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Brain endothelial cell activation and dysfunction associate with and contribute to the development of enlarged perivascular spaces and cerebral small vessel disease

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Summary. Multiple injurious stimuli to the brain's endothelium results in brain endothelial cell activation and dysfunction (BECact/dys) with upregulation of inflammatory signaling cascades and a decrease in bioavailable nitric oxide respectively. These injurious stimuli initiate a brain injury and a response to injury wound healing genetically programed cascade of events, which result in cellular remodeling of the neurovascular unit and blood-brain barrier with increased inflammation and permeability. These remodeling changes also include the perivascular spaces that become dilated to form enlarged perivascular spaces (EPVS) that may be identified noninvasively by magnetic resonance imaging. These EPVS are associated with and considered to be a biomarker for cerebral small vessel disease (SVD) and a dysfunctional glymphatic system with impaired removal of neurotoxic waste, which ultimately results in neurodegeneration with impaired cognition and dementia. The penultimate section discusses the understudied role of venous cerebral circulation in relation to EPVS, SVD, and the vascular contribution to cognitive impairment (VCID). The focus of this review will be primarily on BEC*act/dys* that associates with and contributes to the development of EPVS, SVD, and impaired glymphatic system efflux. Importantly, BEC*act/dys* may be a key piece of the puzzle to unlock this complicated story of EPVS and SVD. Multiple transmission electron micrographs and illustrations will be utilized to depict anatomical ultrastructure and allow for the discussion of multiple functional molecular cascades.

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Abbreviations. AC, astrocyte; ACef, astrocyte endfeet; AQP4, aquaporin-4; BBB, blood–brain barrier; BBB***dd***, BBB dysfunction and/or disruption; BEC(s), brain endothelial cell(s); BEC*act*, BEC activation; BEC*act/dys*, brain endothelial cell activation/dysfunction; BEC*dys*, BEC dysfunction; ***bec***CC, brain endothelial cell cytokines chemokines; BECecGCx, brain endothelial cell endothelial cell glycocalyx; BG, basal ganglia; BM(s), basement membranes; CAA, cerebral amyloid angiopathy; CBF, cerebral blood flow; CCL2, Chemokine (C-C motif) ligand 2; CCL5, Chemokine (C-C motif) ligand 5; CID, cognitive impairment and dysfunction; CL, capillary lumen; CMB(s), cerebral microbleed(s); ***cns***CC, central nervous system cytokines chemokines; CSF, cerebrospinal fluid; CSO, central semiovale; eNOS, endothelial nitric oxide synthase; EPVS, enlarged perivascular spaces; DG, dystroglycan; EC, brain endothelial cell; EPVS, enlarged perivascular spaces; GS, glymphatic system; IPAD, intramural periarterial drainage; IR, insulin resistance; ISF, interstitial fluid; ISS, interstitial space; LPS, lipopolysaccharide; MCP-1, chemoattractant protein 1 (MCP1); MetS, metabolic syndrome; MGCs, microglia cells; MMP-2,-9, matrix metalloproteinase-2,-9; MRI, magnetic resonance imaging; MS, multiple sclerosis; mT, microthrombus; MVPVS, MRI visible perivascular spaces; PD, Parkinson's disease; NADPH oxidase, nicotinamide adenine dinucleotide phosphate oxidase; NO, nitric oxide; NVU, neurovascular unit-neuro-glia-vascular unit; Pc, pericyte; Pcfp, pericyte foot process; *pns*CC, peripheral nervous system cytokines and chemokines; pvACef; perivascular astrocyte endfeet; PVS, perivascular spaces; PVS/EPVS, perivascular spaces/enlarged perivascular spaces; rACs, reactive astrocytes; rMGCs, reactive microglia cells; RONSS, reactive oxygen, nitrogen sulfur species; rPVMΦ, resident perivascular macrophages; SAS, subarachnoid space; RANTES, regulated on activation, normal T cell expressed and secreted; SVD, cerebral small vessel disease; T, Tesla; T2DM, type 2 diabetes mellitus; TEM, transmission electron microscopy; TI/AJs, tight and adherens junctions; TIAs, transient ischemic attacks; VAD, vascular dementia; VCI, vascular cognitive impairment; VCID, vascular contributions to cognitive impairment and dementia; WMH, white matter hyperintensities.

Key words: Blood-brain barrier, Brain endothelial cell, Brain endothelial cell activation and dysfunction, Cerebral small vessel disease, Enlarged perivascular spaces, Glymphatic system, Neurovascular unit, Perivascular spaces, Transmission electron microscopy

Introduction

Brain endothelial cells (BECs) are unique in their lack of fenestrations, a paucity of pinocytotic vesicles with decreased transcytosis, and their increased barrier functional strength, to prevent paravascular passage of molecules, ions, solutes, water, and cells compared with their peripheral systemic endothelial cell (EC) counterparts (Daneman and Prat, 2015; Hayden et al., 2018; Hayden, 2023a; Alahmari, 2021; McConnell and Mishra, 2022; Liao, 2013). BECs have tight and adherens junction (TJ/AJ) complexes, junctional adhesion molecules (JAMs) and vascular endothelial cadherins (VE cadherins) that comprise the blood-brain barrier (BBB) of the neurovascular unit (NVU), which contribute to increased transendothelial electrical resistance (TEER) (McConnell and Mishra, 2022) (Fig. 1).

BECs are known to secrete multiple injurious species and neurotoxins including BEC-derived cytokines and chemokines (***bec***CC), such as interleukin-1 beta (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8) and tumor necrosis factor-alpha (TNFα), and also include the chemokines MCP-1 or CCL2, CCL5 or RANTES, plus others that, in turn, stimulate the brains' reactive microglia and astrocytes (rMGCs, rACs) to produce even more central nervous system cytokines and chemokines (***cns***C/C). Additionally, BECs, rMGCs, and rACs are also capable of actively secreting reactive oxygen species (ROS), resulting in the creation of the reactive species interactome (RSI) of reactive oxygen, nitrogen, sulfur species (RONSS), which in turn activate local matrix metalloproteinases -2, -9 (constitutive MMP-2 and inducible MMP-9) (Yuan et al., 2023) (Fig. 2).

BEC activation (BEC*act*) implies a proinflammatory BEC surface by activating the response to injury wound-healing mechanisms to synthesize vascular cellular adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and endothelial leukocyte adhesion molecule (ELAM or E-selectin) in order to call up peripheral leukocytes for adherence via the activation of the more central BEC nuclear transcription factor: nuclear factor- kappa B (NF-κB) (De Caterina et al., 1995; Liao, 2013; Yuan et al., 2023). Whereas, BEC dysfunction (BEC*dys*) implies reduced bioavailability of the protective vasodilator gasotransmitter nitric oxide (NO) (De Caterina et al., 1995).

It is very interesting that microvessels from human individuals with late-onset Alzheimer's disease (LOAD) were found to secrete an as-yet-unidentified heat-labile, dose and time-dependent soluble protein(s) when cultured with neurons, which resulted in neuronal death (Grammas et al., 1999). These findings prompted Grammas et al. to coin the term "endothelial-mediated

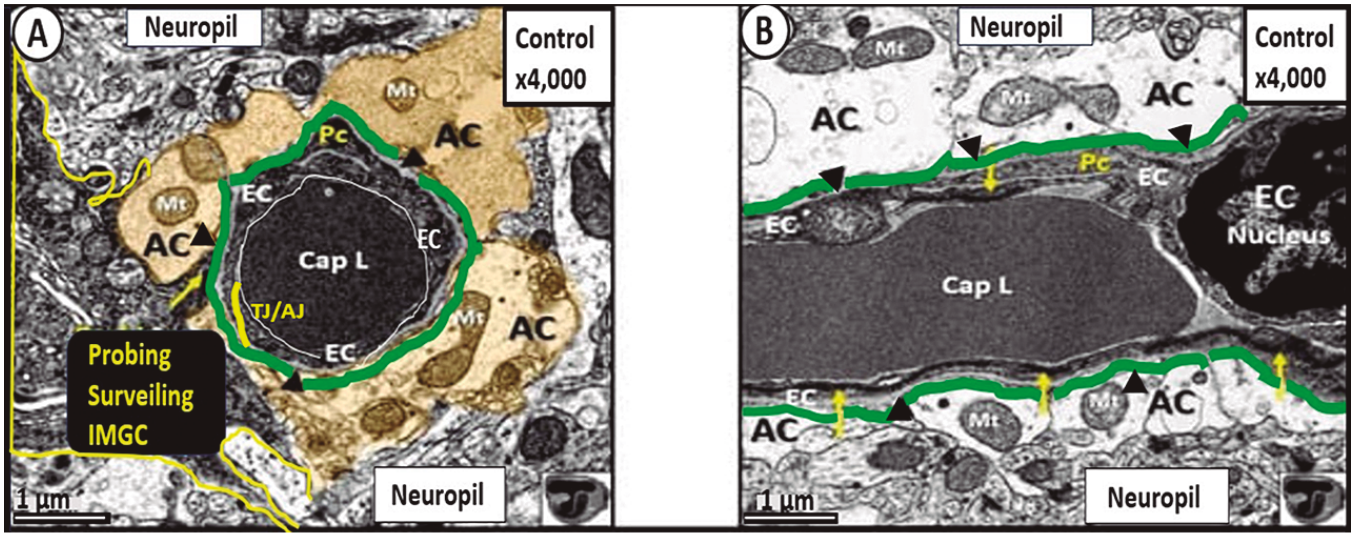

Fig. 1. Ultrastructure transmission electron microscope (TEM) cross and longitudinal sections of the true capillary neurovascular unit (NVU) from a control C57BL/6J female mouse. **A.** demonstrates a TEM cross section of a true capillary NVU and note brain endothelial cell(s) (EC) with their tight and adherens junctions (TJ/AJ yellow line), astrocyte (AC) (pseudo-colored golden to indicate their importance), glia limitans perivascularis (cyan green line and arrowheads), and interrogating microglia (IMGC) are identified. **B.** demonstrates the true capillary NVU in longitudinal view and note again the green cyan line that represents the glia limitans. Note that the ACs are not pseudo-colored and when original they represent the AC clear zone. Further, note that the pia mater membrane follows and appear to fuse with the AC endfeet basement membrane and are lost at the level of the true capillary and also is not present at the level of the postcapillary venules, venules, and veins of the perivascular unit. Reproduced image provided with permission by CC 4.0 (Hayden et al., 2018).

neurodegenerative hypothesis". While the use of the term neurotoxins may be too harsh, they were certainly able to demonstrate that BECs were capable of creating an injurious microenvironment for neurons by secreting either a molecule or molecules with neuronal noxious toxic-like properties (Grammas et al., 1999).

BEC*act/dys* and its consequences may be observed via ultrastructure transmission electron microscopy (TEM) studies in brain layer III cortical structures in obese, insulin resistant, female diabetic *db/db* mice at 20 weeks of age and in lipopolysaccharide (LPS)-treated male CD-1 mice at 10-14 weeks of age (Erickson et al., 2023; Hayden, 2023a,b; Shulyatnikova and Hayden, 2023) (Figs. 3, 4).

BECs are the crucial first cells to come into contact with the systemic circulation and peripheral multiple injurious stimuli, as in Figure 2. When BECs are injured, they are known to respond with a brain injury and response to injury wound-healing mechanism(s) with BEC*act/dys* that initiates i) hemostasis, ii) inflammation (peripheral leukocytes, innate microglia, and perivascular macrophage cells), iii) proliferation (astrogliosis), iv) remodeling, and v) repair and resolution phases if the injurious stimuli are removed. However, if the injurious stimuli remain chronic, this process does not undergo the normal resolution phase and, therefore, a persistent response to injury response develops with tissue and cellular remodeling with numerous aberrant remodeling phenotypes (Hayden, 2023c). Over the past decade, the author has accumulated evidence of at least 10 persistent reoccurring ultrastructural TEM aberrant remodeling changes in obese, insulin-resistant, diabetic, and hypertensive preclinical models that have been associated with BEC*act/dys*, which are presented for those who do not utilize TEM studies regularly (Fig. 5) (Shulyatnikova and Hayden, 2023).

BEC*act/dys* is known to play a key role in the pathogenesis of cerebral small vessel disease (SVD), which functionally occurs prior to or concurrently with

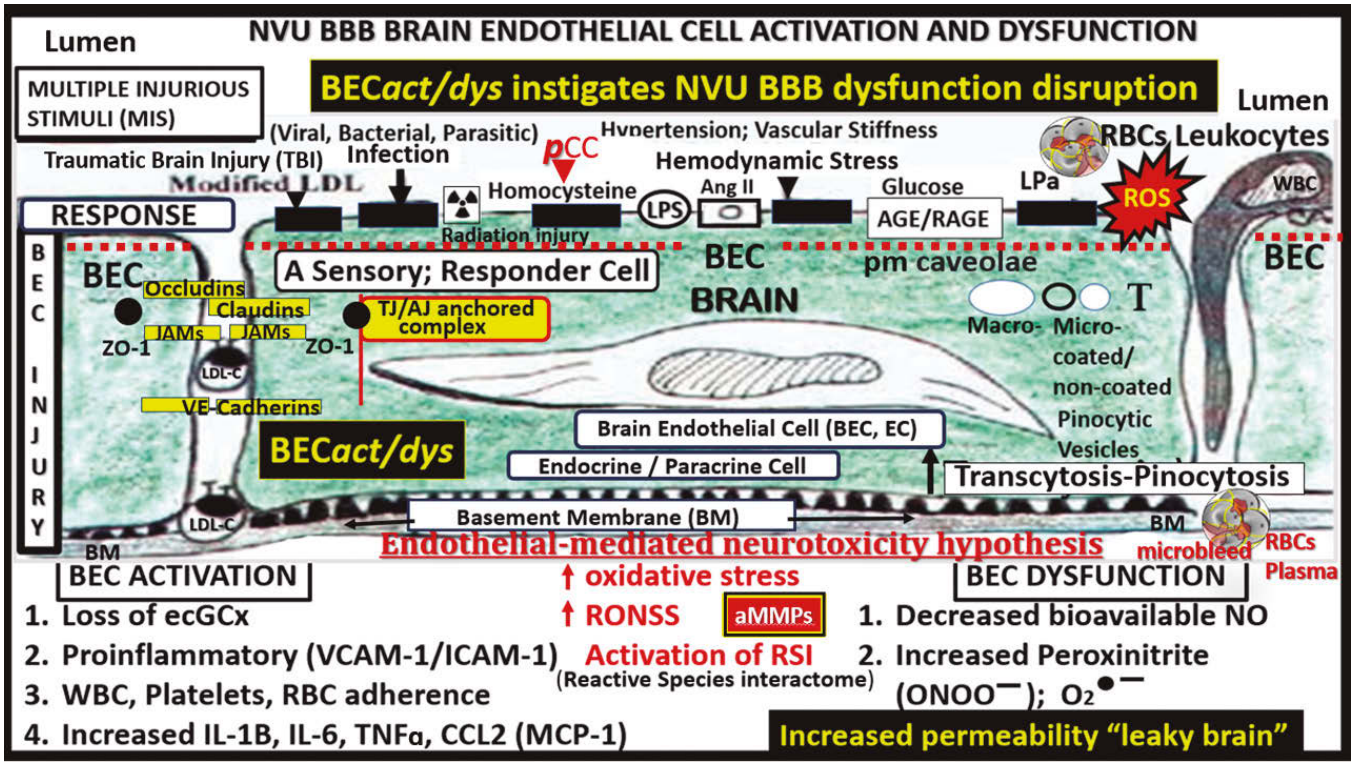

Fig. 2. Multiple injurious species from the peripheral circulation provide the stimulus and signal brain endothelial cell (BEC) to become activated and dysfunctional (BEC*act/dys*). Please note that pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) are not shown: however, they are also responsible for BEC*act/dys*. Also, BH4 uncoupling (BH4 must be totally reduced in order to run the eNOS reaction to produce nitric oxide, and thus, oxidative stress decreases the production of NO by the eNOS reaction) and eNOS uncoupling are not depicted. This highly modified image provided with permission by CC 4.0 (Hayden, 2023a). AGE/RAGE, advanced glycation end-products and receptor for AGE; Ang II, angiotensin two; BBB, blood-brain barrier; BEC, brain endothelial cell; BECact/dys, brain endothelial cell activation/dysfunction; BH4, tetrahydrobiopterin; CCL2, chemokine (C-C motif) ligand 2; ecGCx, endothelial glycocalyx; ICAM-1, Intercellular Adhesion Molecule 1; IL-1β, interleukin-1β; IL-6, interleukin-6; cellular adhesion molecule; JAMs, junctional adhesion molecules; LDL, low density lipoprotein cholesterol; LPa, lipoprotein little a; MCP-1, monocyte chemotactic protein-1; NO, nitric oxide; ONOO–, peroxinitrite; pm, plasma membrane; *pns*CC, peripheral nervous system cytokines and chemokines; NVU, neurovascular unit; RBC, red blood cell; RONSS, reactive oxygen, nitrogen, sulfur species; ROS, reactive oxygen species, RSI, reactive species interactome; Superoxide, $O_2^{\bullet-}$; T, transcytosis; TNFα, tumor necrosis factor alpha; VCAM-1, vascular cellular adhesion molecule-1; WBC, white blood cell.

BBB dysfunction and/or disruption (BBB***dd***) with increased BBB permeability (Rajani et al., 2018; Quick et al., 2021; Bai et al., 2022). Also, features of BEC dysfunction appear as a common theme underlying pathological changes in genetic as well as sporadic SVD (Quick et al., 2021).

Normal BEC function promotes the production of the gasotransmitter NO, and once synthesized by the BEC endothelial nitric oxide synthase (eNOS) enzyme, NO will then diffuse to the vascular smooth muscle cells

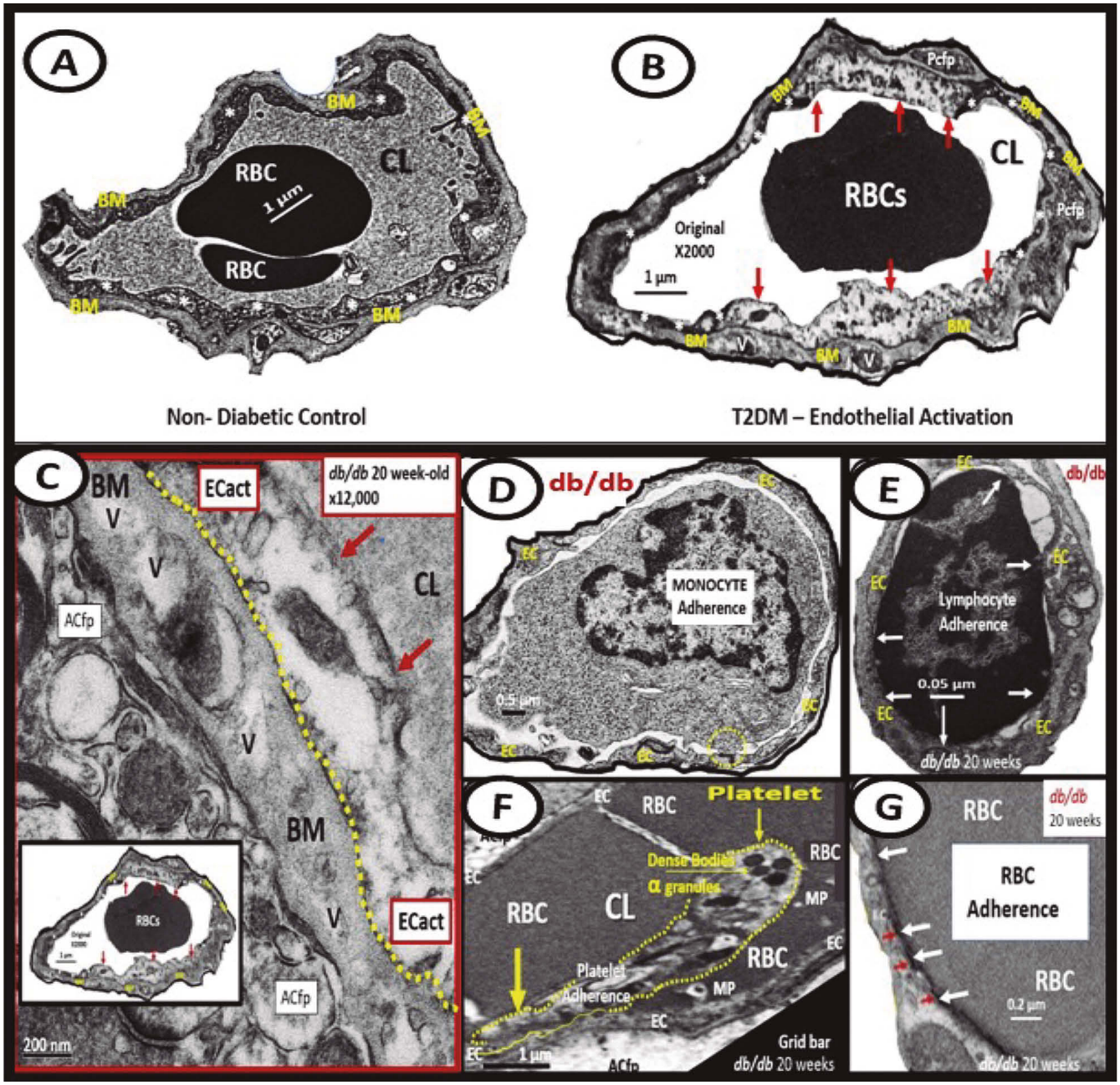

Fig. 3. Ultrastructural images of brain endothelial cell activation/dysfunction (BEC*act/dys*) in 20-week- old obese diabetic *db/db* female models. **A** demonstrates the normal neurovascular unit (NVU) blood-brain barrier (BBB) capillary and note the thinness of the moderate electron dense cytoplasm. **B** depicts regions of abrupt thickened electron lucent (red arrows) with vacuole-like bodies within basement membrane (BM) in obese diabetic female *db/db* models with BEC*act/dys* as compared to control panel A. **C** depicts BM thickening with increased vacuole-like bodies (V). **D, E, F and G** depict monocyte **(D)**, lymphocyte **(E)** leukocytes, platelet **(F)**, and red blood cell **(G)** adherence to the plasma membrane (with adherence plaques that help to secure their adhesion) to brain endothelial cells in BEC*act/dys db/db* models. Images reproduced with permission by CC 4.0 (Shulyatnikova and Hayden, 2023). Original magnification: ×2000; scale bar: 1 µm. ACfp, astrocyte foot processes; Cl, capillary lumen; EC, brain endothelial cells; MP, microparticle of the platelet.

(VSMCs) of small arteries and arterioles and the pericytes of precapillary arterioles to activate guanylate cyclase, resulting in cyclic guanylyl cyclase (cGMP)-mediated relaxation of the vessel (Liao, 2013). Reduced release of NO occurs in dysfunctional ECs (decreased bioavailable NO) and, as a result, will undergo pathological vasoconstriction with impaired regional blood flow, and ultimately tissue hypoxia with decreased delivery of glucose and nutrients to regional neurons will ensue. A functional healthy brain endothelium is a net producer of NO; however, the BEC*act/dys* endothelium becomes a net producer of reactive oxygen species (ROS) (peroxynitrite and superoxide), capable of damaging the surrounding neuropil and activating matrix metalloproteinases-2, -9 (MMP-2, -9) contributing to further NVU BBB disruption via the degrading effect of MMP-2, -9 on TJ/AJ barrier proteins. Therefore, with BEC*act/dys*, regional neuropils will experience not only neuroinflammation but also regional ischemia and increased oxidative stress, resulting in impaired cognition and possible neurodegeneration (Rajani et al., 2018; Quick et al., 2021; Bai et al., 2022).

One of the overarching findings during this past year of reading about the evolution of enlarged perivascular spaces (EPVS) is that NVU BEC*act/dys* and BBB***dd*** results in the remodeling of pathologic EPVS from normal perivascular spaces (PVS) within the PVUs of postcapillary venules, venules, and veins with impaired waste removal by the glymphatic system (GS), which allows toxic waste to accumulate within the brain. This is largely due to NVU BBB***dd***, developing a marked increase in its permeability to various neurotoxins and the attenuation/loss of its BEC glycocalyx (ecGCx) and attenuation, malpositioning, or loss/degradation of its TJ/AJ barrier with loss of function (Wardlaw et al., 2017). Further, Wardlaw et al. provided evidence that the NVU BBB increased permeability and leakage was definitely an important precursor, and likely an early key pathological event in the development of SVD-associated brain damage.

SVD may be defined simply as a condition wherein damage to small arteries, arterioles, capillaries, postcapillary venules, venules, and veins are predominant findings, which lead to reduced or interrupted perfusion (hypoperfusion) of the affected regions of the brain, resulting in global brain disease that

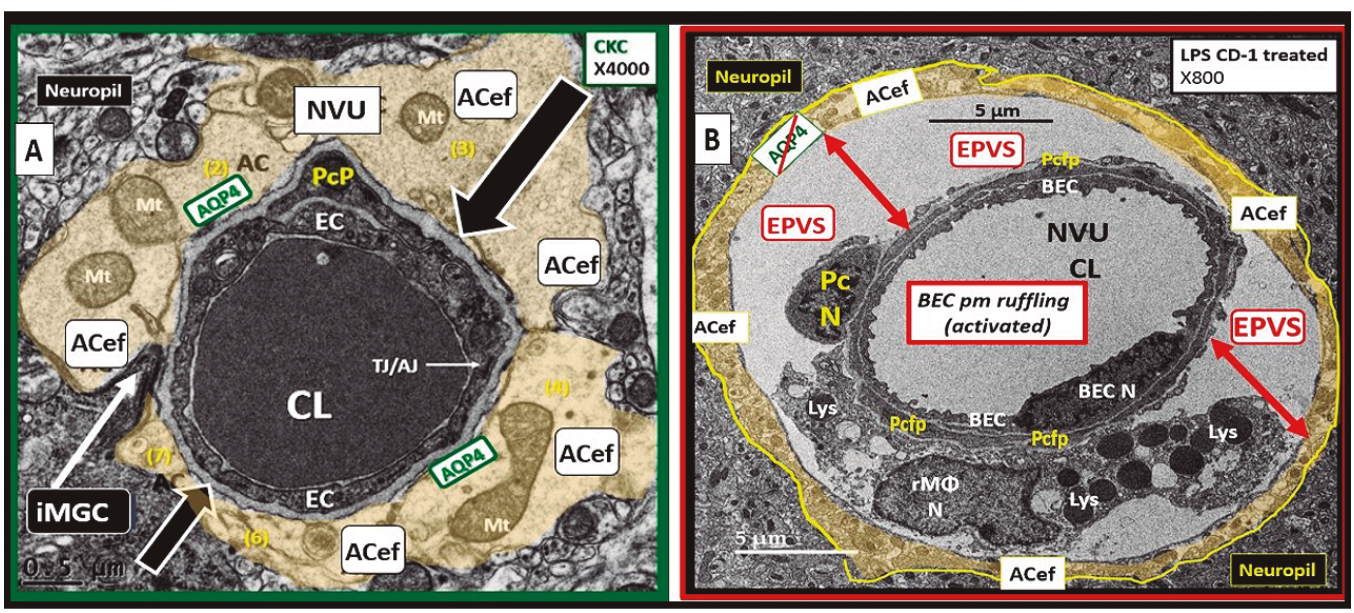

Fig. 4. Brain endothelial cell activation and dysfunction (BEC*act/dys*) is associated with lipopolysaccharide (LPS) administration and enlarged perivascular space (EPVS) development. **A** demonstrates a control true capillary in a non-LPS treated model. Note how the perivascular astrocyte endfeet (pvACef) tightly adhere to the basement membrane (BM) (black open arrows) of the brain endothelial cell (BEC) and pericyte foot processes (PcP) and the interrogating quiesent microglia cell (iMGC). Note the important location of basilar polarization of the aquaporin 4 water channel (AQP4) (green lettering) at the apical plasma membrane. **B** depicts a postcapillary venule that is known to have a perivascular space (PVS) that is usually ≤ one micrometer (µm) in width; however, in the LPS treated model, as depicted, the PVS becomes dilated and is termed an enlarged perivascular space (EPVS) within the perivascular unit spaces of 5 µm. The detachment and separation of the ACef from the NVU BM (double red arrows) along with its apical AQP4 water channel (crossed out to indicate dysfunction and or loss) also contributes to this dilated space. Note the large resident, reactive perivascular macrophage cell (rMΦ) containing multiple lysosomes (Lys), vesicles, and vacuoles (suggesting polarization with increased reactivity) inferior to the NVU that is capable of antigen presentation, which supports the ongoing perivascular neuroinflammation that is also referred to as perivascular macrophage (CD-163 staining not shown). The intra-perivascular space rPVMΦ is capable of migrating within the PVS/EPVS and may contribute to the development of EPVS as it digests debris within the PVS and contributes to stalling, stagnation, and obstruction of the interstitial fluid flow within the perivascular space, resulting in EPVS. Importantly, there is a close proximity between rPVMΦ and BECs and PcPs. Image reproduced with permission by CC 4.0 (Hayden, 2023b). AC, astrocyte; CL, capillary lumen; EC, BEC, brain endothelial cell; iMGC, interrogating microglia; Mt, mitochondria; N, nucleus; PcP, pericyte process; TJ/AJ, tight and adherens junction(s).

causes cognitive impairment, ischemic or hemorrhagic stroke with mobility problems, and neuropsychiatric symptoms. Generally, focal white and deep grey matter lesions on brain magnetic resonance imaging (MRI) are found (Chojdak-Łukasiewicz et al., 2021). Further, SVD lesions (white matter hyperintensities (WMH), lacunes, microbleeds, and EPVS) on MRI studies are associated with cognitive decline and dementia (Cuadrado-Godia et al., 2018). SVD is responsible for stroke incidents, gait disturbances, cognitive impairment, depression, and dementia that occurs especially in the elderly and contributes to about 20% of strokes, including 25% of ischemic strokes and 45% of dementias (Li, et al., 2018). Commonly recognized causes of SVD include arteriolosclerosis, cerebral amyloid angiopathy (CAA), genetic small vessel angiopathy, inflammation, immune-mediated small vessel diseases, and venous collagenosis (Schneider et al., 2007; Rahimi and Kovacs, 2014; Li, et al., 2018). Currently, it is more widely accepted that SVD frequently coexists with late-onset or sporadic age-related neurodegenerative Alzheimer's disease (LOAD) as a co-occurrence or mixed dementia (Pantoni, 2010; Wardlaw, 2020; Duperron, 2023). Also, SVD can exacerbate cognitive deficits, physical disabilities, and other symptoms of neurodegeneration (Li et al., 2018). Additionally, SVD is the most common vascular cause of dementia (Hainsworth et al., 2024) and is a major contributor to mixed dementia, which is responsible for about one-fifth of all strokes worldwide. Importantly, Alzheimer's disease and SVD share common risk factors and both lead to cognitive decline and dementia (Li et al., 2018).

Signs of SVD on MRI include recent small subcortical infarcts, lacunes, EPVS, white matter hyperintensities, cerebral microbleeds (CMBs), and atrophy (Li et al., 2018; Hayden, 2023a-d). SVD is known to be quite heterogeneous; recently Pantoni subcategorized these different pathologies and etiologies into at least six basic categories, taking into account several mechanisms. These six categories comprise: i) arteriolosclerosis, also known as age-related or vascular risk factor-related small vessel disease; associated with aging, diabetes, and hypertension with a loss of VSMCs with thicker and stiffer walls (vascular stiffening) associated with hyaline deposition and narrower lumens; ii) CAA, an accumulation of immunoreactive protein (β-amyloid) in the walls of arteries and arterioles. In severe cases, this leads to vessel disruption and leakage from the circulation into the parenchyma with associated microbleeds. Sporadic or hereditary forms that also occur in LOAD and Down's syndrome; iii) inherited and/or genetic forms distinct from CAA, of which cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) and Fabry's disease are the most common; iv) vasculitis diseases that may be characterized by inflammatory cells in the vessel walls that are typically systemic and the pathology is inflammatory and immunologically mediated; v) venous collagenosis, wherein veins and venules close to lateral ventricles have increased wall thickness, mainly due to excess collagen, resulting in narrowed lumens and, sometimes,

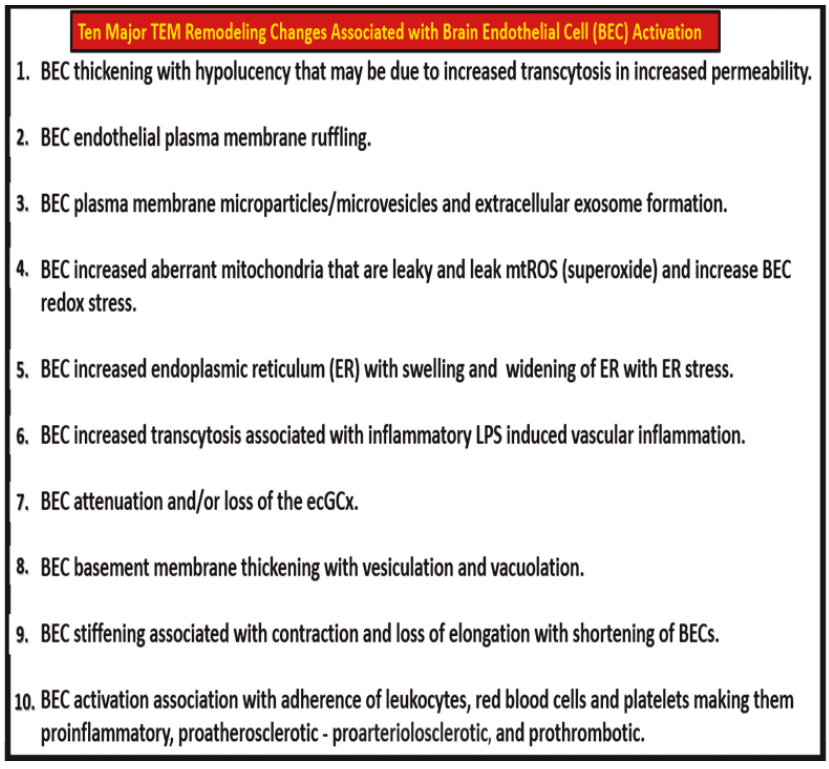

Fig. 5. Ten major transmission electron micrograph (TEM) aberrant remodeling changes associated with brain endothelial cell activation and dysfunction to better understand the various brain endothelial cell response to injury wound healing phenotypes. Image reproduced with permission by CC 4.0 (Shulyatnikova and Hayden, 2023).

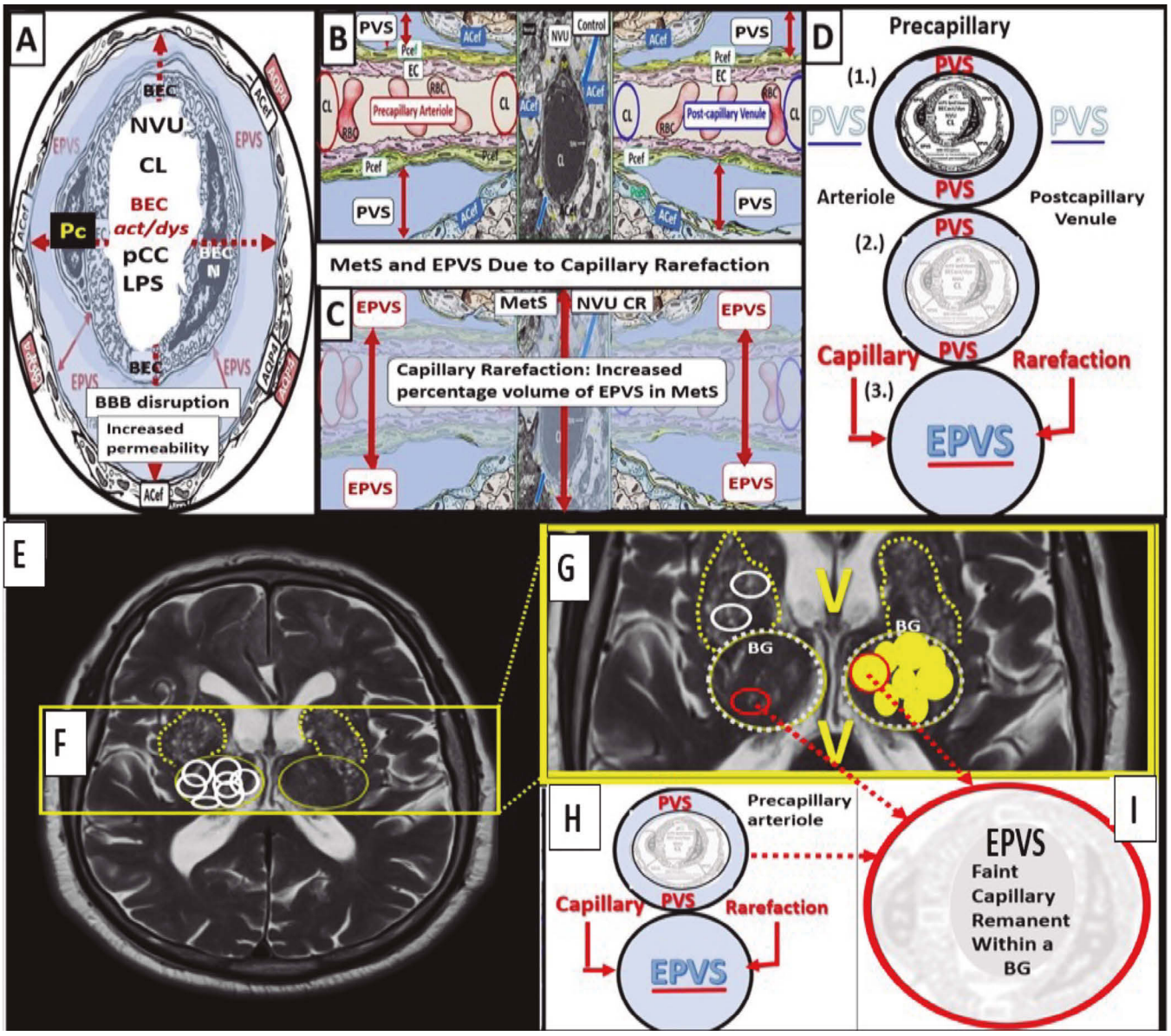

Fig. 6. Capillary rarefaction of microvessels may result in enlarged perivascular spaces. The images presented are illustrations and are not to scale. **A-D** represent precapillary arterioles and postcapillary venules capillary rarefaction illustrated in cross and longitudinal sections within perivascular spaces (PVS) of the perivascular unit (PVU). **A** illustrates a capillary microvessel that is surrounded by a PVS (solid double red arrows with light blue background) representing capillary rarefaction and note the associated red bars to mark the dysfunctional aquaporin 4 (AQP4) water channels. **B** demonstrates a longitudinal control precapillary arteriole, postcapillary venule and neurovascular unit (NVU) capillary that runs through a PVS (light blue). **C** depicts a longitudinal view of microvascular capillary (CR) and note that the volume of the PVS increases its total percentage volume once the capillary has undergone rarefaction. **D** illustrates a control normal precapillary arteriole and postcapillary venule PVS to evolve to EPVS once the capillaries have undergone rarefaction that permits for an increase in its total percentage volume of the PVS (1.-3.). The image in D3 depicts complete rarefaction of the capillary that results in the increase total volume as compared prior to capillary rarefaction. Reproduced with permission by CC 4.0 (Shulyatnikova and Hayden, 2023). **E-I** illustrate an MRI with basal ganglia (BG) and how capillary rarefaction might interact in these areas to result in the development of EPVS. **E and F**. MRI at the level of the BG demonstrating multiple EPVS in a symmetrical manner. **G** is an exploded image of F allowing for better visualization of the EPVS that are residing within the BG. **H** represents a capillary that is undergoing rarefaction and **I** illustrates a EPVS that has developed due to the rarefaction of the only faintly noted capillary that was once now there but now gone leaving a PVS that has now remodeled into an EPVS. Panels EFG provided with permission by CC 4.0 (Hayden, 2023d). ACef, perivascular astrocyte endfeet; AQP4, aquaporin 4; BBB, blood-brain barrier; BEC, brain endothelial cells; BEC*act/dys*, brain endothelial cell activation and dysfunction; BG, basal ganglia; CL, capillary lumen; EC, brain endothelial cell; EPVS, enlarged perivascular space; MetS, metabolic syndrome; N, nucleus; NVU, neurovascular unit; Pc, pericyte; Pcef, pericyte endfeet; PVS, perivascular space; V, ventricle. White circles, identify EPVS within BG.

occlusion; and vi) other SVDs such as post-radiation angiopathy, wherein hyaline deposition leads to vessel wall thickening after irradiation therapy and subsequent degeneration of myelin sheaths (Pantoni, 2010).

There are at least three basic types of SVD: Type 1: sporadic arteriolosclerosis associated with aging and systemic hypertension (especially systolic hypertension and T2DM); Type 2: sporadic hereditary CAA; and Type 3: inherited or genetic forms (non-CAA), most commonly CADASIL (Hayden, 2020a,b). Also, cerebral structural capillary rarefaction has been determined to precede the development of WMH in genetic models of cerebral ischemic SVD (Kwan et al., 2021). Further, Shulyatnikova and Hayden previously hypothesized that structural capillary rarefaction may be associated with the evolution of EPVS in addition to ischemic SVD (Fig. 6) (Díez-Sainz, 2022; Shulyatnikova and Hayden, 2023).

Recently, some researchers have shared their observations from higher-resolution MRIs and have discussed the presence of residual and faint capillary remnants in the EPVS identified within the basal ganglia, as depicted in Figure 6 (Wardlaw et al., 2019).

From BEC*act/dys* to EPVS and SVD

BEC*act/dys* initially develops as a result of the multiple peripheral injurious stimuli to the endothelium, as depicted in Figure 2. While each of these stimuli is capable of injuring the endothelium, they may also act synergistically in contributing to peripheral nervous system cytokines/chemokines (***pns***CC) and exosomes arising from dysfunctional dysbiotic gut microbiome and dysfunctional perivascular adipose tissue of the aorta and omental tissues in obese, insulin-resistant, metabolic syndrome, and diabetic meta-inflammation individuals and models (Poggesi et al., 2016; Brown et al., 2018; Wardlaw et al., 2019; Clancy et al., 2021; Bai et al., 2022). BEC*act/dys* results in NVU BBB disruption with increased permeability (Fig. 7).

Recently, BEC*act/dys* has been considered to be a key mechanism in the pathogenesis of not only EPVS but also SVD, vascular dementia (VaD), vascular cognitive impairment (VCI), and vascular contributions to cognitive impairment and dementia (VCID) (Snyder et al., 2015; Sweeney et al., 2018, 2019; Wardlaw et al.,

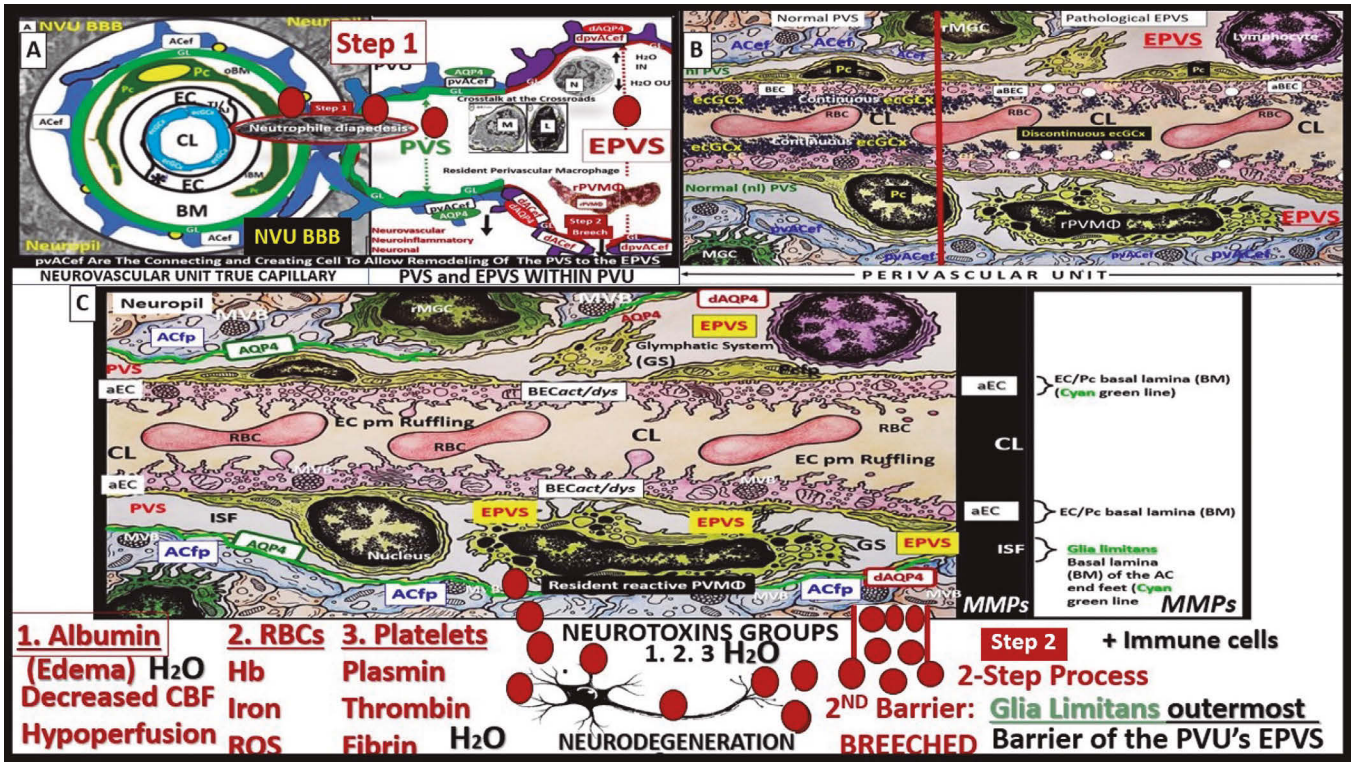

Fig. 7. Neurovascular unit BBB disruption with increased permeability, leakage of leukocytes, peripheral cytokines chemokines, exosomes, and neuroinflammation. Note that the red circles emanating from the enlarged perivascular spaces (EPVS) depict various neurotoxin groups that are divided into three groups (1., 2., 3.), which contribute to neuroinflammation, neurodegeneration, cerebral microbleeds and impaired cognition. Image reproduced with permission by CC 4.0 (Hayden, 2023d). aBEC, activated brain endothelial cell; ACef, astrocyte endfeet; AQP4, aquaporin 4; BEC, brain endothelial cell; BEC*act/dys*, brain endothelial cell activation/dysfunction; BM, basement membrane; CBF, cerebral blood flow; CL, capillary lumen; dACef, dysfunctional astrocyte endfeet; dpvACef, dysfunctional astrocyte endfeet; EC, brain endothelial cell; ecGCx, brain endothelial cell glycocalyx; EPVS, enlarged perivascular spaces; H_2O, water; Hb, hemoglobin; ISF, interstitial fluid; L, lymphocyte; M, monocyte; MGC, microglia cell; MMPs, matrix metalloproteinases; N, neutrophile; Pc, pericyte; PvACef, perivascular astrocyte endfeet; PVS, Perivascular Spaces; PVU, perivascular unit; RBC, red blood cell; rPVMΦ, resident, reactive perivascular macrophage; ROS, reactive oxygen species.

2019). Subsequent neurovascular uncoupling and decreased cerebral blood flow (hypoperfusion) with hypoxia and ischemia, increased permeability-hyper-permeability, increased fluid delivery, proinflammatory leukocytes, ***pns***CC, and neuro-inflammation, along with multiple other injurious stimuli, as presented in Figure 2, with resulting central nervous system cytokines/chemo-kines (***cns***CC) and neuroinflammation. Additionally, this NVU BBB***dd*** is known not only to associate with BEC*act/dys*, but also with EPVS and SVD in addition to their evolution (Fig. 8) (Snyder et al., 2015; Sweeney et al., 2018, 2019; Wardlaw, 2019; Yang et al., 2019; Zeng et al., 2022; Hayden, 2023e; Luo et al., 2023; Zhou et al., 2023).

Role of obesity, metabolic syndrome (MetS), insulin resistance, and type 2 diabetes mellitus (T2DM) with accelerated atherosclerosis and arteriolosclerosis in the development of EPVS, and SVD

Metabolic syndrome (MetS), obesity, insulin resistance (IR), and Type 2 Diabetes Mellitus (T2DM) are known to increase with age, and our current population is not only the oldest global population in recorded history but it will also continue to grow at least over the next two decades (Rhea et al., 2022). They are considered metabolic disorders that seem to parallel the increasing prevalence of obesity and are associated with a known cluster of risk factors, including obesity, hyperlipidemia, hyperinsulinemia, hypertension, and hyperglycemia, which may be associated with or without overt T2DM. The four core risk factors are associated with IR and, importantly, MetS is known to increase the risk of developing cerebrocardiovascular disease with accelerated atherosclerosis and arteriolosclerosis, and T2DM, which are associated with increased risk of micro-macrovascular disease, including SVD (Fig. 9) (Hayden, 2020a,b, 2023b,c; Rhea et al., 202).

It is important to note that BEC*act/dys* and IR are placed centrally within the large X with the four arms depicting obesity hyperlipidemia, compensatory hyperinsulinemia and hyperamylinemia, hypertension and vascular arteriolar stiffness, and hyperglycemia, with or without overt T2DM. Importantly, IR has been emerging as a driver for the evolution of SVD for some time (Doubal et al., 2010). Recently, Yang et al. shared

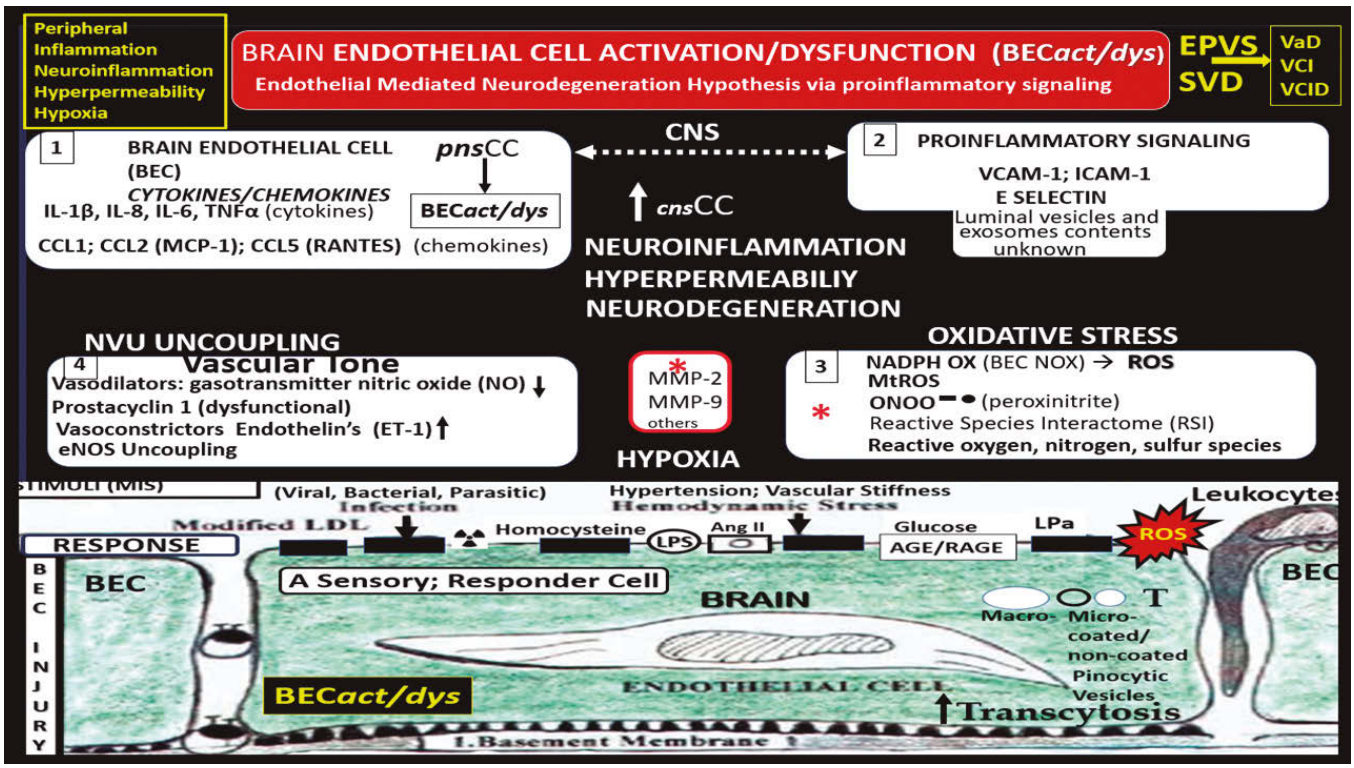

Fig. 8. Brain endothelial cell activation and dysfunction (BEC*act/dys*) associates with and contributes to the evolution of enlarged perivascular spaces (EPVS) and cerebral small vessel disease (SVD). BEC*act/dys* ultimately results in hyperpermeability, neuroinflammation, and hypoxia to result in EPVS and SVD via the above mechanisms (1 through 4). AGE, advanced glycation end-products; Ang II, angiotensin II; asterisks, denotes that reactive oxygen species are responsible for the activation matrix metalloproteinases (MMPs); BECact/dys, brain endothelial cell activation and dysfunction ; BEC, brain endothelial cell; eNOS, endothelial nitric oxide synthase; ICAM-1, intercellular adhesion molecule; LDL, low density lipoprotein-cholesterol; Lpa, lipoprotein little a; LPS, lipopolysaccharide; MMPs, matrix metalloproteinases; MtROS, mitochondrial reactive oxygen species; NADPH Ox, nicotinamide adenine dinucleotide phosphate reduced oxidase; ***pns***CC, peripheral nervous system cytokines chemokines RAGE, receptor for AGE: T, transcytosis; VCAM-1. vascular cellular adhesion molecule.

that IR is an independent risk marker associated with increased severity of the SVD burden and independent of other clinical risk factor burdens in populations of the elderly, non-diabetic, and healthy individuals (Yang et al., 2019).

Previously, in this section peripheral IR has been primarily discussed concerning EPVS and SVD. Even though IR is known to be associated with both aging and LOAD, it is not settled as to whether peripheral IR or brain IR predominates in one or the other or both. Therefore, at this point in time, it may be best if we think of peripheral and brain IR as independent risks but as interacting processes (Benjamin et al., 2018).

SVD presents clinically as lacunar strokes and frequently also demonstrates WMH, EPVS, and CMBs. SVD is responsible for at least 20% of ischemic strokes and also represents a major cause of VaD, VCI, and VCID. EPVS are a biomarker and prominent feature of both SVD and VaD, which are known to associate with lacunar stroke and WMH (Hayden, 2019, 2023c; Sweeney et al., 2019; Paavonsalo et al., 2020; Wu, 2020; Barisano, et al., 2021). Once identified by MRI, EPVS associate with WMH and lacunes. IR has been independently correlated with EPVS in the basal ganglia of the healthy non-diabetic elderly population (Liu et al., 2018). Cerebral capillary rarefaction in obesity and metabolic diseases depends on IR and decreased NO bioavailability (Brundel et al., 2014).

The essential role of the intact brain endothelial cell glycocalyx (ecGCx)

The role of the ecGCx is not to be underestimated and serves as the first barrier of the NVU BBB. LPS is one of the most common methods to induce and/or accelerate neuroinflammation utilized in animal models and is a commonly secreted portion of gram-negative bacteria plasma membranes residing in the gut, which is elevated in clinical meta-inflammation originating from the gut (GBD 2016 Disease and Injury Incident and Prevalence Collaborators, 2017; Powers et al., 2019; Murphy and Werring et al., 2020).

Erickson et al., have recently utilized intraperitoneal LPS injections to identify the following remodeling changes to NVU BBB BECs and PVS including: aberrant BEC mitochondria, BEC plasma membrane

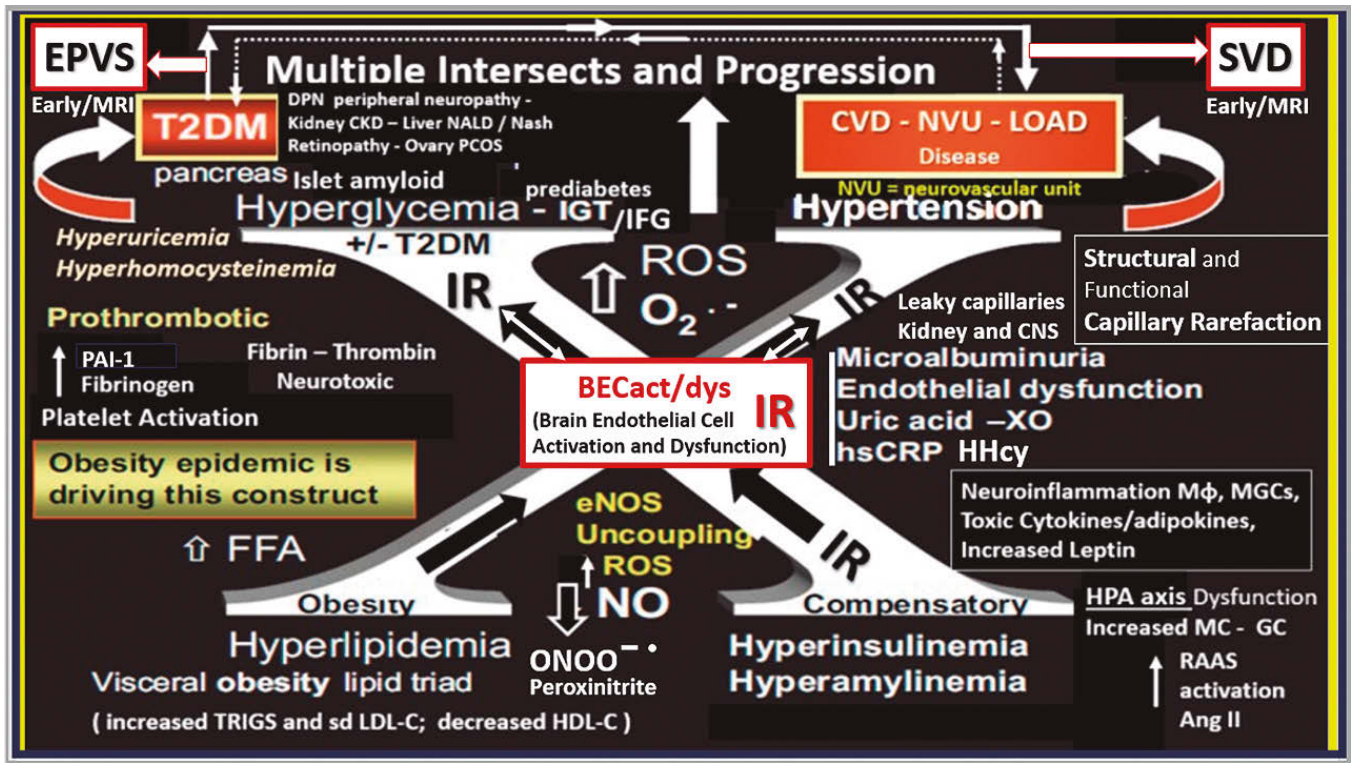

Fig. 9. Obesity, MetS, T2DM, brain endothelial cell activation/dysfunction (BEC*act/dys*) cerebrocardiovascular disease (CCVD), EPVS, SVD. Note the importance of BEC*act/dys* and IR being placed centrally within the white letter X as its effects may be driving both the EPVS as well as SVD development and evolution via accelerated atherosclerosis and arteriolosclerosis. Ang II, angiotensin two; CKD, chronic kidney disease; CNS, central nervous system; CVD, cerebrovascular disease; DPN, diabetic peripheral neuropathy; EPVS, enlarged perivascular spaces; eNOS, endothelial nitric oxide synthase; FFA, free fatty acids; GC, glucocorticoid; HHcy, hyperhomocystemia; HPA, hypothalamic pituatary adrenal axis; hsCRP, highly sensitive C-reactive protein; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; IR, insulin resistance; LOAD, late-onset Alzheimer's disease; MC, mineralocorticoid; MGC, microglia cells; MRI, magnetic resonance image; NALD, non-alcoholic liver disease; NASH, non-alcoholic steatohepatitis; NO, nitric oxide; $O_2^{\cdot -}$, superoxide; PAI-1, plasminogen activator inhibitor-1; PCOS, polycystic ovary syndrome; RAAS, renin-angiotensin-aldosterone system; ROS, reactive oxygen species; SVD, small vessel disease; XO, xanthine oxidase.

ruffling, increased extracellular microvesicles and small exosome formation, and increased transcytosis with intact TJ/AJ (Erickson et al., 2023). Aberrant pericytes revealed Pc nucleus rounding and retracted cytoplasmic extensions, which attracted microglia cells to the NVU, perivascular astrocyte endfeet (pvACef) retraction and

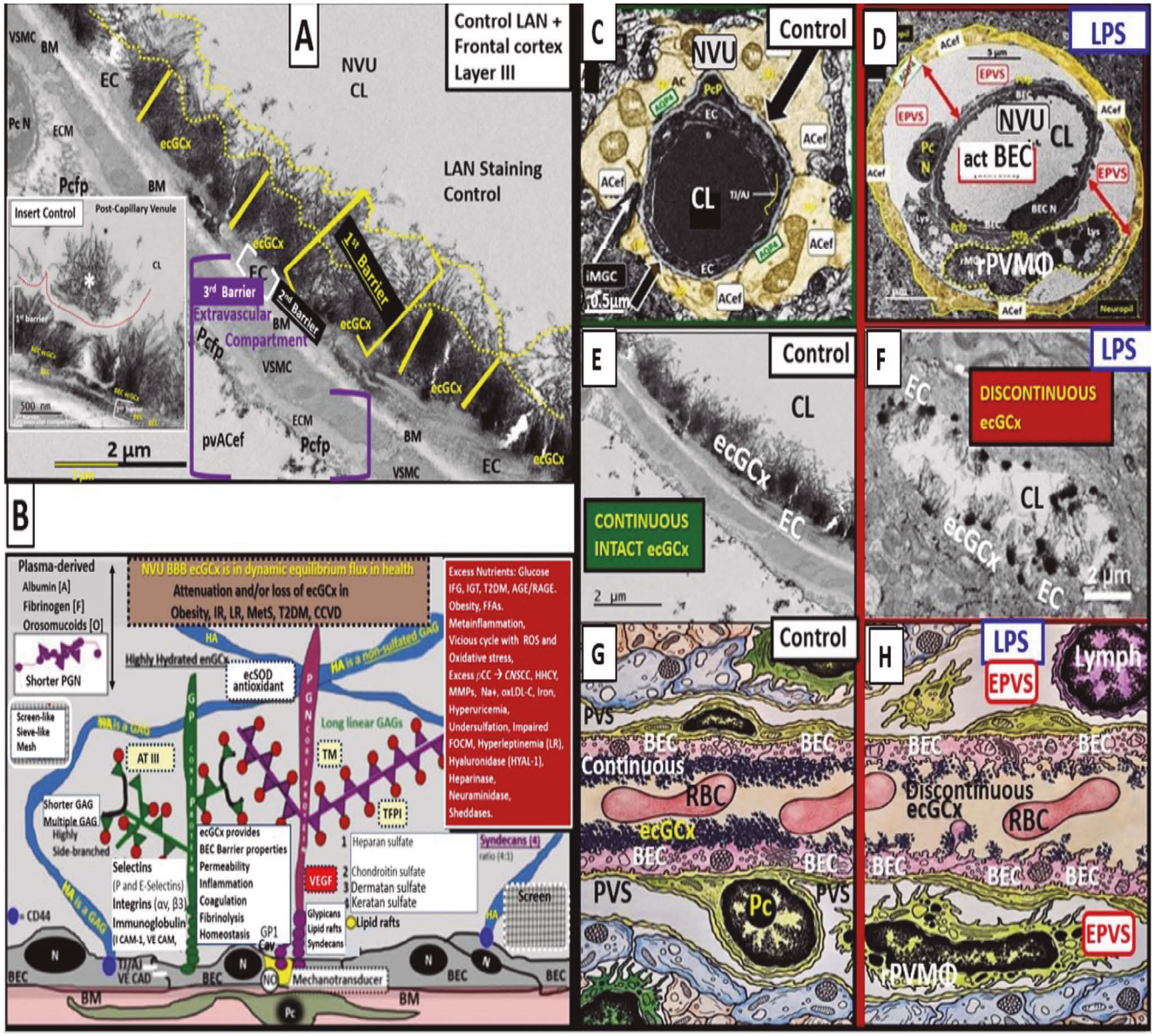

Fig. 10. The brain endothelial glycocalyx (ecGCx) transmission electron microscopy (TEM) ultrastructure and molecular functions. **A** demonstrates the ecGCx lanthanum nitrite (LAN)-stained ecGCx in the control male 10-week-old CD-1 model from cortical layer III. The ecGCx is the first barrier of the neurovascular unit blood-brain barrier (NVU BBB). Note the electron dense staining of the first barrier (boxed-in yellow), the BEC itself is the 2nd barrier along with the shared basement membrane of the BEC and pericyte (Pc) - vascular smooth muscle cell (boxed-in white), and the third barrier that is comprised by the extracellular compartment (boxed-in purple color). Note the insert lower left (lined in white color) depicting the intermittent separation of the most superficial ecGCx layer (red dashed line). **B** illustrates the molecular structure of the endothelial glycocalyx with color coding of the individual molecular components. Scale bar: 2 µm; insert scale bar: 500nm (panel A). **B** depicts three primary proteins: proteoglycan (PGN, purple), glycoprotein (GP, green) with their various side chains and note the sulfur terminals (red dots) that provide for the highly electron negative charge of the ecGCx, and hyaluronan (HA blue color). Not to scale. **Panels C, G, and E** demonstrate the appearance of the normal control ecGCx as compared to the lipopolysaccharide (LSP) treated models **(D, F, and H)**, which demonstrate an attenuation, discontinuous, ecGCx with intact scale bars. **G and H** represent a continuous perivascular unit with a normal PVS in **G** and an EPVS in **H** where the ecGCx becomes interrupted and discontinuous. Act BEC, activated brain endothelial cell; BEC, brain endothelial cell; Acef, astrocyte endfeet; AQP4. aquaporin 4; CL, capillary lumen; EC, brain endothelial cell; EPVS, enlarged perivascular space; iMGC, interrogating microglia cell; LAN, lanthanum nitrite; LPS, lipopolysaccharide; N, nucleus; NVU, neurovascular unit; Pcfp, pericyte foot process; Pc, pericyte; PVS, perivascular space; RBC, red blood cell; rPVMΦ, reactive perivascular macrophage; TJ/AJ, tight and adherens junction(s).

separation, and attenuated discontinuous ecGCx, which was associated with the development of EPVS (Erickson et al., 2023). Additionally, TEM remodeling changes in this model are depicted (Fig. 10A, C, D, E, F) (Erickson et al., 2023). Importantly, the attenuation and/or loss of the first barrier, the ecGCx, may be the earliest ultrastructural remodeling change associated with BEC*act/dys* and the subsequent development of EPVS and SVD (Fig. 10A).

Global burden of SVD and specifically lacunes, lacunar infarcts, and stroke

Stroke may be defined as an acute focal neurologic deficit caused by a vascular injury (infarction or hemorrhage) to the brain, and is the second leading cause of death and disability worldwide (Gorelick, 2019). Most strokes are ischemic (85-87%) due to SVD arteriolosclerosis, large arterial atherothrombotic thromboembolic, or cardioembolic phenomena with the remainder being hemorrhagic, with up to 80% due to SVD such as CAA (Gorelick, 2019).

As an overview, one may examine stroke and even transient ischemic attacks (TIAs) from the following perspectives of genetic and environmental factors, which may predispose to various disease processes, such as atherosclerosis, SVD, and cardiac diseases with embolic potential like atrial fibrillation. The associated risk factors include advanced age, hypertension, obesity, MetS, T2DM, smoking, and hyperlipidemia. Hematological pro-coagulative disorders that share the mechanisms of large artery embolism, SVD including lacunar stroke with occlusion and or rupture (with ischemia or hemorrhage), cardiac embolism, hemodynamic injuries such as hypertension and multiple injurious stimuli as in Figure 2, and now BEC*act/dys*. When viewing Strokes and TIAs from this type of perspective it is obvious once either of these two clinical diagnoses are made that they are just the starting point for further rational clinical investigation and long-term treatment.

Stroke is the fifth leading cause of death in the United States and lacunar strokes are the most common among ischemic strokes, constituting up to 25% (Katan and Luft, 2018; Lammie et al., 1998; Wardlaw et al., 2013; Caplan, 2015). Further, lacunar strokes are a type of ischemic stroke that are small and located in subcortical white matter regions of the brain.

SVD findings on conventional MRI include lacunes, EPVS, WMH, CMBs, recent small subcortical infarcts, and atrophy such that it is important to compare their similarities and differences (Table 1) (Pantoni, 2010; Wardlaw et al., 2013).

Three types of lacunar infarcts—lacunes—have been described by histopathology: Type 1 is classified as an ischemic infarct, Type 2 as a small hemorrhage, and Type 3 includes dilated PVS (Fischer, 1968; Iadecola, 2013). Further, Caplan discussed both the pathology and pathophysiology of lacunar infarction, which includes two main types (Caplan, 2015): 1) thickened arterial-arteriole media and 2) narrowing or obstruction of the penetrating vessels by the parent intimal plaques. These lesions tend to involve basal ganglia, pons, thalami, and white matter and leak fluid resulting in edema and reactive astrocytes with astrogliosis in white matter tracts. Also, MMPs are activated, which results in

Table 1. Comparing similarities and differences between the 'four horsemen' of small vessel disease (SVD). 1. Lacunes (footprint of stroke); 2. Enlarged perivascular spaces (EPVS) (biomarker of SVD); 3. White matter hyperintensities (WMH) (footprint of ischemia); 4. Cerebral microbleeds (biomarker of SVD, hemorrhage). Note that 'recent small subcortical infarcts were not included in this table because they are very similar to lacunes parameters; however, they are known to have a greater flair as compared to lacunes, which suggest a more recent occurance. (+), positive; mm, millimeter.

	Lacunes	EPVS	WMH	Cerebral Microbleeds
Location	Upper portions of Basal Ganglia (BG), thalamus, internal and external capsule, pons, and periventricular white matter	BG **type I** Centrum semiovale (CSO) **Type II** Midbrain **Type III**	Periventricular, deep white matter distinct from periventricular regions.	Cortico-subcortical junction and deep grey or white matter in the cerebral hemispheres, brainstem, and cerebellum.
Morphology Shape	Irregular shapes, sharp edges, or wedged shapes.	Well-defined, round, oval, tubular.	Sharp edges, linear, and frequently follow the outlines of the adjacent ventricle. Elongated.	Primarily rounded, oval spherical, smooth edges.
Symmetry	Asymmetrical	Symmetrical	Asymmetrical	Asymmetrical
Size	3-15 mm diameter	1-3 mm diameter.	3-12 mm. Usually elongated.	2-5 mm sometimes up to 10 mm areas of void.
FLAIR (fluid-attenuated inversion recovery)	(+) FLAIR (usually reflect siderosis or gliosis)	Primarily non-FLAIR	(+) FLAIR	Hypointense, black oval circles on T2*-weighted gradient echo (GRE) or susceptibility weighted magnetic resonance imaging (MRI) sequences

vascular barrier rupture with further vascular leakage with continuous gliosis and white matter atrophy (Fisher, 1968; Caplan, 2015). Of great interest is the startling fact that 20% of strokes and about 40% of dementias may be attributed to SVD, which is associated with BEC*act/dys* (Pantoni, 2010; Iadecola, 2013; Wardlaw et al., 2015; Shi and Wardlaw, 2016). Globally, about 15 million people have a stroke, of which each year 6 million die and 5 million are left permanently disabled (Lozano et al., 2013). Furthermore, 35.6 million people worldwide are estimated to be living with dementia, and this is expected to triple by 2050 (Lozano et al., 2013; Wardlaw et al., 2015).

Role of brain endothelial cell activation and dysfunction (BEC*act/dys*) and the glymphatic system and its potential role in the evolution of SVD

The GS is a brain-wide perivascular fluid transport system consisting of PVS and is thought to be analogous to the peripheral tissue lymphatic system, which is essential to deliver cerebral spinal fluid (CSF) and clear waste fluids, and toxic waste protein accumulation from the interstitial spaces-interstitial fluid (ISS-ISF) from the brain to the CSF to exit via the dural venous sinus lymphatics to the cervical lymph nodes and the peripheral venous circulation (Iliff et al., 2012, 2013; Benveniste et al 2019; Benveniste and Nedergaard, 2022). Further, it is essential to understand, that long-term stagnation with decreased waste and water clearance is decreased and represents a key aspect in the pathogenesis of SVD (Benveniste and Nedergaard, 2022).

The hallmark findings on MRI studies of SVD are EPVS and WMH, and the fact that fluid accumulation is associated within WMH may either subside or persist and remodel into the findings of lacunar infarcts is of utmost importance (Benveniste and Nedergaard, 2022). Importantly, Benveniste and Nedergaard proposed that the failure of brain waste fluid transport by the GS may play a key role in the initiation and even progression of SVD (Benveniste and Nedergaard, 2022). Their primary case for this concept was that PVS are utilized as waterways for CSF influx, and when the GS becomes impaired, there would be impaired clearance with the stagnation of glymphatic fluid transport, which could instigate loss of brain fluid homeostasis leading to transient white matter edema, PVS dilation, and ultimately demyelination (Benveniste and Nedergaard, 2022).

The understanding and appreciation of the role of GS has grown exponentially among clinicians and researchers during the past 12 years since its introduction (Iliff et al., 2012; Benveniste and Nedergaard, 2022; Ang et al., 2024). There clearly exists an overlap and bidirectionality between SVD and impaired GS function (Mathiisen et al., 2010; Verkman et al., 2011; Jessen et al., 2015; Jiang et al., 2017; Mestre et al., 2017; Nedergaard and Goldman, 2020; Kaur et al., 2021; Xu et al., 2022; Ang et al., 2024). Even though more work needs to be conducted in this field of study to prove the interconnection between impaired GS and the pathogenesis of SVD, the importance of this dynamic relationship is felt by the author and others (Benveniste and Nedergaard, 2022; Ang et al., 2024) to be of great importance at this point.

BEC*act/dys* contributes to EPVS, SVD, and relates to impaired molecular functions

While this review has largely focused on aberrant structural remodeling changes, this section intends to point out the concurrent impaired molecular function, which occurs as presented by others (Shakhov et al., 2022; Vorotnikov et al., 2022; Ziganshina et al., 2022; Khapchaev et al., 2024). Some of these impaired molecular functions include those associated with elevated saturated free fatty acids (palmitate) (Khapchaev et al., 2024), glucotoxicity and lipotoxicity associated with T2DM (Vorotnikov et al., 2022; Khapchaev et al., 2024), cytoskeleton actin remodeling and molecular dysfunction with BEC contraction and shrinkage with increased BBB disruption and permeability (Shakhov et al., 2022), and preeclampsia with ecGCX attenuation and/or loss (Ziganshina et al., 2022).

Figure 2 illustrates multiple molecular injurious stimuli that result in BEC*act/dys* and further molecular dysfunction with excessive prooxidative peroxynitrite production, eNOS uncoupling, oxidative stress with excessive BEC activation and molecular dysfunction via increased ICAM-1, VCAM-1, and E-Selectin production, and BEC dysfunction with decreased NO with loss of its anti-inflammatory, antithrombotic, and antioxidant protective properties, which associate with increased NVU BBB permeability. Figure 5 depicts the 10 most common aberrant TEM BEC remodeling changes, of which, numbers 4, 7, 9, and 10 each directly relate to molecular dysfunction. Figure 8 incorporates a sequence of numbered events (8.1. through 8.4.), which depict multiple dysfunctional molecular changes that result in EPVS and SVD. Further, Figure 8 illustrates that once multiple injurious stimuli (including peripheral nervous system cytokines and chemokines - ***pns***CC) have activated NVU BECs, they undergo ***cns***BEC*act/dys* with the synthesis and secretion of dysfunctional molecular proinflammatory CNS-derived cytokines/chemokines (***cns***C/C), such as IL-1β, IL-8, IL-6, TNF-α (cytokines), and CCL1, CCL2 (MCP-1), CCL5 (RANTES) (chemokines), as in Figure 8.1. In turn, these ***cns***CC and proinflammatory VCAM-1 ICAM-1, and E SELECTIN can further increase BEC*act/dys*, as in Figure 8.2. Also, the neuroinflammation seen in Figures 8.1. and 8.2. can result in oxidative stress via increased BEC-derived nicotinamide adenine dinucleotide phosphate oxidase (NADPH oxidase - NOX), superoxide, peroxynitrite, and Mt ROS, which are capable of activating MMP-2, -9, as in Figure 8.3. The

molecular dysfunction created due to BEC*act/dys* includes decreased bioavailable NO and increased endothelin-1 (ET-1) with a proconstrictive NVU that is associated with NVU and eNOS uncoupling with decreased cerebral blood flow and hypoxia, which may increase the risk of neurodegeneration, as in Figure 8.4.

It is essential to provide some of the molecular perspectives in the development of EPVS that lead to the development of SVD. In regards to 1) *NVU BBB BECact/dys and BEC dysfunction*, some important molecular perspectives include oxidative stress, inflammation, and specifically neuroinflammation with dysregulation of NO signaling pathways with decreased NO bioavailability; 2) *NVU BBB disruption and increased permeability* with some important molecular perspectives, which include activation of ***pns***CC and ***cns***CC with subsequent increased oxidative stress and matrix MMPs -2, - 9 with dysregulation, malpositioning, and/or degradation of TJ/AJs; 3) *Vascular remodeling (arteriolar and venular) fibrosis*, which includes molecular mechanisms consisting of CNS renin-angiotensin-aldosterone system (RAAS) activation with elevation of endothelin-1 and TGF-beta that promote VSMC proliferation and hypertrophy, extracellular matrix (ECM) deposition and expansion with BEC basement membrane remodeling and thickening with eventual microvessel narrowing and fibrosis, in addition to venous collagenosis; 4) *CAA and deposition of amyloid-beta (Aβ) protein* in the outer vascular wall and interstitium of small cerebral vessels, in which the molecular mechanisms include dysregulation of Aβ accumulation with disruption of vascular integrity, which promotes inflammation and impairs vascular function and molecular mechanisms including dysregulation of increased Aβ production and decreased clearance, as well as activation of inflammatory pathways by Aβ toxic oligomers; 5) *Molecular mechanisms involved* in neuroinflammation include reactive microglia and astrocytes, release of pro-inflammatory ***cns***CC, and recruitment of immune cells into the brain via the PVU and EPVS (Fig. 11).

Additionally, this section relates to the important association between structure and function, and while Linus C. Pauling (one of the strongest promoters of structure as it relates to molecular function/dysfunction) supported the concept that structural abnormalities of proteins resulted in molecular dysfunction, which occurs in multiple neurological clinical diseases (Bland, 2018, 2019). For example, when Pauling was asked what was the most important paper he had written, he pointed to the paper entitled: "The Nature of the Chemical Bond" (Pauling, 1931). Further, he commented that this paper was important because it developed a pathway for a new concept of the time, which related to the association between molecular structures and their functions (Bland, 2018, 2019). The importance of the structure/function concept may be viewed differently by histologists, pathologists, and molecular biologists. However, the author feels that they are equally important and it is difficult in any research situation to say that one is more important than the other, as all appear to be equally important and inseparable as regards their importance in the development of clinical neurologic diseases.

The venular side of the cerebral circulation and its role in the evolution of EPVS and SVD

The low-pressure, low-velocity, and large-volume venous circulation of the brain plays critical roles in the maintenance of brain homeostasis. However, despite its physiologically important role in homeostasis, the role of age-related alterations of the brain's venous circulation in the pathogenesis of vascular cognitive impairment and dementia is much less understood and studied compared with the arterial circulation.

While the arterial side of cerebral circulation received the greatest attention in regard to the development and evolution of EPVS and SVD in the existing literature, the venular side is becoming more and more important and interesting with the evolution of the GS concerning waste removal by the necessary and essential intact venous PVS for waste removal from the ISS containing the ISF excess water and metabolic waste (Verkman et al., 2011; Jessen et al., 2015; Benveniste and Nedergaard, 2022; Ang et al., 2024). When PVS become dilated, they are known to be EPVS (1-3 mm on MRI) and are associated with impaired removal of metabolic waste due to stalling and sluggish flow and are known to become associated with and contribute to EPVS and SVD (Mathiisen et al., 2010; Verkman et al., 2011; Jessen et al., 2015; Mestre et al., 2017; Benveniste and Nedergaard, 2022; Ang et al., 2024).

ECs lining the brain blood vessels have long been under-appreciated as players in brain diseases: they not only control blood flow and blood-brain barrier function, but also, their cellular interactions influence surrounding brain tissue, such as NVU coupling and cerebral blood flow, and they are also are important for signaling their surrounding cells to result in a response to injury wound-healing mechanisms that result in remodeling, leading not only to EPVS but SVD also. SVD is a common form of VaD and often accompanies neurodegenerative diseases, where BEC*act/dys* may lead to surrounding white matter changes, an early feature of SVD. EPVS are associated with aging, lacunar stroke, and WMH lesions and should, therefore, be considered as another magnetic resonance imaging marker of SVD (Hainsworth et al., 2015). BEC*act/dys* and its brain injury response to vicious cycles of injury wound healing associates with and contributes to the development of EPVS, SVD, and impaired GS neurotoxic waste and fluid clearance (Nezu et al., 2015; Shaaban, 2017; Brown et al., 2018; Fulop et al., 2019; Hayden, 2020a,b, 2023a,b; Lahna et al., 2022). While the postcapillary venular system has not been studied nearly as extensively as the arterial system in cerebrovascular and neurodegenerative disease, it nevertheless plays a huge role as regards the PVU,

which contains both the PVS dilates to become EPVS, which are located immediately adjacent to the true capillaries within the postcapillary PVU (Diez-Sainz et al., 2022; Shulyatnikova and Hayden, 2023).

Venular collagenosis and increased tortuosity are both known to occur and play an important role in the development of SVD, in that they could lead to reduced cerebral blood flow (CBF) and result in SVD (Barisano et al., 2021; Garcia et al., 2023). Additionally, collagenosis and tortuosity singularly or in combination may lead to reduced CBF and increased upstream resistance to exacerbate arterial pathology. Also, inflammatory changes in postcapillary venules, venules, and veins could contribute to additional tortuous venous collagenosis and thickened basal lamina in aging, hypertension, LOAD, and SVD. Notably, this tortuosity has been reported, upon postmortem examination, to be in very close proximity to WMH (Brown et al., 2002; Keith et al., 2017).

Concurrently, with the development of venular collagenosis and tortuosity, inflammatory cascades would be capable of further damaging the venous vasculature, reducing CBF, and even compromising the BBB to allow increased permeability to the neuropil. These phenomena become apparent as remodeled morphological changes, such as tortuosity, collagenosis, and thicker basal lamina occur. Such changes have been seen *in vivo* in LOAD and postmortem studies, and they

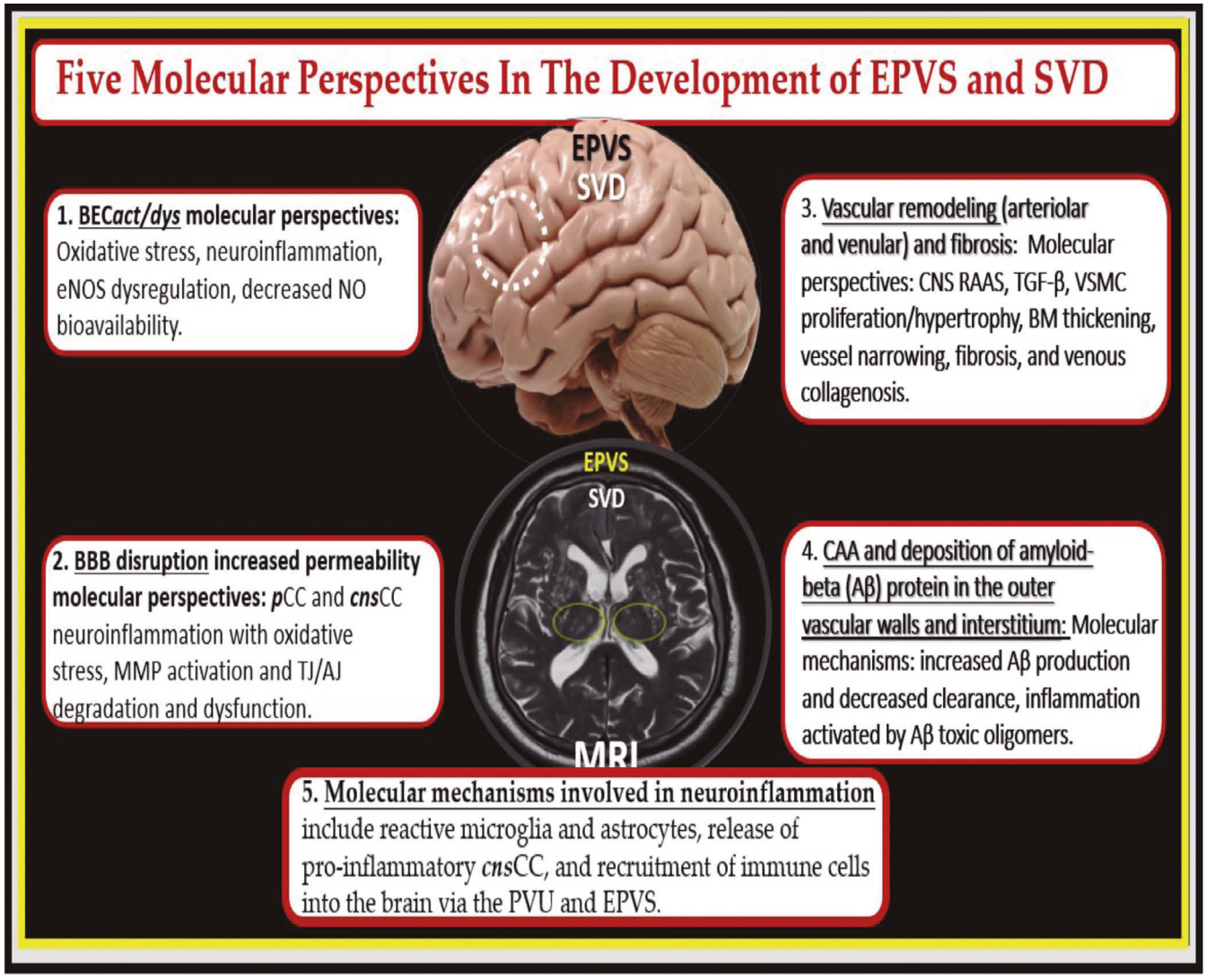

Fig. 11. Five molecular perspectives in the development of enlarged perivascular spaces (EPVS) and cerebral small vessel disease (SVD). The five molecular perspectives (1 - 5) are explained in this figure. BM, basement membrane; CAA, cerebral amyloid angiopathy; CNS, central nervous system; ***cns***CC, central nervous system cytokines/chemokines; dashed circle, location of ultrastructure studies; eNOS, endothelial nitric oxide synthase; MMP, matrix metalloproteinase; MRI, magnetic resonance images; NO, nitric oxide; ***pns***CC, peripheral nervous system cytokines/chemokines; RAAS, renin-angiotensin-aldosterone system; TGF-β, transforming growth factor beta; TJ/AJ, tight and adherence junctions; VSMC, vascular smooth muscle cell.

appear more commonly with HTN, advancing age, and frequently in close proximity to regions with WMH. With aging and HTN, obesity, MetS, and T2DM cerebral arteries and arterioles are known to undergo arterial vascular stiffening (Rhea et al., 2022).

Fulop et al. recently reviewed the low-pressure, low-velocity, and large-volume venous circulation and the critical role it plays in maintaining homeostasis in the brain (Fulop et al., 2019). They discussed that venous collagenosis and increased tortuosity were increased in brains that manifested leukoaraiosis and WMH, and suggested that the venous system was a contributor to the development of WMH of SVD, which associates with aging and neurodegenerative diseases, as occurs in LOAD, among others (Brown et al., 2002; Kilic and Akakin, 2008; Makedonov and Black, 2013; Hartmann et al., 2017; Keith et al., 2017). They also pointed out that intracerebral veins possessed no valves, and that their walls are extremely thin and vulnerable due to a paucity of a well-developed vascular smooth muscle layer (Kilic et al., 2008; Li et al. 2023). Also, aging is known to alter the structure of cerebral capillaries, promoting structural abnormalities of the basement membrane, increasing perivascular collagen deposits, and leading to basement membrane thickening with collagenosis and increased tortuosity in venules and veins (Duvernoy et al., 1981; Kilic et al., 2008; Hartman et al., 2017, 2018). In general, SVD and WMHs are hypoperfused compared with normal white matter, which strongly suggests that ischemia may be playing an important role in their pathogenesis (Duvernoy et al., 1981; Kilic and Akakin, 2008; Zedde et al., 2023). Interestingly, Fulop et al. made a strong case for the potential contribution of elevated venous pressure to increase the incidence of WMH in elderly patients with congestive heart failure (Fulop et al., 2019); additionally, Li et al. demonstrated that congestive heart failure in the elderly is associated with BEC*act/dys*, which may contribute to the decreased CBF and perfusion as well as the development of WMH and SVD (Kilic and Akakin, 2008; Li et al., 2023; Zedde et al., 2023).

Hartmann et al. and Duvernoy et al. previously described how penetrating venules formed vascular units that were surrounded by rings of penetrating arterioles (Duvernoy et al., 1981; Hartman et al., 2018). Exact ratios were not specified in their studies; however, a typical penetrating venule appeared to drain blood supplied by ~ 4–5 penetrating arterioles (Kilic and Akakin, 2008; Keith et al., 2017). Importantly, even though the venular accumulation of beta-amyloid fragments has been identified in both animal models and humans, the role of the venous network and venous dysfunction induced by the venular accumulation in SVD and CAA will need to be further explored and studied (Li et al., 2023). The Hartmann and Duvernoy venular unit hypothesis illustrates the comparison between postcapillary venous and precapillary arteriole single microthrombosis and their respective effects on the relatively vulnerable ischemic neural regions affected by a single penetrating microthrombus (Fig. 12) (Duvernoy et al., 1981; Hartmann et al., 2018).

Venular accumulation of amyloid beta fragments in both human and animal models has been identified (Weller et al., 1998; Revesz et al., 2002; Dorr et al., 2012; Cohen et al., 2013; Michaud et al., 2013; Joo et al., 2017; Hartmann et al., 2018; Klakotskaia et al., 2018; Rotta et al., 2021). According to Rotta (Rotta et al., 2021), the role of the venous network and venous

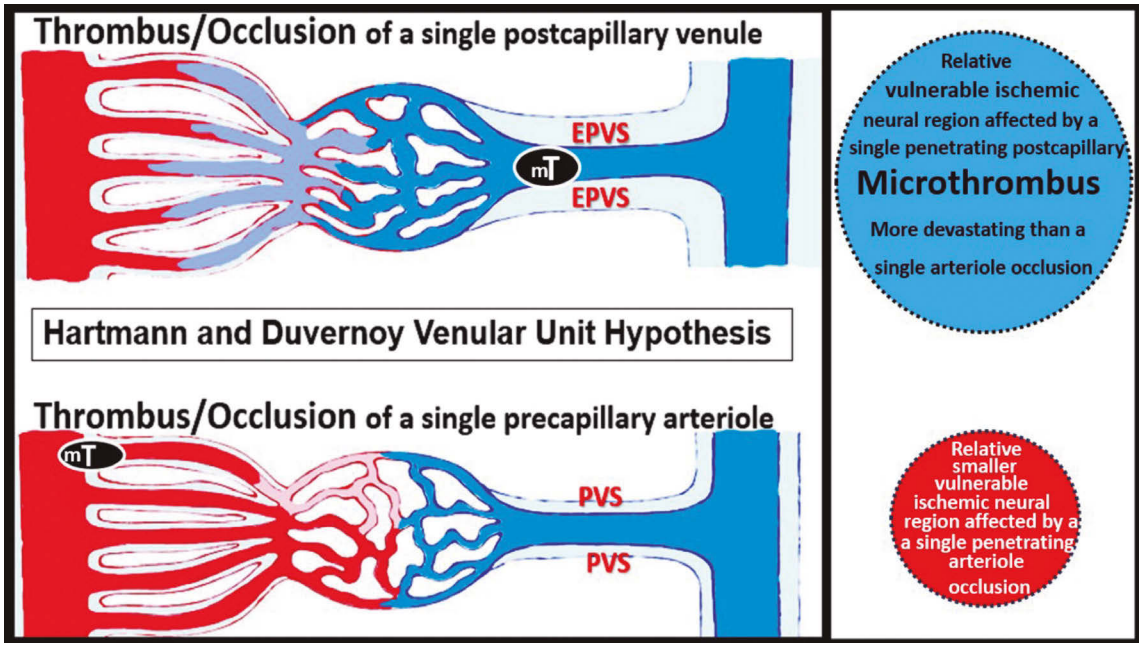

Fig. 12. Single microthrombus in venular system verses arterial system. Note that the single penetrating postcapillary venular microthrombus (mT) may have a more devasting effect to the neural regional blood flow (top panel) as compared to the single penetrating arteriole mT (bottom panel) thrombotic occlusion. EPVS, enlarged perivascular spaces; PVS, perivascular spaces.

dysfunction induced by amyloid accumulation in SVD and CAA will need to be further studied. Notably, as regards the venular system, should the findings of Hartman and Duvernoy (Duvernoy et al., 1981; Hartmann et al., 2018) be verified by others, this would certainly help to explain the importance of the venular neurovasculome (Iadecola et al., 2023) and cerebral venular microinfarcts that play such an important role in the development of cerebral regional ischemia and impaired CBF (Smith et al., 2012). Additionally, recent studies demonstrated that blockage of a single venule in mice increased microinfarcts and vastly impaired cerebrovascular structure and function (Hartmann et al., 2018).

Thus far, a central overarching finding in the venular system associated with EPVS and SVD was a frequent observation of venular collagenosis and venular stiffening by multiple researchers with reduced pulsatility and decreased efflux (Moody et al., 1995; Brown, et al., 2002; Zhou et al., 2015; Keith et al., 2017). As we continue to study and explore the GS and the venular side of the brain's circulation in greater depth, it is felt that there will also be a gain in our knowledge of the venular system's role in the development of EPVS, SVD, and impaired neuronal function with impaired neurocognition, such as VCID, especially with higher-resolution MRI such as 7 Tesla.

Conclusion

SVD may be defined as the sum of all neuropathological processes, which affect small vessels of the brain including small arteries, arterioles, capillaries, venules and small veins (Pantoni et al., 2010). The pathogenesis of SVD is thought to be multifactorial; however, the one mechanism that seems to play a central and key important role is BEC*act/dys* with NVU BBB***dd*** and its associated increased permeability (hyperpermeability), hypoxia, and neuroinflammation as presented in figure 12. Indeed, BEC*act/dys* co-occur (Liao, 2013) and are currently considered to be a key mechanism in the etiopathogenesis of not only EPVS but also SVD, VaD, VCI, and VCID (Poggesi et al., 2016; Wardlaw et al., 2017; Brown et al., 2018; Bia et al., 2022). Further, others have discussed the role of endothelial dysfunction or more specifically BEC*act/dys* (Gimbrone et al., 1997; Wardlaw et al., 2017, 2020; Hainsworth et al., 2015; Nezu et al., 2015; Quick et al., 2021; Garcia et al., 2023), which results in increased morbidity, mortality, and economic burden to our aging population. BEC*act/dys* along with its associated NVU BBB***dd*** are increasingly being recognized as important players in multiple brain diseases and specifically in SVD, since they could be the initial drivers responsible for the pathogenesis and progression of this disease (Cuadrado-Godia et al., 2018).

Herein, the author has sought to describe how to identify BEC*act/dys* at a structural and functional molecular level and how these changes directly affect the BBB in producing BBB***dd*** and the surrounding cells including the ECM, Pc's, pvACef cells, resident perivascular macrophages, glia cells (reactive microglia and astroglia) with neuroinflammation and reactive astrogliosis, and neurons with their effects on the development and progression of EPVS and SVD. Still, much remains to be clarified, both in terms of better understanding of the processes of BEC*act/dys* in addition to what is known such as biomechanical activation, cytokine induced (TNFα), superoxide anion, modified-oxidized low-density lipoprotein cholesterol, hyper-cholesterolemia (Ohara et al.,1993), hyperhomocysteinemia, and systemic and local brain renin-angiotensin-aldosterone system (RAAS) activation with hypertension role in BEC*act/dys* and SVD (De Silva et al., 2021) as depicted in figure 2. Also, it is important to note its impact on the blood vessel itself as well as the surrounding neuronal tissues and their supporting cells. Advances in transcriptomics, particularly at the single cell level, may help resolve some of the questions of heterogeneity in BECs and provide an even more solid BEC dysfunction signature phenotype, which may vary somewhat from the signature obtained from BECs at the regions of BBB disruption. Importantly, the use of TEM has helped to better understand some of the associated aberrant remodeling signature phenotypic changes associated with BEC*act/dys* as presented in figure 5. These phenotypic remodeling changes may aid in a better understanding of novel approaches regarding hypotheses as to how we might better approach the treatment for impaired GS function that associates with EPVS and SVD.

Current treatments for SVD include antiplatelet, antihypertensive (specifically endothelial stabilizing antihypertensive such as angiotensin converting enzyme inhibitors), and statin therapies (especially in regards to their multiple pleiotropic effects of increasing NO, decreasing BEC neuroinflammation via its RANTES receptor blockade to decrease the activation of the proinflammatory NFkappa-B independent of its cholesterol lowering effects, and lifestyle modification such as increased exercise and. decreased salt intake Additionally, there are some medications that are already available that aid to normalize BEC*act/dys* (Tong and Hamel, 2015; Freitas et al., 2017; Rajani et al., 2018; Becker et al., 2019; Quick et al., 2021).

The primary intent of this review was to familiarize the reader with the importance of BEC*act/dys* as it relates to the pathogenesis of not only EPVS but also to the pathogenesis and progression of SVD and impaired GS removal of excess waste and water as it relates to the surrounding brain structures remodeling changes that exists in SVD. Importantly, as we come to use higher resolution MRI such as 7Tesla or higher we may come to better understand a more complete role that the venular side of the circulation may have in regards to how it affects the surrounding tissues in the response to injury wound healing mechanisms in multiple chronic recurrent

brain injuries.

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