to intrinsic optical imaging; translational and clinical modalities include positron emission tomographic (PET), single-photon emission computed tomography (SPECT), and magnetic resonance imaging (MRI).<sup>127-130</sup> These techniques can be applied to study structural and functional modifications of the NVU during aging and epileptogenesis, such as BBB

permeability, cerebral blood flow (CBF) alterations, inflammation, neuronal and axonal changes, as well as gradual, pathological processes such as the presence of amyloid and tau-related markers.<sup>6,99,130-135</sup> Recent developments include multi-photon laser scanning microscopy (MPLSM) and functional ultrasound imaging (fUSi).<sup>128,136</sup> MPLSM can be used for *in vivo* imaging of cellular dynamics and morphology, together with CBF.<sup>128</sup> fUSi is an innovative imaging modality based on Doppler ultrasound, capable of recording vascular brain activity over large scales (eg, tens of cubic millimeters) at unprecedented spatial and temporal resolution for such volumes (up to 10  $\mu$ m pixel size at 10 kHz).<sup>128</sup> By merging these two technologies, researchers may have access to a more detailed view of the various processes at the neurovascular level.<sup>128</sup> Although these techniques were developed and tested in head-fixed animals, fUSi is also feasible in freely moving animals.<sup>128</sup>

Longitudinal MRI studies in rats after status epilepticus show that BBB dysfunction in the piriform network is a sensitive and specific predictor for epilepsy,<sup>112,137</sup> whereas diffused pathology is associated with a lower risk.<sup>112</sup> A multi-parametric MRI combined with machine-learning analysis revealed distinct pathological modifications of BBB permeability, blood volume fractions, and apparent diffusion coefficient in the hippocampal epileptogenic zone and seizure-propagating brain regions devoid of lesion.<sup>40</sup> In a TLE model, these imaging identifiers spatiotemporally overlapped with trajectories of brain inflammation, amyloidogenic, and tau-hyperphosphorylation changes unfolding over time.<sup>138</sup> These results are coherent with the evidence showing that vascular dysfunction is an element favoring neurodegenerative modifications over time, advocating for a regional cerebrovascular link between seizures and associated comorbidities.<sup>97</sup> Significant effort is currently devoted toward the prediction and risk assessment of AD and the transition from mild cognitive impairment to AD,<sup>139</sup> as well as in epilepsy, to define epileptogenic and seizure propagation regions based on BBB measures in experimental TLE.<sup>40</sup>

Considering PET, there are multiple imaging agents, including small molecules, peptides, affibodies, aptamers, antibodies, and nanoparticles.<sup>130</sup> Novel agents are being developed or under investigation, which may help identify NVU alterations at the molecular level. The gap between experimental models and patients for these novel imaging modalities is significant; the field of brain imaging is advancing swiftly, with the explicit goal of targeting increasingly larger volumes of tissue with high spatial and temporal resolution.<sup>128</sup>

Blood or peripheral fluids biomarkers of NVU dysfunction represent another emerging tool with diagnostic and prognostic values. In conditions of increased



BBB permeability, a panel of astrocyte, neuronal, and pericyte proteins can exit the brain into the peripheral blood, where they can be detected (see Ref. 126 for review). Although blood biomarkers of BBB permeability have not been studied in the specific context of LOE, significant indications exist for neurodegenerative and aging conditions.<sup>72</sup> In blood, phosphorylated tau (p-tau181, p-tau217) represents a biomarker for diagnosing or staging AD patients.<sup>126</sup> CSF A $\beta$ 42:A $\beta$ 40 ratio can discriminate AD from other neurological disorders. Furthermore, blood and CSF neurofilament light chains and GFAP correlate with AD staging.<sup>126</sup> A few studies focused on pediatric or young adults with epilepsies.<sup>41,42,44</sup> A systematic review indicated an increase of blood S100B, a BBB permeability biomarker.<sup>140</sup> MRI T1 peri-ictal imaging in drug-resistant epileptic patients correlates with blood S100B, indicating BBB permeability during a seizure.<sup>141</sup> Increased S100B blood levels in pediatric TLE were reported minutes after a complex partial seizure.<sup>142</sup> Blood S100B levels were increased in a pediatric population of intractable focal epilepsy.<sup>143</sup> Furthermore, accumulating evidence outlined specific blood microRNAs (miRNA) as biomarkers of neurovascular and neuroinflammatory modifications across epilepsies (see Ref. 144–146 for a comprehensive review). These emerging approaches are essential for identifying biomarkers of NVU dysfunction, with a prospect application to epilepsies in the aging population.

## 6 | NVU PHARMACOLOGY IN EPILEPSY: AVAILABLE MOLECULES FOR THE HOMEOSTATIC CONTROL OF BRAIN INFLAMMATION

Considering the ramifications of NVU dysfunction in seizure and neurodegenerative disorders, restoring cerebrovascular integrity within a broader control of brain inflammation could represent a strategy to prevent, curb, or halt disease progression. The pharmacological management of epilepsy can be age-dependent. Here, we hypothesize that treatments aimed at restoring NVU functions might reduce or prevent seizures during aging. Past studies point to a plethora of candidate molecules, novel or repurposed, exerting BBB protective and general anti-inflammatory effects. Here, we examine this existing evidence and the possible benefits of contemporary molecules in seizure disorders, although recognizing that pre-clinical studies performed during aging are unavailable. [Table 1](#) identifies four categories: pro- and anti-inflammatory equilibrium pathways, PDGF/TGF pathways, oxidative stress pathways, and matrix metalloproteinases. BBB

permeability and neuroinflammation intersect during seizures and broad-spectrum (e.g., corticosteroids<sup>147,148</sup>), or specific anti-inflammatory molecules (e.g., anakinra/VX-765<sup>149–152</sup> or antibodies against integrins,  $\alpha$ 4 or VCAM-1<sup>153</sup>), as well as the immunosuppressant and mTOR inhibitor rapamycin<sup>137,154,155</sup> can ameliorate BBB integrity, and reduce ictogenesis or epileptogenesis in experimental models. Some promising results exist for specific compounds in clinical studies with drug-resistant epilepsies ([Table 1](#)). Boosting the endogenous anti-inflammatory mechanisms represents an emerging approach, using peripheral T-regulatory cells<sup>156</sup> or Annexin-A1.<sup>157–159</sup> Other therapeutic approaches are PDGF/TGF targeting (using PDGF-BB,<sup>100</sup> Imatinib,<sup>44</sup> IPW,<sup>6</sup> or losartan,<sup>112,160–165</sup> antioxidant treatment vitexin<sup>166</sup> or carveol<sup>167</sup>), or matrix metalloproteinase inhibition<sup>95,168</sup> that were shown to reduce BBB permeability and brain inflammation, ictogenesis, or epileptogenesis.<sup>169</sup> However, the efficacy of these molecules in curbing age-related pathological changes or aggravations remains to be investigated.

## 7 | FUTURE DIRECTIONS AND CONCLUSIONS

Although the incidence of seizures in the aging population increases, pre-clinical studies on this phenomenon lag behind. A caveat of the proposed framework is that NVU dysfunctions occur in various pathologies, not necessarily leading to epilepsy, and substantial research is needed to fully understand how the NVU modifies during aging and how this can contribute, or not, to seizures or epileptogenesis. It is also essential to develop minimally invasive, clinically applicable methods for diagnosing the extent and localization of NVU dysfunction. Pre-clinical studies have shown that detecting and quantifying NVU alterations is feasible. Novel developments are being implemented in the clinic (e.g., quantitative contrast-enhanced MRI), which may help identify novel biomarkers for epileptogenesis or treatment response in the aging population. Existing and urgent questions are: whom should we treat? When should we start treatment, at which dose, and for how long? Coupling accurate diagnosis to specific therapy will allow tailoring the type, dose, and duration of treatment to each patient, increasing the likelihood that personalized medicine could become a new approach for the prevention and treatment of epilepsy in the elderly. Essential factors to be considered are lifestyle, diet, exercise, and being cognitively active. The latter elements and their impact on the NVU functions and age-related pathologies remain to be fully studied.

In conclusion, we have presented an overview of the pathophysiological intersections between the NVU, brain

**TABLE 1** NVU and inflammation-homeostatic targets applicable to epilepsies

Drug	Group	Specific mechanism	Reported effects in experimental studies	Experimental model	Epilepsy-related clinical trials or usage
Rapamycin	Target Inflammatory Mediators or Boosting	Immunosuppressant	Reduces BBB permeability and epileptogenesis	Rat electrical <sup>154</sup> and kainic acid SE model <sup>137,155</sup>	Tuberous sclerosis complex <sup>170-173</sup>
Anakinra/VX-765	Endogenous Anti-inflammatory Mechanisms	Human interleukin-1 receptor antagonist (IL-1Ra)	Reduces BBB permeability, inflammation, ictogenesis, epileptogenesis	Rat pilocarpine SE model, <sup>149,150</sup> mouse kainic acid SE model, <sup>151</sup> guinea pig ex-vivo brain model <sup>149,152</sup>	Febrile infection-related epilepsy Syndrome (FIRES), <sup>174-176</sup> Temporal Lobe Epilepsy <sup>177</sup>
Dexamethasone	Glucocorticoid	Decreased <i>JMJD3</i> gene expression. Suppression of MMP-2, MMP-3, and MMP-9 gene activation	Reduces BBB permeability, ictogenesis, and epileptogenesis Preserves tight junctions' integrity	Rat pilocarpine SE model, <sup>148</sup> rat febrile seizure model <sup>147</sup> In vitro BBB model <sup>178-180</sup>	Epileptic encephalopathies, <sup>181,182</sup> FIRES <sup>183</sup>
Annexin-A1 (ANXA1)	Endogenous glucocorticoid anti-inflammatory player	Inhibition of the small G protein RhoA	Restores cell polarity, cytoskeleton integrity, and paracellular permeability	Mouse kainic acid SE model, <sup>157</sup> rat pilocarpine SE model, <sup>158</sup> ANXA-/- mice <sup>159</sup> In vitro BBB model <sup>159</sup>	None
Integrins, $\alpha_4$ or VCAM-1 antibody	Modulation of vascular adhesion molecules	Blockade of T-cells vascular interplay	Reduces BBB permeability, ictogenesis, and epileptogenesis	Mouse pilocarpine SE model <sup>153</sup>	None
T-regulatory cells	Endogenous anti-inflammatory CD4 <sup>+</sup> , CD25 <sup>+</sup> FoxP3 <sup>+</sup> leukocytes	Suppress activation, proliferation and cytokine production from T cells	T-cell depletions increase seizure activity and inflammation	Mouse kainic acid SE model <sup>156</sup>	None

(Continues)

TABLE 1 (Continued)

Drug	PDGF/TGF Targeting	Group	Specific mechanism	Reported effects in experimental studies	Experimental model	Epilepsy-related clinical trials or usage
PDGF-BB	PDGF/TGF Targeting	PDGFR agonist	Pericyte trophism during acute damage	Reduces pericyte loss and vascular pathology after SE Restores BBB function in an endothelial monolayer	Mouse kainic acid SE model <sup>100</sup> In vitro BBB model <sup>184</sup>	None
Imatinib		Kinase inhibitor	Inhibition of PDGF signaling. Anti-inflammatory/pericyte	Reduces vascular fibrosis	Organotypic hippocampal culture with kainic acid <sup>44</sup>	Epilepsy with chronic myeloid leukemia, <sup>185</sup> glioblastoma <sup>186,187</sup>
IPW		TGF- $\beta$ receptor inhibitor	Inhibition of TGF $\beta$ R signaling	Reduces transient paroxysmal slow wave events, ictogenesis, and TGF $\beta$ signaling improve cognition	Serum albumin brain infusion in mice <sup>6</sup>	None
Losartan		Antihypertensive	Angiotensin II antagonist	Reduces BBB permeability, inflammation, oxidative stress, neuronal loss, ictogenesis, and epileptogenesis	Albumin or bile salt application on the cortex of rats <sup>160</sup> rat pilocarpine SE model, <sup>161,162</sup> rat amygdala kindling model, <sup>163</sup> rat pentylenetetrazol kindling model, <sup>164</sup> rat kainic acid SE model, <sup>165</sup> rat paraoxon SE model <sup>112</sup>	None
Vitexin	Oxidative Stress Targeting	Flavonoid	Antioxidant	Reduces BBB permeability, inflammation, ictogenesis	Rat hypoxia-ischemia model <sup>166</sup>	None
Carveol		Terpenoid	Antioxidant	Reduces BBB permeability, inflammation and ictogenesis	Rat pentylenetetrazol kindling model <sup>167</sup>	None

TABLE 1 (Continued)

Drug	Group	Specific mechanism	Reported effects in experimental studies	Experimental model	Epilepsy-related clinical trials or usage
IPR-179 (or ACT-03)	Extracellular matrix Targeting	Matrix metalloproteinase inhibitor	Reduces MMP9 expression, and ictogenesis, and epileptogenesis	Rat hippocampal kindling model and mouse kainic acid SE model <sup>95</sup>	None
Doxycycline		Matrix metalloproteinase inhibitor	Reduces perineuronal net loss, synaptic reorganization, neuronal loss, ictogenesis	Rat amygdala kindling model <sup>168</sup>	None

Abbreviations: BBB, blood-brain barrier; MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; PDGF-BB, platelet-derived growth factor-BB; PDGFR, platelet-derived growth factor receptor; SE, status epilepticus; TGF, transforming growth factor; VCAM, vascular cell adhesion molecule.

inflammation, epilepsy, and seizures during aging. The discovery of new disease mechanisms will pave the way to original opportunities for biomarkers predicting epilepsy onset and related cognitive dysfunction. It is essential to develop novel therapeutic strategies to curb or prevent seizure onset and epilepsy in the aging population.

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. Neither of the authors has any conflict of interest to disclose.

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