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Physiological and pathological roles of caveolins in the central nervous system

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Abstract

Caveolins are a family of transmembrane proteins located in caveolae, small lipid raft invaginations of the plasma membrane. The roles of caveolin-enriched lipid rafts are diverse, and include mechano-protection, lipid homeostasis, metabolism, transport, and cell signaling. Caveolin-1 (Cav-1) and other caveolins were described in endothelial cells and later in other cell types of the central nervous system (CNS), including neurons, astrocytes, oligodendrocytes, microglia, and pericytes. This pancellular presence of caveolins demands a better understanding of their functional roles in each cell type. In this review we describe the various functions of Cav-1 in the cells of normal and pathological brains. Several emerging preclinical findings suggest that Cav-1 could represent a potential therapeutic target in brain disorders.

Caveolins and lipid rafts in the CNS

Caveolin proteins are associated with caveolae ('little caves'), which are enclosed lipid raft (LR) invaginations enriched in sphingolipids and cholesterol. The roles of caveolae include facilitating transport, encapsulation, and scaffolding of diverse regulatory proteins. The association of caveolins with LRs suggests functions related to membrane stabilization and additional roles in caveolae functioning. The functions of caveolins in the peripheral nervous system (PNS) and the CNS have been ascribed to a wide range of physiological activities, such as endocytosis (clathrin-independent), mechano-sensing, lipid control, metabolism, and

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cell signaling. Caveolae have been found in all major cell types of the CNS, including neurons [1] and glial cell types (microglia, astrocytes, and oligodendrocytes) [2,3].

The broad functions and cellular distribution of caveolae and the considerable uncertainties as to their precise roles complicate a single encompassing review. In sum, caveolins and their associated LRs have major cellular and molecular functions, but understanding these functions is evolving and many outstanding questions remain. This review summarizes the known wide-ranging functions of caveolae with a particular emphasis on the brain vasculature, neuronal signaling, and neuroinflammation. We further expand on how loss or dysfunction of caveolins are implicated in CNS pathology, including Alzheimer's disease (AD) and acute injuries (e.g., stroke).

Caveolae and caveolins across CNS cell types

Membrane LRs, which exist as different classes, are complex aggregations of cholesterol, glycolipids, and membrane-spanning proteins that can be loosely characterized as caveolae and planar rafts. Planar rafts are aligned horizontally within the membrane, while the caveolae subset of LRs are small invaginations of the mammalian plasma membrane characterized by the presence of two proteins: cavins and caveolins. Caveolae are present in many cells throughout the body with ubiquitous functions (Figure 1, Boxes 1 and 2) [4–7]. Notably, they are involved in cell signaling via direct binding of molecules to their scaffolding domain, the caveolin scaffolding domain (CSD) (Figure 1C, Box 3).

Caveolins exist as three isoforms in the CNS – caveolin-1 (Cav-1), caveolin-2 (Cav-2) and caveolin-3 (Cav-3) – present in different cell types, including endothelial cells (ECs), neurons, astrocytes, oligodendrocytes, microglia, smooth muscle cells, and pericytes [8]. Cav-1 was first observed in ECs of the CNS [9], but now its diverse expression raises questions about its functional roles within each cell type. *In vivo* and *in vitro* experiments have provided some evidence for cell-specific roles of Cav-1 and confirmed its critical role in physiological processes. Global Cav-1 knock-out (KO) mice exhibit a neurodegenerative phenotype, and recent work points toward a potential protective role of Cav-1 in aging and CNS injuries [10]. By contrast, the absence of Cav-1 in neurons of *Caenorhabditis elegans* prolonged their lifespan [11]. Thus, and as discussed in more detail in the following sections, conflicting findings for Cav-1 and other caveolins in normal and pathological brains complicate current insights into the precise functions of caveolins.

Caveolins in brain ECs regulate cerebrovascular properties

In brain ECs, Cav-1 and Cav-2 are predominantly localized in caveolin-enriched LRs [9]. Cav-1 is involved in the regulation of vascular tone in large blood vessels (Figure 2A), integrity of the blood–brain barrier (BBB) under physiological conditions (Figure 2B), and cellular pathophysiology (Figure 3A,B). In several brain disorders, Cav-1 has divergent effects that can be beneficial or detrimental, depending on the disorder and time of the pathophysiology (Figure 3A,B).

Cav-1 regulation of vascular tone is mediated by endothelial nitric oxide synthase (eNOS)

Cav-1 is involved in the regulation of eNOS activity via the CSD binding motif, and NO produced by eNOS is a potent regulator of vascular tone in peripheral and brain blood vessels [12–14]. The CSD domain (Figure 1C, Box 3) of Cav-1 associates with eNOS and maintains it in an inactive conformation by preventing its phosphorylation, leading to downregulation of eNOS activity and decreased NO production in ECs from mouse lungs [15]. Direct binding of eNOS by Cav-1 can regulate temporal and spatial NO production in ECs [13]. In human pulmonary arteries a reciprocal regulatory relationship has been proposed where (i) Cav-1 inhibits eNOS and mutually eNOS-derived NO production, leading to Cav-1 phosphorylation, thereby increasing its affinity for, and inhibition, of eNOS, and (ii) sustained NO levels lead to ubiquitination and degradation of Cav-1 [16]. Caution is urged in extrapolating these periphery-related findings to the CNS, as inhibitory roles of Cav-1 on eNOS are likely tissue-, cell- and time-dependent.

eNOS has a key role in maintaining cortical baseline cerebral blood flow (CBF) [17] and in neurovascular coupling in response to metabolic demand [18]. Neurovascular coupling mechanisms link metabolic demand to vascular delivery of oxygen and nutrients. In brain arteriole ECs, Cav-1 regulates neurovascular coupling independently of eNOS activation [19] (Figure 2B). Arterioles have more caveolae than capillaries [19] due to the presence of the major facilitator superfamily domain-containing protein 2 (Mfsd2a) in capillaries. Mfsd2a is a lipid transporter at the luminal plasma membrane of brain ECs that delivers the essential omega-3 fatty acid docosahexaenoic acid (DHA) to the brain [20]. The expression of Mfsd2a in brain capillaries inhibits the formation of caveolae and transcytosis [21]. Mfsd2a acts as a lipid flippase transporting DHA to the inner plasma membrane leaflet, which results in displacement of cholesterol and Cav-1, thereby inhibiting caveolae formation [22]. The role of caveolae in neurovascular coupling is not fully clear, but caveolae cluster important ion channels and transporters in ECs for signaling in smooth muscle cells [19]. Cav-1 and Cav-3 also appear to form tubes after fusion of multiple lipid rafts in human and mouse cardiac and skeletal muscle cells [23,24] and in human EC cultures [25]. Caveolar tube formation may regulate vesicular transport, angiogenesis, and connectivity between plasma membranes, and might be a mechanism to increase the brain EC surface area during vasodilation, modulating neurovascular coupling.

Roles of caveolins in BBB integrity

Caveolin-enriched LRs constitute as much as 30% of the total surface of ECs in peripheral capillaries [26], but appear to be rare in brain capillary ECs [19,22]. In peripheral ECs, caveolae/caveolin-enriched LRs may mediate mechano-protection and control lipid homeostasis [27], and may contribute to transcellular transport of macromolecules across ECs [28–30]. By contrast with peripheral ECs, caveolar transcytosis is limited in cerebral capillary ECs as part of a functional BBB [28,29]. However, caveolae/caveolin-enriched LRs mediate transport of specific macromolecules – including albumin, lipids, and a growing list of pathogens (viruses, bacteria and associated toxins, fungi, prions) – in brain ECs [31,32].

Cav-1 is central to maintenance of structural BBB integrity by its direct interaction with tight junction (TJ) proteins such as claudins, occludins, and junctional adhesion molecules

[33,34]. The association of Cav-1 and zona-occludens1 with preformed occludin and claudin-5 homodimers facilitated the transport and assembly of TJs in rat brain ECs [34].

Permeability glycoprotein (P-gp) is an important efflux transporter present in brain ECs and is a significant drug efflux pump in the BBB. Cav-1 directly interacts with and modulates P-gp transport activity in brain ECs [35]. Its activity is enhanced by tyrosine-14 phosphorylation of Cav-1 [36], directing P-gp trafficking from the nucleus to the plasma membrane [37]. In caveolin-enriched LRs, P-gp also interacts with Cav-2 to form large molecular complexes, whereas loss of Cav-1 from caveolin-enriched LRs reduces P-gp transport activity [35]. P-gp plays an important role in β-amyloid and tau clearance [38,39] suggesting a potential role for Cav-1 in the BBB in health, aging, and neurological disease. Downregulation of Cav-1 after repeated mild traumatic brain injury (TBI) resulted in decreased tau removal and accumulation within the injured tissue, favoring chronic traumatic encephalopathy in a transgenic mouse model containing human tau [40]. Cav-2 increases were reported in brain ECs of aged mice [41]. Since Cav-1 and Cav-2 can form homodimers, it is conceivable that Cav-2 could also contribute to β-amyloid and tau clearance, but additional studies are required.

Brain endothelial Cav-1 in BBB hyper-permeability and cerebrovascular repair

The roles of Cav-1 in BBB properties after brain injury are divergent and depend on the pathology and time of assessment, with detrimental or beneficial effects (Figure 3A,B). Brain ischemia studies are illustrative; some studies showed increased Cav-1 at 24 h after brain ischemia, but decreased claudin-5 and VE-cadherin coinciding with BBB dysfunction in three mouse models [42]. By contrast, other ischemia studies showed protective roles on cerebral blood vessels for Cav-1. After brain injuries in rodents, increased Cav-1 expression has been described [43–45] without influencing claudin-5 expression [8]. Global Cav-1 KO mice recovered poorly compared with wild-type (WT) mice after brain injuries (i.e., ischemia, TBI) [43,44,46], while Cav-1 overexpression attenuated brain edema by inhibiting TJ degradation [47]. These somewhat disparate findings appear to depend on the time after brain injury. In hyper-acute (hours) ischemic reperfusion, cortical spreading depolarization waves, and the consequences of potassium neuronal depolarization, resulted in increased caveolae formation, Cav-1 expression, and a higher transcytosis rate, which contributed to early BBB hyperpermeability in the mouse cortex [48–50]. At later times, Cav-1 can be involved in BBB integrity and vascular repair. In fact, Cav-1-maintained BBB function was linked to changes in NO and metalloproteinase (MMP) activation (Figure 3B) [51,52]. Cav-1 expression in brain ECs was downregulated temporally after ischemia reperfusion, while MMP9 and MMP2 activation was increased. In global Cav-1 KO mice, absence of Cav-1 inhibited MMP-activity, protected TJ-proteins and maintained BBB integrity [51,52] (Figure 3B). Cav-1 also activated the vascular endothelial growth factor (VEGF) pathway leading to angiogenesis, promoting tissue repair in rat brain, as seen with treadmill exercise after ischemia [53,54]. Similarly, EC Cav-1 overexpression restored oligodendrogenesis after chronic brain ischemia in mice, where white matter damage was associated with decreased expression of EC Cav-1 and a gradual impairment of oligodendrogenesis [25]. Collectively, evidence points towards a protective role for Cav-1 in cerebral pathologies by blunting BBB breakdown and facilitating blood vessel remodeling (Figure 3B).

In rodent models of TBI (closed head injury, cold injury, and controlled cortical impact injury), the mechanical impact elicited an increase in Cav-1 mRNA, protein levels, and phosphorylation within days [8,42,55] which then reduced by 12 months [40,56]. Cav-1 increases can be beneficial or detrimental (Figure 3). Cav-1 contributed to MMP activation, inflammatory processes, and neuronal loss after intracerebral hemorrhage in mice [57] and cerebral small-vessel injury in humans [58]. After TBI, the level of expression of Mfsd2a was decreased while Cav-1 and transcytosis were increased in mouse brain ECs, facilitating entry of albumin [59]. In brain tissue from patients with hypertension, the β-site amyloid precursor protein-cleaving enzyme 1 (BACE1) was elevated in cerebral microvessels [58] alongside increased cleavage of occludin and Cav-1, resulting in enhanced Cav-1-mediated endocytosis, TJ protein degradation, attenuation of eNOS activity, and EC dysfunction [58]. In high-altitude cerebral edema, the upregulation of Cav-1 by hypoxia was associated with loss of the vascular TJ proteins with ensuing increased permeability in the mouse brain [60]. Methyl-β-cyclodextrin (MβCD) treatment disrupted LRs by removing cholesterol, improving BBB function and decreasing claudin-5 internalization by modulating Cav-1 function [60].

In summary, Cav-1 and caveolae are increased transiently hours to days after brain injury leading to BBB permeability [8,49,50], but at later times are decreased, improving BBB integrity. One cannot exclude the possibility that increases in Cav-1 and caveolae are an innate protective mechanism against mechanical stress on blood vessels. Elevations of caveolin may impart a trophic role (via the VEGF pathway) in vascular repair and neo-angiogenesis [8,43,44,61].

Neuronal Cav-1 participates in cell signaling

Cav-1 signaling in synaptic plasticity and neuronal survival

Cav-1 protein is expressed at low levels in neurons [1,62], and to our knowledge there is no evidence for caveolae in neurons [63]. Indeed, absence of morphological caveolae in neurons led to the assumption that neurons did not express caveolins [64]. However, Cav-1 and Cav-3 were later reported in axonal and dendritic terminals of mouse hippocampal neurons [65]. In hippocampal neuronal cultures, punctate labeling patterns were speculated to be neuronal caveolae [1], but without electron microscopy (EM) imaging, this finding remains debatable [64]. LRs were observed in the neuronal spines at postsynaptic densities in the mouse [66]. Further, neuronal Cav-1 was found to scaffold glutamate receptors to LRs, suggesting potential involvement in synaptic plasticity, neuronal development, and excitotoxicity [67].

Hypothetical neuronal Cav-1 functions may include: (i) regulating neurogenesis, (ii) compartmentalizing neuronal signaling molecules via Cav-1 interactions with cytoskeletal components, and (iii) contributing to synaptic maintenance and stabilization [68–70] (Figure 2). Neuronal Cav-1 regulation of neurotrophin signaling has been described *in vitro* and *in vivo* [71,72]. In immature cortical neurons, Cav-1 is expressed predominantly at neuronal maturation sites, and short-hairpin RNA (shRNA) targeting and ablating Cav-1 disrupted immature neurite pruning, leading to process elongation and subsequent impairment of neuronal migration in the mouse [73]. Cav-1 overexpression in neurons enhanced LR

formation, receptor-mediated cAMP production, Trkb receptor expression and signaling, as well as increased dendritic arborization [71,72]. Experiments in human neurons derived from induced pluripotent stem cells (iPSCs) demonstrated that Cav-1 phosphorylation is critical for axonal growth [74] (Figure 2). Cav-3 was shown to play a role in estrogen receptor trafficking in the arcuate nucleus in female rats by modifying metabotropic glutamate receptor signaling [75]. How neuronal-Cav-1 regulates molecular signaling events is still under investigation.

Protective roles of neuronal Cav-1 signaling in neurodegenerative diseases

In the mouse brain, Cav-1 expression was found to increase with age [76], while in peripheral organs and systems, Cav-1 regulated cellular senescence and contributed to aging phenotypes in human and mouse skin cells [77]. Cav-1 RNAi-mediated knockdown in *C. elegans* led to an extended lifespan and mitigated toxic protein aggregation by modulating expression of aging genes [11]. The life extension in nematodes is at odds with experiments of global Cav-1 KO mice (3–6 months of age) that showed signs of premature neuronal aging and degeneration (i.e., increased β-amyloid, p-Tau, astrogliosis) compared with WT mice [10]. Decreased Cav-1 was associated with abnormal p-Tau after β-amyloid application on rat neuronal cultures [78,79]. Cav-1 and associated signaling complexes (neurotransmitter receptors) were decreased in postmortem brains from chronic traumatic encephalopathy (CTE) patients diagnosed by abnormal p-Tau [78,79]. Concordantly, neuron-targeted overexpression of Cav-1 in the hippocampi of adult and aged mice resulted in improved learning and memory [72]. Neuron-targeted Cav-1 overexpression in the PSAPP [presenilin PS and amyloid precursor protein (APP) overexpression] transgenic mouse model of AD preserved TrkB signaling protein localization to LRs, conserved neuronal and synaptic function, maintained mitochondrial function, and mitigated cognitive dysfunction [80,81]. Application of synthetic Cav (SynCav) to spinal cord motor neurons preserved motor neurons and neuromuscular junctions, delayed disease onset, and extended survival in the $hSOD1^{G93A}$ mouse model of amyotrophic lateral sclerosis (ALS) [82]. Similarly, neuronal Cav-1 depletion led to development of pathological AD features in type-II diabetic mice, but restoration rescued recognition memory and ameliorated AD pathology [83]. Overall, the literature supports the notion of a protective role of neuronal-Cav-1 in aging and senescence [84,85] (Figure 3).

Nevertheless, there are also findings suggesting that decreased Cav-1 expression is beneficial for neuronal survival. In neuronal cultures, Cav-1 small interfering RNA (siRNA) knockdown limited neuronal cell death after hemin application [57]. A transgenic mouse model with 150 CAG repeats ‘knocked in’ to the endogenous allele of the Huntingtin gene was used as model of Huntington’s disease. In these mice, absence of Cav-1 expression contributed to delayed onset of Huntington’s disease, and motoneuron loss via caveolar-related cholesterol trafficking in neurons [86]. Cav-1 overexpression in neuronal cell cultures from A53T α-synuclein overexpressing transgenic mice facilitated uptake of misfolded synuclein proteins, propagation of α-synuclein from cell to cell, and formation of Lewy-body-like inclusions, which in Parkinson’s and Huntington’s diseases contribute to the development of pathology [76]. In the A53T human α-synuclein transgenic mouse model of Parkinson’s disease, increased Cav-1 expression was mainly observed in neurons

[76]. More research is needed to better clarify the potential neuroprotective roles of Cav-1 phosphorylation and its relevance for neuronal survival in neurodegenerative conditions.

Roles of Cav-1 have also been explored in animal models of TBI and schizophrenia. In a mouse model of TBI, neuron-specific Cav-1 overexpression improved motor function and preserved memory via the involvement of caveolae LRs and associated roles in cell signaling and protein trafficking [66]. The disrupted in schizophrenia (DISC1) gene in rodent neurons and in human neurons derived from iPSCs from individuals with schizophrenia is regulated by neuronal Cav-1 [87]. In summary, these results suggest a pivotal role for caveolin-enriched LRs and caveolins in neurodegenerative processes (Figure 3).

Role of caveolins in neuroinflammation

Astrocyte caveolins involved in cell signaling and morphological remodeling

Cav-1 and Cav-2 are described primarily in astrocyte cultures, whilst astrocytes *in vivo* preferentially express Cav-3 [2,88,89]. In astrocytes *in vivo*, Cav-1 and Cav-3 were observed in perilesional brain tissue after ischemic stroke [44] and juvenile TBI in rodent models [8]. Despite limited ultrastructural assessment, current findings support the existence of caveolin-related membrane LR microdomains in glial cells. One might predict that, in astrocytes, Cav-1 and Cav-2 form stable hetero-oligomer complexes where Cav-1 assists in the subcellular transport of Cav-2, and Cav-2 modulates Cav-1-mediated caveolae assembly (for review see [3]). Astrocytes contain morphologically identifiable caveolae that likely participate in transcellular transport functions [9]. Little information is available on the role of caveolins in astrocytes under physiological conditions, but we speculate a mechanical role for caveolae in astrocytes related to volume regulation (Figure 2). The transient receptor potential vanilloid 4 (TRPV4) channel was proposed to act as a volume sensor rather than as an osmo-sensor [90], and its channel opening is coupled with aquaporin 4 (AQP4) present on astrocyte end-feet. AQP4 expression was decreased in astrocytes of Cav-1 KO mice after brain ischemia [91], suggesting a role for Cav-1 in volume regulation. Additional research is needed on the physiological roles of caveolins in astrocytes (Figure 2).

In pathological conditions, the functions of astrocytic caveolins are somewhat clearer. When astrocytes respond to injury and alter their morphology [92], Cav-1 contributes to caveolae flattening, leading to a reduction in surface tension, and acts to buffer plasma membrane rupture [6]. Recent work found that Cav-1 can provide mechano-protection to cells via caveolae in the SH-Sy5y cell line differentiated into neurons [93]. Cav-1 stabilized non-caveolar invaginations named dolines, and buffered plasma membrane tension in the presence of mechanical stimulation, such as osmotic stress [93]. Caveolar flattening also led to the dissociation of Cavin-1 from Cav-1, thereby increasing the amount of freely diffusing Cav-1 at the inner face of the plasma membrane [94,95] (Figure 3). One could speculate that membrane reservoirs of caveolae and dolines contribute to enabling astrocytes to change shape and volume during brain activity and in response to injury.

In support of a beneficial role of Cav-1, downregulation of Cav-1 using siRNA resulted in increased damage in primary astrocyte cultures exposed to oxygen/glucose deprivation (OGD) [96], while treatment with Cav-AP, a peptide containing the CSD coupled to the

antennapedia (AP) internalization sequence, attenuated the OGD-induced cell damage [96]. After ischemia in Cav-1 KO mice, the astrocytes in the peri-lesion had a decreased length of processes and branch number compared with WT mice [44], suggesting a distinct role for Cav-1 in astrogliosis after acute brain injury. We hypothesize that Cav-1 facilitates the extension of astrocyte processes by mobilizing LRs to elongate the plasma membrane. The astrocyte phenotypic changes in the absence of Cav-1 [44] contributed to a worse outcome in several brain injury models, including spinal cord injury, TBI, and stroke [92]. After stroke, Cav-1 endocytosis facilitated the entry of matrilin-3, an astrocytic extracellular protein shown to decrease neuroinflammation and neuroprotection in rodent transient middle cerebral artery occlusion models [97].

Expression of Cav-1 and Cav-3 was increased in astrocytes after juvenile TBI compared with astrocytes from control animals, and the expression co-localized with phospho-eNOS [8]. In astrocyte cultures, Cav-1 and eNOS were proposed to interact [98]. Further, increased Cav-1 expression was associated with a decrease in expression of the glutamate transporter GLT1 [99], suggesting divergent signaling likely leading to altered glutamate uptake and excitotoxicity. In AD patients and in AD transgenic mice overexpressing APP, increased expression of Cav-3 was observed in astrocytes encircling senile plaques [100]. Cav-3 interacts physically with APP in caveolin-enriched LRs [100]. Similarly, increased astrocytic Cav-3 expression was concomitant with increased Connexin 43 (Cx43) and swollen perivascular astrocyte end-feet after envenomization with *Phoneutria nigriventer* spider venom, a model of neuroinflammation [101]. Primary astrocyte culture experiments further confirmed a role for Cav-3 in Cx43-positive gap-junction communication involving activation of iNOS [102]. In sum, these results highlight roles for Cav-1 and Cav-3 in multiple astrocyte cell signaling cascades.

Cav-1 also has roles in various signaling pathways controlling survival, proliferation, migration, and invasiveness of glioblastoma cells [5]. The roles of Cav-1 in glioblastoma are paradoxical, because Cav-1 can act as both a tumor suppressor and an oncogene [5] in the development of glioblastoma. Cav-1 may facilitate caveolae formation for transport, epidermal growth factor receptor (EGFR) signaling, urokinase-type plasminogen activator (uPAR) activation, and integrin activation [5]. The increased expression of Cav-1 in glioblastoma cells mediated inhibition of the P-gp transporter, an efflux pump that may participate in increasing sensitivity to chemotherapeutic agents (for review see [5]). High Cav-1 expression in glioblastoma tissue samples was an independent negative biomarker of patient survival [103]. In other CNS tumor types, such as meningiomas, increased Cav-1 expression was associated with the biological aggressiveness of the tumor, resulting in a worse prognosis [104]. These disparate results of Cav-1 in cell signaling in brain tumoral cells highlight the complexity of Cav-1 functioning in promoting or inhibiting cell cycle progression in dividing cells.

Microglial caveolins contribute to neuroinflammation

Cav-1 and Cav-3 have been identified in microglia. Cav-1 proteins were reported in low quantities in the plasmalemma and cytoplasmic vesicles of inactive microglia, while active (amoeboid-shaped) microglia showed increased Cav-1 expression [105]. It has been

suggested that the scaffolding ability of caveolins in microglia can regulate the cytoskeletal polymerization state of microtubules and actin. Cav-1 was shown as a key component of microglial activation via internalization of the plasma membrane sodium-vitamin C cotransporter 2 (SVCT2) [106]. The phagocytic activity of microglia in hypoxia involved Cav-1, with BBB disruption in a mouse model of high-altitude cerebral edema [107]. Cytokine and chemokine production and lesion volume were enhanced in Cav-1-deficient mice after TBI [46], suggesting that a lack of Cav-1 enhances neuroinflammation. Further evidence of caveolin importance in microglial functioning was that global Cav-1 and Cav-3 KO mice had increased levels of the microglial protein IBA1 [46]. Overall, caveolins appear to be a critical component of microglia and regulation of upstream/downstream regulatory elements in inflammation. Emerging evidence for microglial regulation of brain vasculature is intriguing and may suggest cross-talk between caveolin-containing cell types (brain ECs, microglia, astrocytes) in regulating blood flow and response to inflammation [108].

Caveolins in cell signaling and myelination processes in oligodendrocytes

In the CNS, Cav-1 has been identified in Schwann cells [109], in the myelin sheaths of oligodendrocytes [110], and in oligodendrocyte progenitors [111] in rodents. Oligodendrocyte Cav-1 is clustered in LR domains alongside cell signaling proteins (e.g., estrogen receptor) [110], nerve growth factor (NGF) [112], and oligodendrocyte myelin glycoprotein, a ligand of neuronal Nogo receptors [113]. Oligodendrocyte progenitors contain Cav-1 in LR-like domains that contribute to calcium signaling microdomains by clustering proteins involved in calcium signaling [111]. Absence of Cav-1 in oligodendrocytes reduced process formation after NGF application, limiting cell signaling and cellular growth of oligodendrocytes [114]. Pharmacological treatment of oligodendrocyte cultures with Cav-AP suppressed NGF-induced phosphorylation of TrkA as well as the activation of p42/44 mitogen-activated protein kinase (MAPK) signaling cascades known to be involved in NGF-stimulated oligodendrocyte-process growth [115].

During myelination, Cav-1 expression was increased in Schwann cells and oligodendrocytes [116]. A conserved purine complex upstream of the Cav-1 gene locus was identified as a genomic susceptibility locus in the pathophysiology of multiple sclerosis (MS) [116]. In a mouse model of MS (experimental autoimmune encephalomyelitis, EAE), the presence of Cav-1 exacerbated disease pathogenesis by promoting selective trafficking via caveolae of Th1-positive lymphocytes across the BBB, allowing cell infiltration without tight junction remodeling [117]. Conversely, in this model application of CSD-peptide reduced inflammatory cell infiltration, contributing to improved BBB function. Blockade of VEGF-A signaling using the CSD peptide targeting eNOS also protected against neurological deficits in the EAE MS mouse model [118]. In summary, caveolins play a significant role in oligodendrocyte maturation and in diseases affecting white matter, but our current understanding is incomplete and further research is warranted.

Concluding remarks and future perspectives

In this review we have described the expression of caveolins in the major cell types of the CNS and their diverse physiological roles. In glial, neuronal, and endothelial cell

populations, caveolins play roles in a broad range of physiological processes (Figure 2). Despite the growing understanding of caveolins and their associated LRs, our knowledge of the precise physiological functions of caveolins, in particular Cav-1, is incomplete. Controversies and open questions (see Outstanding questions) remain and will need to be investigated further to expand current understanding of how caveolins integrate and modulate a host of physiological processes. One approach is to query caveolins' roles under pathophysiological conditions along temporal continuums after injury (Figure 3).

To facilitate research on caveolins in the CNS and to elucidate their roles, we recommend emphasis on the following research and methodological goals: (i) improving transgenic mouse models targeting caveolin deletions in specific cell types, (ii) developing new and better pharmacological tools to probe caveolin function, as the current armamentarium is limited, and (iii) improving methods (i.e., antibodies) to identify cellular localization. These recommendations could be extended to the therapeutic potential of targeting caveolins. Indeed, therapeutic applications of the CSD peptide report reduced inflammation, atherosclerosis, and dampening tumor angiogenesis. Despite these promising protective roles of Cav-1, direct delivery of the CavAP peptide after brain injury is in the early stages. Similarly, neuron-targeted Cav-1 (i.e., SynCav1) gene therapy may also be a potential therapeutic approach for neuroprotection.

In conclusion, current evidence points to major functional roles of caveolins in health and disease in all brain cells. We believe that fine-tuning caveolin expression has a promising role as a therapeutic strategy to minimize neuropathology, but this awaits further experimental exploration.

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Highlights

Caveolae are small lipid raft domains present in various cells of the central nervous system with roles in the normal and pathophysiological brain.

Caveolae and caveolins participate in cerebral blood perfusion and blood–brain barrier properties.

Caveolae and caveolins play a role in synaptic plasticity, brain aging, and neurodegenerative diseases.

Caveolae and caveolins are found in multiple cell types, including astrocytes, with a potential role in inflammation.

Caveolin represents a future target for drug development.

Box 1.

Overview of caveolae

Omega-shaped structures were described morphologically using electron microscopy in the 1950s in ECs of blood capillaries in skeletal muscle, heart, intestine, and pancreas [119]. Similar structures were later reported in mouse gall bladder epithelial cells, and were named caveolae due to their ‘little cave-like’ appearance [119]. Caveolae have a defined diameter of 60–80 nm and an omega or U-shaped form depending on the electron microscopy technique used for visualization [6]. Caveolae engage in transcytosis, endocytosis, and exocytosis, and compartmentalization of molecules to prevent their action. Caveolae are also a special type of lipid raft (LR). They are raft-like aggregates of cholesterol and sphingolipids in the outer leaflet of the plasma membrane (Figure 1 in main text) and contribute to the heterogeneous distribution of lipids in cell membranes [4,120]. LRs represent a subtype of lipid microdomains and serve as platforms for signaling complexes by congregating multiple signaling components for cellular signal transduction. LRs assemble receptors for communication support between the extracellular and intracellular milieu. The activity of these signaling platforms depends on the interaction between the caveolar organization and the clustering of the LRs, or dynamic rearrangement of the cytoskeleton [121]. Three-dimensional electron microscopy has revealed complex interactions between caveolae and cytoskeletal constituents such as actin filaments and microtubules [94]. The intermediate filaments are involved in the regulation of caveolae formation and elimination, being part of the mechano-sensitive role of caveolae, and modulate interactions between the different cytoskeletal components. An often overlooked role of caveolae is their ability to respond physically to mechanical stress, as caveolae can serve as mechano-sensors and mechanical transducers to facilitate shape changes in response to mechanical stress [94]. Cav-1 can modulate membrane tension and constitute a reserve within membranes that allows expansion in response to mechanical stress, a feature noted in a variety of cell lines and organisms ranging from zebrafish to mice [122].

Box 2.

Caveolins and cavins

Caveolins and cavins are the main proteins involved in caveolae formation [94] (Figure 1B in main text). Cav-1, Cav-2, and Cav-3 are distinct gene products with unique molecular masses in the range 18–24 kDa. Cavins [123] are adapter proteins that oligomerize as trimers in the outer coat complex to remodel the membrane into caveolae [124]. Cavins localized in caveolae play important roles in formation and function of caveolae. The cavin family is composed of four isoforms, all cytosolic proteins, and includes polymerase I and transcript release factor complex (PTRF or Cavin-1), serum deprivation protein response (SDPR or Cavin-2), sdr-related gene product that binds to C-kinase (SRBC or Cavin-3) and muscle-restricted coiled-coil protein (MURC or Cavin-4). These proteins are 31–47 kDa in size and are usually present as large hetero-oligomeric complexes to stabilize caveolae in their interaction with caveolin [94]. Cavin complexes underlie the striated appearance of caveolae observed in electron microscopy (EM) images. In the absence of Cavin-1, there is no caveolae formation [122].

Box 3.

Caveolin 1 and caveolin scaffolding domain: role in cell signaling

There is ample evidence for an extra-caveolar role of caveolins. Cav-1 modulates protein and vesicle trafficking, and angiogenic and inflammatory signaling [125]. Cav-1 is an integral membrane protein divided into four distinct domains (Figure 1C): an N terminal domain, a caveolin-scaffolding domain (CSD) composed of 20 amino acids, a transmembrane domain, and a C terminal domain [126]. The CSD facilitates interaction and organization of signaling molecules to help provide coordinated and efficient signal transduction. A 20-amino-acid peptide derived from the scaffolding domain of Cav-1 (residues 82–101) was first characterized *in vitro* as a potent inhibitor of heterotrimeric G proteins in GTP hydrolysis assays, providing evidence of direct signaling modulation [127]. The protein structure binding to CSD was studied by screening a synthetic peptide library using a CSD fusion protein. Most synthetic CSD-binding peptides had one of the following motives: ΦXΦXXXXΦ, ΦXXXXΦXXΦ, or ΦXΦXXXXΦXXΦ, where Φ is an aromatic residue (Phe, Tyr, or Trp) and X is any amino acid. Tyrosine and serine/threonine kinases have such a motif and are inhibited by the synthetic CSD peptide. Cav-1 can be either an inhibitor or an activator depending on whether it is located in caveolae or not [94]. Cav-1 inhibits eNOS in caveolae but activates eNOS outside of caveolae [94], a dual role which complicates disentanglement of roles of Cav-1, CSD, and caveolae in physiological and pathophysiological conditions. Many of the proteins that interact with or transcriptionally repress, or that Cav-1 inhibits, fall under proliferative, oncogenic, anti-apoptotic, pro-survival and pro-growth categories of molecules.

Outstanding questions

Cav-1 is involved in caveolae formation in the peripheral endothelium and cell signaling (e.g., eNOS regulation). What is/are the function(s) of Cav-1 in brain endothelial cells? The role of Cav-1 may vary depending on the location in the vascular tree (large vessels versus capillaries), and these differences may contribute to the divergence regarding the roles of Cav-1 in the BBB after injury.

Can the potentially beneficial effect of increased Cav-1 and caveolae be explained by facilitated transport through endothelial cells? An increase in Cav-1 and caveolae is observed after brain injury. This increase enhances transcytosis, which might facilitate the removal of cell debris from the brain to the blood stream, as well as promoting the entry of molecules (e.g., cytokines) from the blood to the brain to resolve local dysfunction.

How can more knowledge of the role of Cav-1 in neurons be gained? Presence of Cav-1 in neurons has been suggested to be protective, and its absence appears to accelerate aging, possibly in relation to the synaptic plasticity. *In vivo* studies in specific brain regions and neuronal subtypes over the lifespan would help to better determine the neuronal functions of Cav-1.

How do Cav-1 and caveolae generate morphological changes in astrocytes and influence astrocyte cell signaling? The presence of caveolae in astrocytes may serve as a reservoir of bilipid membrane that can rapidly be mobilized in different physiological conditions: additional bilipid membranes can contribute to changes in morphology with longer or larger processes. Caveolae may act as volume sensors jointly with AQP4 and TRPV4. Cav-1 may also contribute to astrocyte cell signaling under certain physiological and pathophysiological conditions.

Do the roles of Cav-1 differ among the different cell types found in the neurovascular unit? The use of viral vector constructs *in vitro* and *in vivo* might address the specific cellular roles of Cav-1.

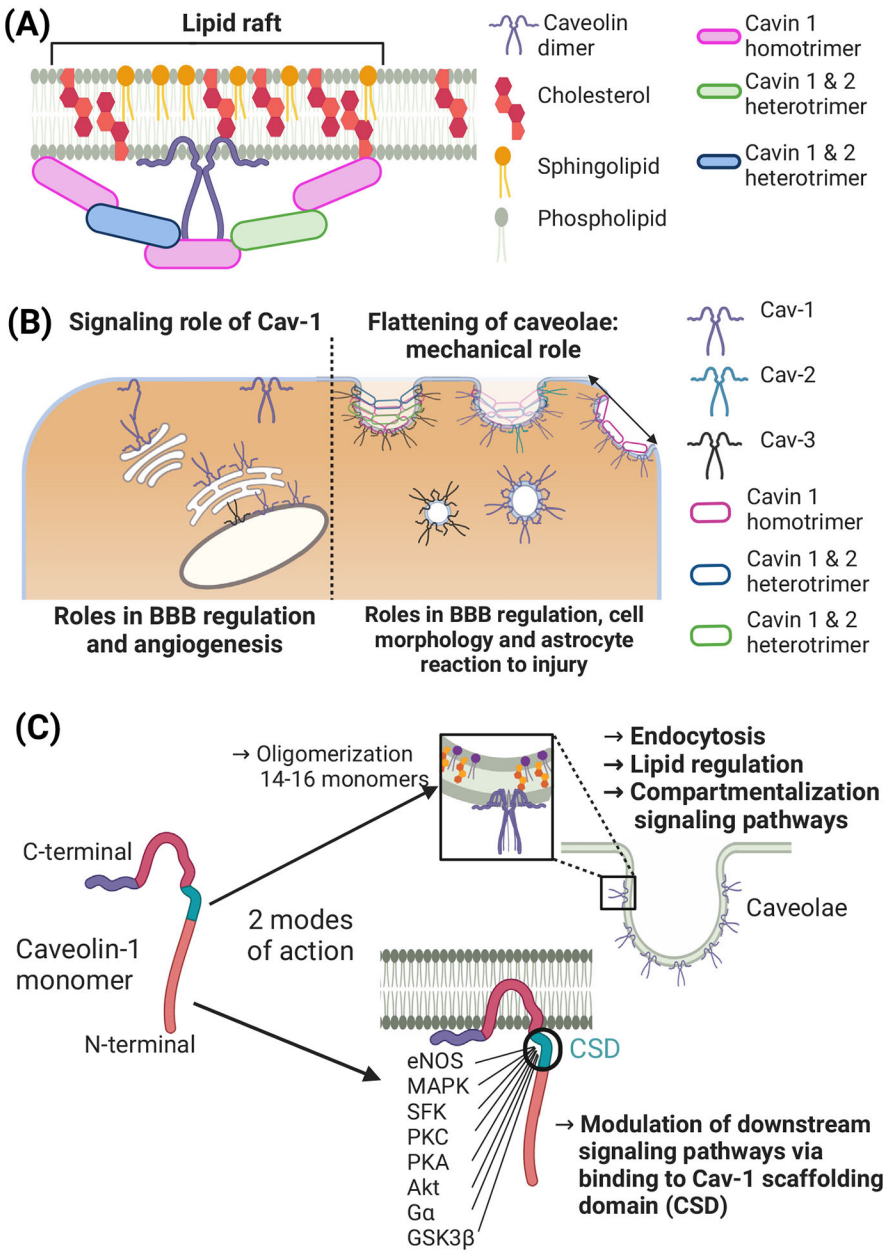

Figure 1. Schematic illustrations of planar and caveolae lipid rafts (LRs) and caveolin-1 (Cav-1) structure.

(A) LR domains are composed of caveolin-enriched phospholipid membranes with sphingolipids, cholesterol, and cavin proteins. (B) Summary of putative physiological actions of caveolins and cavins across central nervous system (CNS) cells. (C) Cav-1 has two primary modes of action. Caveolae formation via oligomerization of Cav-1 or Cav-3 homo-oligomers and/or Cav-1–Cav-2 hetero-oligomers and signaling modulation via direct binding of molecules to the caveolin scaffolding domain (CSD, in blue). The transmembrane domain is in pink, the C-terminal domain purple, and the N-terminal domain orange. Abbreviations: BBB, blood–brain barrier; eNOS, endothelial nitric oxide synthase; Gα, Gi protein alpha subunit; GSK3β, Glycogen synthase kinase-3 beta; MAPK, mitogen-activated

protein kinase; PKA, protein kinase A; PKC, protein kinase C; SFK, SRC family kinase. Figure created with BioRender.

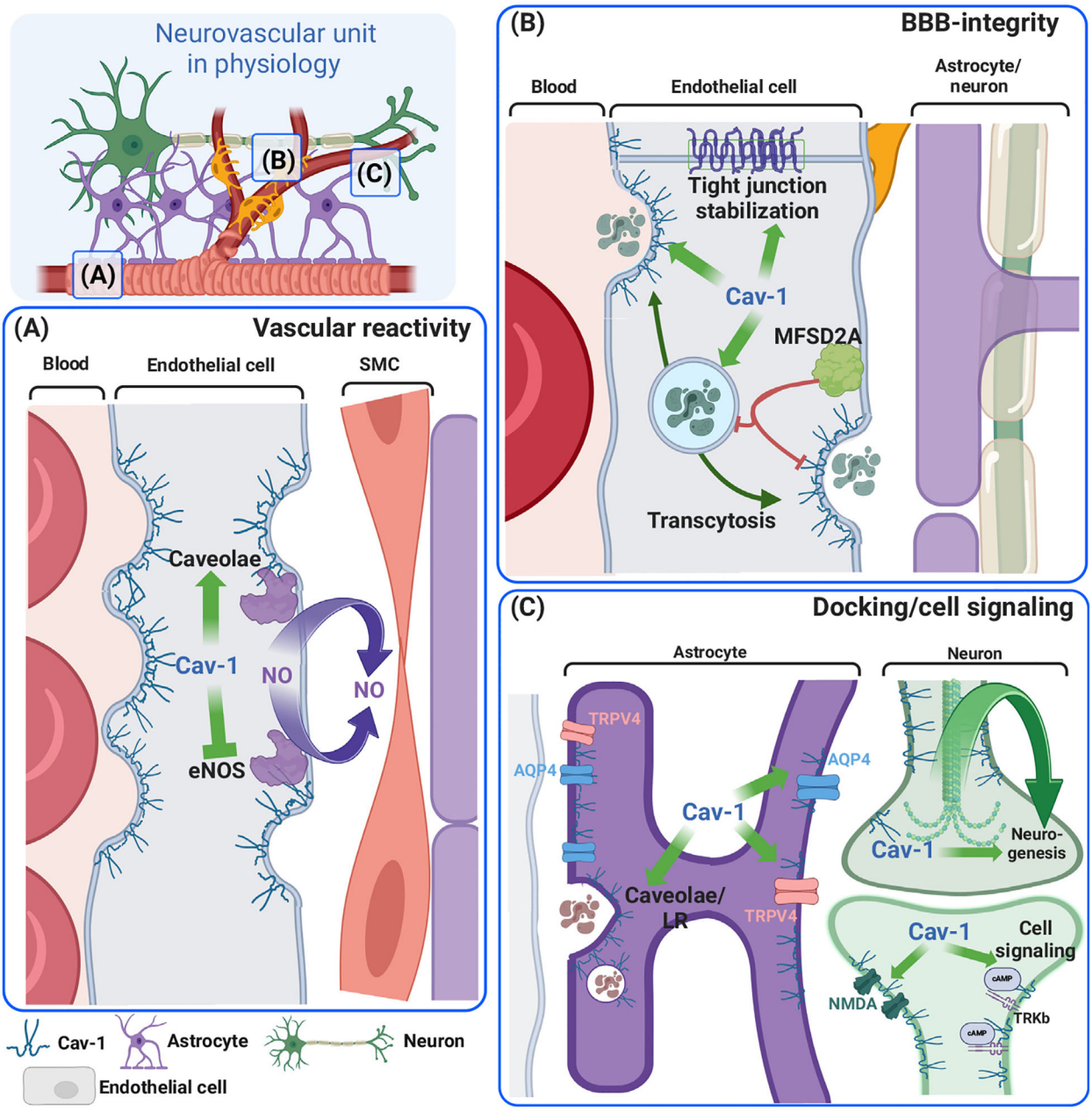

Figure 2. Putative physiological actions of caveolin-1 (Cav-1) in cell types of the neurovascular unit.

(A) Cav-1 is present in endothelial cells (ECs) of large arteries and participates in blood vessel dilation either by caveolae-mediated mechanisms or by regulation of endothelial nitric oxide synthase (eNOS) activity (vascular reactivity). (B) Cav-1 in the capillary bed is possibly involved in blood–brain barrier (BBB) regulation by controlling proteins involved in permeability properties and transcytosis. The level of expression of Cav-1 is under the control of major facilitator superfamily domain-containing protein 2 (Mfsd2a) (capillary and BBB). (C) Astrocytic Cav-1 is involved in caveolae and transcytosis mechanisms via astrocyte channels such as aquaporin 4 (AQP4) and transient receptor potential vanilloid 4 (TRPV4). Neuronal Cav-1 is proposed to interact with the cytoskeleton and participate

in neurogenesis. At the synaptic level, neuronal Cav-1 contributes to cell signaling by interacting with TRKb and NMDA receptors (docking/cell signaling). Figure created with BioRender.

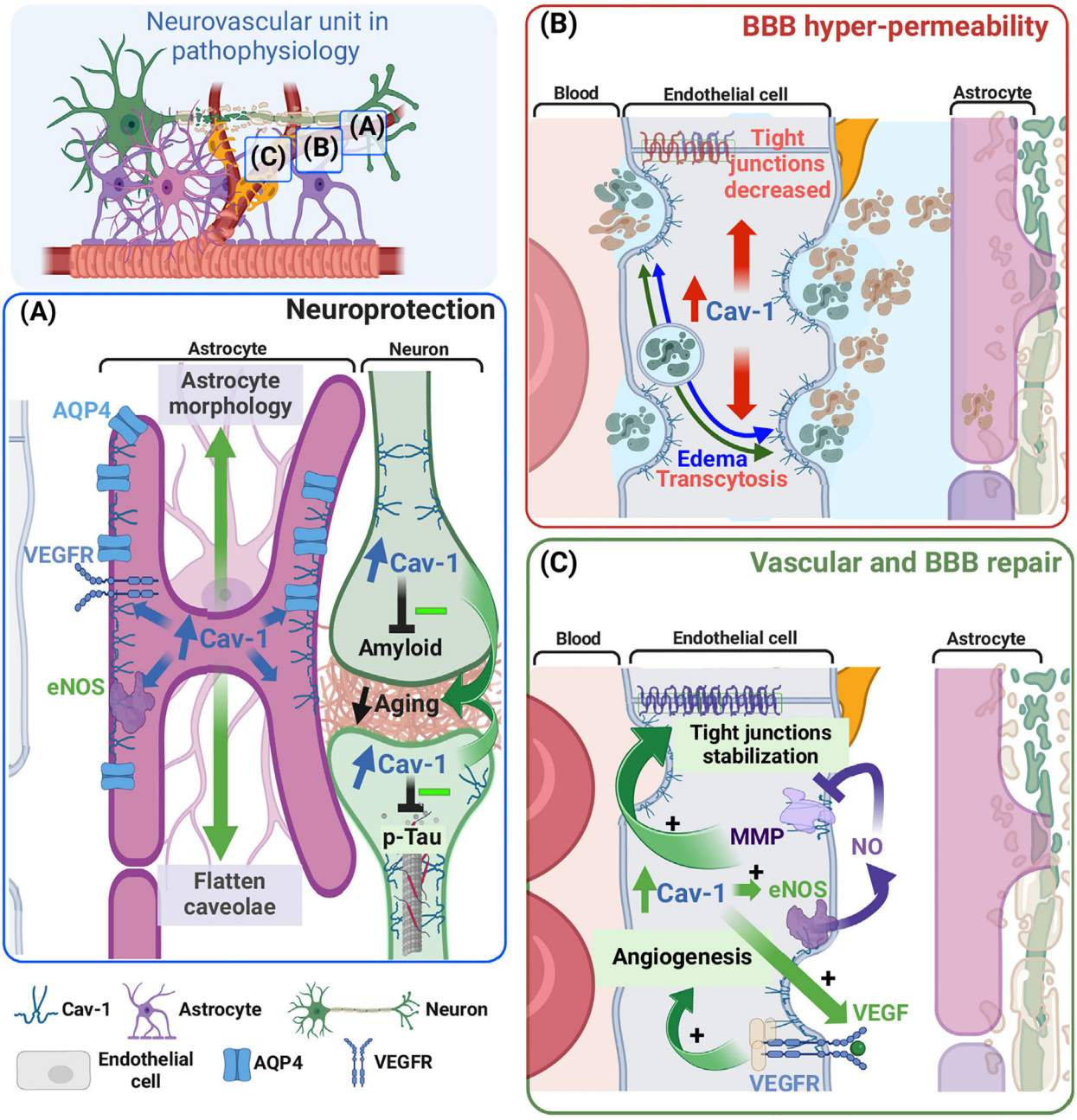

Figure 3. Putative pathophysiological actions of caveolin-1 (Cav-1) in different cells of the neurovascular unit.

(A) Increase in Cav-1 expression in astrocytes coincides with an increase in aquaporin 4 (AQP4), vascular endothelial growth factor receptor (VEGFR), and endothelial nitric oxide synthase (eNOS), a flattening of the caveolae, and an increase in the length of the astrocytic processes. Increased Cav-1 in neurons has been proposed to be beneficial with subsequent inhibition of p-Tau, and β-amyloid release, limiting the neurodegeneration process. However, the role of Cav-1 in aging and neurons is debated. (B) Cav-1 expression in brain blood vessels can be beneficial or detrimental in pathophysiological situations, depending on the preclinical model. Cav-1 promotes the formation of caveolae and

transcytosis, favoring exchange between blood and brain compartments, seen as detrimental when engaged in the edema process in early time after injury. The increase in transcytosis may participate in molecular exchange between both compartments. Some studies have observed a decrease in the tight junctions in relation to an increase in Cav-1 expression. (C) In brain endothelial cells (ECs), Cav-1 interacts with eNOS, leading to inhibition of metalloproteinase 9 (MMP9), thereby stabilizing the tight junctions. Cav-1 binds to VEGFR and promotes angiogenesis and blood vessel repair. Abbreviation: BBB, blood–brain barrier. Figure created with BioRender.