Cells of the Blood-brain Barrier: an Overview of the Neurovascular Unit in Health and Disease

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Abstract

The brain is endowed with highly specialized vasculature that is both structurally and functionally unique compared to vasculature supplying peripheral organs. The blood-brain barrier (BBB) of the cerebral vasculature prevents extravasation of blood products into the brain to protect neural tissue and maintain a homeostatic environment. The BBB also functions as part of a neurovascular unit (NVU), which is comprised of neurons, astrocytes, and microglia in addition to the specialized endothelial cells, mural cells, and the basement membrane of the BBB. Through coordinated intercellular signaling, these cells function as a dynamic unit to tightly regulate brain blood flow, vascular function, neuroimmune responses, and waste clearance. In this chapter, we review the functions of individual NVU components, describe neurovascular coupling as a classic example of NVU function, and discuss archetypal NVU pathophysiology during disease.

Keywords

Neurovascular unit; blood-brain barrier; cerebral blood flow; astrogliosis; neurovascular coupling

Introduction

Vasculature in the central nervous system (CNS) is structurally and functionally unique compared to vasculature that supplies the peripheral organs. To protect neural tissue, which is particularly sensitive to changes in the composition of its interstitial fluid, the cerebral vasculature has evolved to tightly regulate the entry and exit of compounds between the blood and the brain. This blood-brain barrier (BBB) is comprised of several different cell types and encompasses a physical barrier as well as selectively regulated transport mechanisms. Highly specialized endothelial cells line the lumenal surface of blood vessels and abut an ablumenal basement membrane that contains embedded mural cells— pericytes or vascular smooth muscle cells (VSMCs). Astrocytes project endfeet processes that completely ensheathe these components, while their fine processes contact neuronal synapses in the neuropil. Motile microglia are also present within this biological interface.

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Functionally, these components interact to regulate vascular permeability, neuroimmune responses, cerebral blood flow (CBF), and to maintain a homeostatic CNS environment.

With an evolving understanding of the dynamic signaling interplay that occurs between the BBB components and neurons to support neuronal homeostasis and information processing, the BBB is now often considered in the context of a functional neurovascular unit (NVU) (1). The NVU is a fascinating and clinically relevant disease target. Neurovascular dysfunction is present in numerous neurological diseases, yet whether it is a cause or an effect of a particular disease state is currently not well understood (2). The cells of the CNS are specified and differentiated during early development. Thereafter, with few exceptions, these cells neither turn over nor regenerate effectively. This cell longevity renders the CNS susceptible to factors from the periphery that can disrupt cellular function and survival. Furthermore, as a highly energy-dependent organ with minimal energy stores, the brain relies on a near-constant supply of oxygen, glucose, and other nutrients to drive adenosine triphosphate (ATP) production and maintain metabolic homeostasis. Indeed, no cortical brain cell is located further than 25 μm from a capillary (3). Thus, both from a cell survival and metabolic standpoint, the NVU is critical for CNS health and NVU dysfunction is poised to exacerbate or even initiate neurological disease. Herein, we describe the function of NVU components and the role of the NVU in health and disease.

Components of The Neurovascular Unit

NVU components exist along a three-dimensional vascular network of pial and penetrating arterioles, capillaries, venules, and veins. This vascular network densely permeates all brain tissue to ensure adequate nutrient delivery (1, 4) and clearance of metabolic waste products (5, 6). To maintain CNS homeostasis and meet the fluctuating metabolic demands of the brain tissue, NVU components have functional properties that vary by brain region and vascular zone. Although the NVU is present throughout the vascular tree, we focus largely on the capillary NVU as this is where nutrient exchange occurs and the strongest BBB properties are manifest.

We describe non-neuronal components of the NVU radially, beginning with endothelial cells and progressing outward through the layers of basement membranes (noncellular), mural cells, astrocytes, and finally microglia.

Endothelial Cells—A continuous monolayer of specialized endothelial cells lines the blood vessels of the brain. Unlike the endothelial cells lining the peripheral vasculature, brain vascular endothelial cells are completely confluent and interconnected circumferentially by transmembrane tight junction proteins that prevent the passive transfer of cells and molecules between the blood and the brain based on size, surface electrical charge, and lipid solubility (Figure 1). These tight junctions create a strict seal throughout vascular lumenal surfaces giving rise to *in vivo* transendothelial electrical resistances of up to ~1800 $\Omega \bullet cm^2$ and preventing even the flow of water or small ions across the endothelium (7).

Besides this physical barrier, brain vascular endothelial cells maintain a dynamic regulatory system that facilitates bidirectional transport of substances between the blood and the brain.

The transport functions of these cells are mediated and maintained by polarized expression of various lumenal and ablumenal membrane receptors and transporters, such as HCO_3^- exchangers and Na^+/K^+-ATPase (8, 9). As opposed to peripheral vascular systems where substances in the blood are more or less indiscriminately moved across the endothelial lining into the tissue via transcytosis, these pathways are suppressed in brain endothelial cells, reducing their non-specific transport capacity (10, 11). Thus, depending on the chemical entity, transport of substances across the BBB must be actively regulated. This occurs via several mechanisms, including paracellular diffusion (12), carrier and receptor-mediated transcytosis (13, 14), and adsorptive-mediated transcytosis (Figure 2) (15, 16). These specialized properties of brain endothelial cells are reflected in their unique gene expression patterns, a finding highlighted in recent transcriptomic studies (17–19). For example, expression of Mfsd2a, the transcytosis-suppressing protein responsible for lipid transport regulation, is high in endothelial cells of the cerebral microvasculature but very low in lung and heart endothelial cells (18, 19). Although this regulation effectively maintains the delicate ionic milieu and extracellular homeostasis of neural tissue, it also creates a pharmacological bottleneck for CNS drug delivery (15, 20).

Brain vascular endothelial cells are phenotypically heterogeneous and have numerous location-specific functional roles that vary with both their position in the vascular hierarchy and location in the brain (18, 21, 22). For example, the circumventricular organs and hypothalamic regions contain specialized endothelial cells that make their vasculature more permeable than the rest of the brain (21, 22). In addition to their barrier functions, brain vascular endothelial cells dynamically modify permeability in response to inflammatory and immunological stimuli (19) or in a circadian rhythmic fashion (23–25). Further, endothelial cells also help mediate neurovascular coupling (NVC) (26–28) and regulate the spread of vascular responses via endothelial gap junctions, which allow propagation of signals along the vascular tree to mediate changes in regional CBF (Figure 4) (29, 30). The increased mitochondrial load of brain vascular endothelial cells reflects their active role in NVU regulation, but also makes them more sensitive to changes in oxidative stress (31).

Basement Membrane—The cerebrovascular endothelial tube is ensheathed by a non-cellular basement membrane comprised of five major proteins—collagens, laminins, nidogens, perlecan, and agrin—arranged in a compact lattice meshwork. This vascular basement membrane merges with a parenchymal basement membrane in areas along the vasculature lacking mural cell coverage, particularly in capillary regions not covered by pericytes (Figure 1). Vascular basement membrane proteins are secreted largely by endothelial cells while parenchymal basement membrane components are secreted by astrocytes via their vascular endfeet (32). Bidirectional signaling between the cells of the NVU—endothelial cells, pericytes, and astrocytes—and the basement membrane dynamically regulates BBB permeability. For example, pericyte recruitment to endothelial tubes during development is an important stimulus that helps generate the basement membrane matrix (33), but absence of the basement membrane protein laminin can, in turn, reduce pericyte coverage of the vasculature (34).

Structurally, basement membrane proteins form a sheet-like extracellular matrix that stabilizes the BBB, anchors cells, facilitates signal transduction, and enhances vascular

barrier properties (35). The composition and thickness of the basement membrane are dynamic, vary regionally throughout the brain, and can affect cellular interactions and receptor activation states (35, 36). Functionally, the basement membrane contributes to BBB development, endothelial cell tight junction formation, and maintenance of transendothelial electrical resistance (35). It also helps recruit and establish the polarity of astrocyte endfeet, including anchoring of aquaporin 4 to the vessel-facing cell membrane of the endfoot, functions that are likely mediated via interactions of laminin with its receptor dystroglycan in astrocyte endfeet (34). However, as astrocytes are an important source of the parenchymal basement membrane components, astrocyte dysfunction can also result in a weakened basement membrane (37), underscoring the importance of bidirectional crosstalk between BBB components.

Mural Cells: Pericytes—Mural cells called pericytes enwrap capillaries in all vascular beds. Capillaries in the CNS are associated with the highest pericyte coverage in the body, exhibiting a pericyte to endothelial cell ratio of 1:4 (38, 39) compared to, for example, a ratio of 1:10 or more in skeletal muscle (40, 41). This suggests an important role of pericytes in the cerebral circulation. Indeed, brain pericytes play critical roles during both embryogenesis and adulthood by establishing and maintaining BBB integrity (42), orchestrating vascular development (43), and regulating capillary blood flow (44). During brain development, pericytes migrate to vessels and, in concert with endothelial cells and other NVU components, contribute to vascular pruning and angiogenesis (43, 45). A balance of these processes effectively shapes and organizes the cerebral vasculature to ensure an optimal angioarchitecture that facilitates nutrient delivery to brain tissue. Like the endothelial cells and basement membrane, pericytes tune the polarization of astrocyte endfeet along the capillary wall (46, 47). Embedded between the endothelial tube and astrocyte endfeet, pericytes are often described as having a "bump on a log" morphology (Figure 3) (48). This description accurately describes the cell body but may not adequately convey the extensive coverage of the vasculature by pericyte processes, which can contact up to 90% of brain vasculature in mice (49). Although pericytes are fixed in place and do not migrate after development under physiologically healthy conditions, they are structurally dynamic and can extend their processes and remodel to compensate for loss of nearby pericytes or endothelial cell coverage in areas along the vasculature (50). In adult brains, pericytes exhibit contractile responses to vasoactive compounds and therefore control capillary diameter (44, 51, 52). However, not all pericytes are contractile (53–55) and they may be relatively less contractile than the VSMCs on larger vessels. It has been proposed that pericytes may maintain basal CBF equilibrium at the capillary level while arterial VSMCs are responsible for larger, more rapid CBF fluctuations (50).

Pericytes display broad heterogeneity in function, gene expression, and appearance. They are morphologically variable along the vascular tree, showing different structures and vessel coverage at capillary, pre-capillary, and post-capillary vascular zones. Therefore, depending on their location and morphology, pericytes may exert different functions in their regulation of the BBB and CBF at different levels of the vascular tree (48, 56). Pericytes located at branch points between arterioles and capillaries may act as 'precapillary sphincters' to direct flow into or away from a region (51, 57). There is also strong evidence that

pericytes on pre-capillary arterioles and capillaries closer to arterioles participate in NVC (44) and regulate CBF (50, 58). Gap-junctional connections between neighboring pericytes and between pericytes and endothelial cells also help propagate signals along the vascular tree (30), such that a contractile response initiated in one region quickly spreads up- and down-stream to increase blood flow through that region (59, 60). Pericytes in mid-capillary branches may be more important for basal CBF regulation (61, 62) and maintenance of BBB integrity and stability in concert with endothelial cells (48, 56, 63). Very little is known about the pericytes on cerebral venules, but they are hypothesized to regulate immune cell trafficking, as they do in peripheral vascular beds (64, 65).

Mural Cells: Vascular Smooth Muscle Cells—VSMCs are a distinct contractile mural cell type from pericytes (66). They are embedded in the arterial wall where they form several concentric layers around these larger vessels. They have important roles in maintaining and generating basal vascular tone, vascular reactivity, and autoregulation (67, 68). VSMCs express a variety of ion channels, are electrically excitable, and display robust calcium signals and consequent contractile responses; hence, they are traditionally thought to be the principal site of functional hyperemia in the brain (1, 67). Furthermore, VSMCs are gap junction-coupled to each other (69) and also to endothelial cells lining the vasculature, which allows evoked vasomotor responses to propagate along vessels (70) over long distances as calcium waves (71). The polarity and magnitude of these calcium signals determine the degree of evoked vasoconstriction or dilation. Fluctuations between relaxation and contraction states in cerebral VSMCs gives rise to vasomotion, which is postulated to be a mechanism of perivascular drainage, which clears waste from the brain interstitium (72).

Astrocytes—Astrocytes are the most numerous glial cell type in the CNS with a complex, polarized morphology. On the one hand, they have vascular endfeet processes, which almost completely encapsulate the vasculature of the brain, including the endothelial tube and surrounding mural cells (Figure 3) (73). On the other hand, they have numerous fine, ramified processes extending from a stellate cell body which permeate the neuropil (Figure 3). Like neurons, astrocytes are heterogeneous in structure, function, and distribution throughout the brain. Traditionally, astrocytes were classified into two groups: protoplasmic astrocytes found in the well-vascularized grey matter and fibrous astrocytes found in the less vascular white matter. In both grey and white matter areas, astrocytes physically separate synapses or nodes of Ranvier, contact neurons, and cover the vasculature (74). More recent work has revealed considerable heterogeneity even within grey matter astrocytes, with pronounced regional or even sub-regional differences (75–79), some of which are regulated by signals from surrounding NVU components, including neurons (80) and endothelial cells (81, 82). This heterogeneity manifests as variations in astrocyte functions, their interactions with neurons (83, 84), and their response to injuries (85).

Astrocytes play a multifaceted role in maintaining CNS homeostasis. They spatially tile the brain parenchyma and are interconnected by gap junction proteins to form a functional syncytium (86), which allows them to signal over long ranges via calcium wave propagation (87). Astrocytes also express high levels of the inwardly rectifying potassium channel Kir4.1, which is pronounced in their peri-synaptic processes and vascular endfeet. This

allows astrocytes to take up potassium after bouts of neuronal activity and spatially buffer potassium along the syncytium (86). At synapses, astrocytes are active participants in tuning the information transfer between neurons by taking up neurotransmitters at the cleft to spatially and temporally refine synaptic signaling (74).

Astrocyte endfeet processes terminate on and enwrap all capillaries and arterioles in the CNS to form a contiguous gliovascular interface. Astrocytes extend other fine processes to a variety of targets within the CNS, including synapses, nerve cell bodies, and nodes of Ranvier. This polarized morphology positions astrocytes centrally within the NVU to dynamically mediate many regulatory functions, including maintenance of BBB integrity, mechanical and metabolic support, endothelial transport, innate immune responses, and cerebrovascular regulation (88, 89). Via their endfeet processes, astrocytes secrete basement membrane proteins such as laminins (90) to stabilize the BBB. They also signal to pericytes (90) and endothelial cells (91) to maintain the CNS-specific properties of these vascular cells within the BBB. Specialized membrane proteins anchored to astrocyte endfeet processes allow astrocytes to regulate the transport of nutrients and neurotransmitters after they cross the BBB, including glucose and glutamate, which are necessary for healthy brain function (81, 92). Further, selective expression of channels such as Kir4.1 and aquaporin 4 at the endfeet polarize astrocytes and facilitate the directional flow of potassium and water molecules to maintain ion homeostasis and neuropil volume (93–95).

Astrocytes serve important roles as sensors of neuronal activity and stabilizers of the extracellular environment. They are also active players in information processing within the brain. In response to synaptic activity, numerous intracellular signaling pathways are activated in astrocytes, the most studied so far being calcium-dependent. Calcium increases can be transient and localized within microdomains, spread through an entire process, spread throughout the astrocyte territory, or even be sustained and propagated across the astrocyte syncytium (87, 96). This activation of astrocytes results in the release of gliotransmitters (at a spatial extent dictated by the calcium signals), which can then modulate activity ranging from anywhere between individual synapses to whole circuits (25, 87, 97–99). Given that astrocytes also express virtually all neuromodulator receptors, they have been proposed to be the cellular substrates underlying coordinated long-range neuromodulatory networks (100). With their endfeet on vessels for nutrient uptake and their gap junction-coupled fine neuropil processes, astrocytes also form a crucial metabolic supply network that not only regulates glucose delivery to active regions (101) but may also modulate circuit properties that regulate plasticity (102) and behaviors such as sleep (103).

Within the NVU, astrocytes are key regulators of CBF (104). Astrocytes contribute to the resting tone of arterioles through release of vasoactive agents such as prostaglandin E2 (105) and ATP (106, 107). They can mediate signals from active neurons to the vasculature to mediate NVC. This intercellular signaling pathway – from neurons to astrocytes to the vascular mural cells – is the primary pathway regulating capillary NVC (29, 52, 108), but it appears to be less important in regulating arteriolar NVC, where it is engaged only upon sustained or strong activation (Figure 4) (109). Even so, recruitment of astrocytes results in a threefold enhancement of functional hyperemia *in vivo*, suggesting an important role for astrocytes in activity-dependent energy supply to the brain (110).

Finally, astrocytes serve an important role in glymphatic waste clearance. During sleep, aquaporin-4 water transport channels on the vessel-facing endfeet processes of astrocytes facilitate the exchange of perivascular cerebrospinal fluid (CSF) with interstitial fluid at capillaries (111). This exchange results in the drainage of CNS waste products into the CSF, which is then removed at CSF clearance sites. Both sleep deprivation (112) and aging (113) result in glymphatic dysfunction by causing mislocalization of aquaporin 4 on astrocyte endfeet, potentially contributing to the accumulation of toxic waste products in the brain.

Microglia—Microglia are yolk sac-derived myeloid cells that seed the brain early in development (114). During this early period, microglia stimulate angiogenesis, increase vascular complexity, and promote endothelial tip cell fusion within capillary beds (115–117). Conversely, the vascular network may also provide microglia with a migration 'highway' to populate the brain during development (118). Adult microglia are the tissue-resident macrophages of the brain. These highly ramified, motile cells act as phagocytic sentinels, surveilling and probing their environment for any alterations or injury by extending and retracting their processes. Microglia are scattered evenly throughout the brain, in a manner similar to the tiling of astrocytes, and occasionally extend a process directly to the vasculature at locations containing pericytes (73). They are exquisitely sensitive to changes in the neuropil environment or in nearby perivascular homeostasis. Upon sensing insult, microglia take on an ameboid morphology and undergo dynamic transcriptomic and phenotypic changes that are characterized along a spectrum of pro-inflammatory to anti-inflammatory activated states. These activated microglia serve a critical role in phagocytosing debris (119, 120), signaling to peripheral immune cells (120), and regulating reactive astrogliosis (121, 122). Microglia may also modulate the BBB, sometimes enhancing BBB properties and sometimes contributing to BBB breakdown and NVU dysfunction (123–125), depending on the type, extent, and chronicity of the injury (126–128). As with any other component of the NVU, microglial properties are also dynamically modulated by surrounding NVU cells such as astrocytes and endothelial cells (129). Together, astroglial and microglial activation may optimize innate immune responses in the CNS by engaging in coordinated gliotransmitter and cytokine release and metabolite uptake (130).

Neurovascular Coupling as a Classic Example of Intercellular NVU Signaling

The CNS is dependent on a continuous supply of energy substrates and other nutrients from the blood to maintain healthy function. Signaling within the NVU, particularly between astrocytes and mural cells, regulates the resting tone of CNS vessels to help maintain resting blood flow (105, 107). Subcortical projection neurons originating from regions such as the basal ganglia and the brainstem can endow cerebral vessels with vascular tone, which may occur directly, via astrocytes, or via local interneurons (reviewed in (29)). The brain is also protected from large fluctuations in blood flow due to changes in systemic physiology via a process termed cerebral autoregulation, such that blood flow is maintained relatively constant within a physiological range of blood pressure changes. Although autoregulation is generally mediated by myogenic or endothelial factors, a role for astrocytes in a process akin to autoregulation was recently identified, whereby astrocytes sense pressure changes and release vasoconstrictors onto mural cells to maintain vascular tone (106). Layered

upon these processes is NVC, an intercellular signaling mechanism that engages almost all cellular components of the NVU to increase blood supply to regions of increased neuronal activity in a spatially and temporally coordinated manner (Figure 4) (104), and gives rise to a functional hyperemia response in the brain. Neuronal activity and blood flow changes are coupled so tightly that this phenomenon has been exploited by various functional neuroimaging techniques such as blood oxygenation level-dependent functional magnetic resonance imaging (29, 131, 132), positron emissions tomography (133, 134), and the recently developed functional ultrasound (135) to image brain activity in real-time.

The study of pathways regulating NVC has blossomed in the last two decades, revealing complex intercellular interactions at the NVU interface. NVC is initiated by neuronal activity and can be mediated by vasoactive signals released directly from neurons or indirectly via astrocytes, which trigger a change in vascular lumen diameter (Figure 4) (reviewed in (1, 29, 67)). Vasodilatory signals such as nitric oxide (NO), prostaglandins, potassium, and epoxyeicosatrienoic acids released by neurons and/or astrocytes can relax mural cells, thereby dilating blood vessels and increasing CBF. Conversely, vasoconstrictive signals such as ATP and 20-hydroxytetraenoic acids from neurons/astrocytes cause contraction of mural cells, thereby constricting blood vessels and decreasing CBF. Though traditional views held that NVC only involved direct signaling from neurons to the VSMCs to increase CBF at the arteriolar level, recent studies have found significant evidence for a role of pericytes in capillary flow regulation and for astrocytes in mediating signals to capillaries (Figure 5) (52, 108). Indeed, with their fine processes apposed to numerous synapses and their endfeet ensheathing the entire cerebral vasculature (including arterioles and capillaries) astrocytes are ideally positioned to be NVC intermediaries. Current evidence suggests that astrocytes play an obligatory role in capillary NVC (52, 108), while their role in arteriolar NVC may be more modulatory (109, 110, 136). Ultimately, signals from both neurons and astrocytes must alter the contractile state of vascular mural cells—pericytes on capillaries or VSMCs on arterioles—for NVC to successfully produce functional hyperemia. Furthermore, a role for endothelial cells in this intercellular signaling pathway, wherein endothelial cells respond to signals from astrocytes and in turn secrete vasodilators onto VMSCs, has also been discovered (Figure 4) (28). Thus, functional interactions between NVU components are crucial regulators of blood flow and hence energy supply to the brain.

NVU in Disease

Neurovascular dysfunction is a central theme of numerous brain disease states, though whether it is a cause or an effect of a particular disease state is not always well defined. Broadly, neurovascular dysfunction encompasses a range of pathological states that can be attributed to individual NVU components or the unit as a whole. In lieu of a particular disease focus, we instead describe elements of NVU dysfunction that are common to many brain diseases. Critically, NVU dysfunction occurs along a spectrum with variable pathophysiological features depending on the disease state and individual.

As a major function of the NVU is to maintain BBB characteristics and function, a general consequence of NVU dysfunction is often BBB disruption, though this term can reflect distinct and nuanced structural, functional, cellular, and molecular deficits depending

on the particular disease state. For example, NVU cellular responses to hypoxia differ from those occurring in neurodegeneration or traumatic injury. On a structural level, BBB disruption reflects a loss of integrity and an increase in permeability due to altered endothelial cell expression of tight junction proteins (137, 138) and transporters (139), enlarged perivascular spaces (140), or even degeneration of NVU components including endothelial cells (141), pericytes (142–144), astrocytes (145–147), or basement membranes (148). Pathologically impaired waste clearance may also compromise BBB integrity (149). A functional consequence of increased BBB permeability is permissive infiltration of blood products through the vulnerable barrier, including but not limited to peripheral immune cell infiltrates.

Under conditions that deviate from a normal physiological range, astrocytes often become 'reactive' and alter their morphologic and transcriptomic properties. Reactive astrogliosis can also be induced following BBB breakdown via signaling from blood-borne proteins such as albumin, fibronectin, and thrombin (2, 150–152). Reactive astrocytes are characterized by gain of new functions or loss of existing functions, which together alter the overall functions of the region affected (2, 153). They can also release various cytokines that can alter the properties of nearby neurons, microglia, and vascular cells (154), and perhaps even attract or modulate peripheral immune cells to the rescue of the CNS (155–157). The current consensus is that reactive astrogliosis is a context-dependent process engaged to re-establish homeostasis and protect neurons from further damage (122, 158–160); however, in some contexts, they acquire detrimental properties and may amplify injury (121, 161, 162). The central positioning of astrocytes in neurovascular signaling also poises them to subtly or dramatically impair energy homeostasis in the CNS due to an "uncoupling" of neuronal activity and vascular responses during both acute and chronic NVU impairment (2, 104). Indeed, CBF alterations are commonly seen in many brain disease states and can manifest as decreased perfusion to deep brain structures, loss of NVC, and impaired vascular reactivity to flow and pressure changes (163–167).

Conclusion

The mammalian cerebrovascular system is characterized by structurally and functionally unique vessels endowed with a strong biological barrier that selectively separates blood and brain components. Distinct functional features characterize individual NVU components, though they maintain many intimate functional relationships with one another. Further, cells of the NVU can change both functionally and phenotypically, depending on microenvironmental signaling cues.

The presence of the BBB has important implications for neuronal function during physiology and pathology. It protects the CNS extracellular environment from peripheral signals that could destabilize neuronal function. Further, complex intercellular signals between NVU components allows these cells to sense and respond to changes in regional neuronal activity to regulate CBF and meet the energy demands of neurons. The recent development and application of novel and sophisticated neuroimaging paradigms that allow real-time imaging of these physiological processes offers exciting prospects for delineating the contributions of individual NVU components to regional hemodynamic changes.

Finally, NVU dysfunction can precede or exacerbate CNS disease states. With their multifaceted functional roles in physiological processes, dysfunctional NVU components are poised to adversely influence a broad range of metabolic, hemodynamic, and immunologic regulatory responses in the brain. Compelling evidence so far supports this view; however, considerable research will be required to form a complete picture of the NVU's role in neurological diseases.

Acknowledgments

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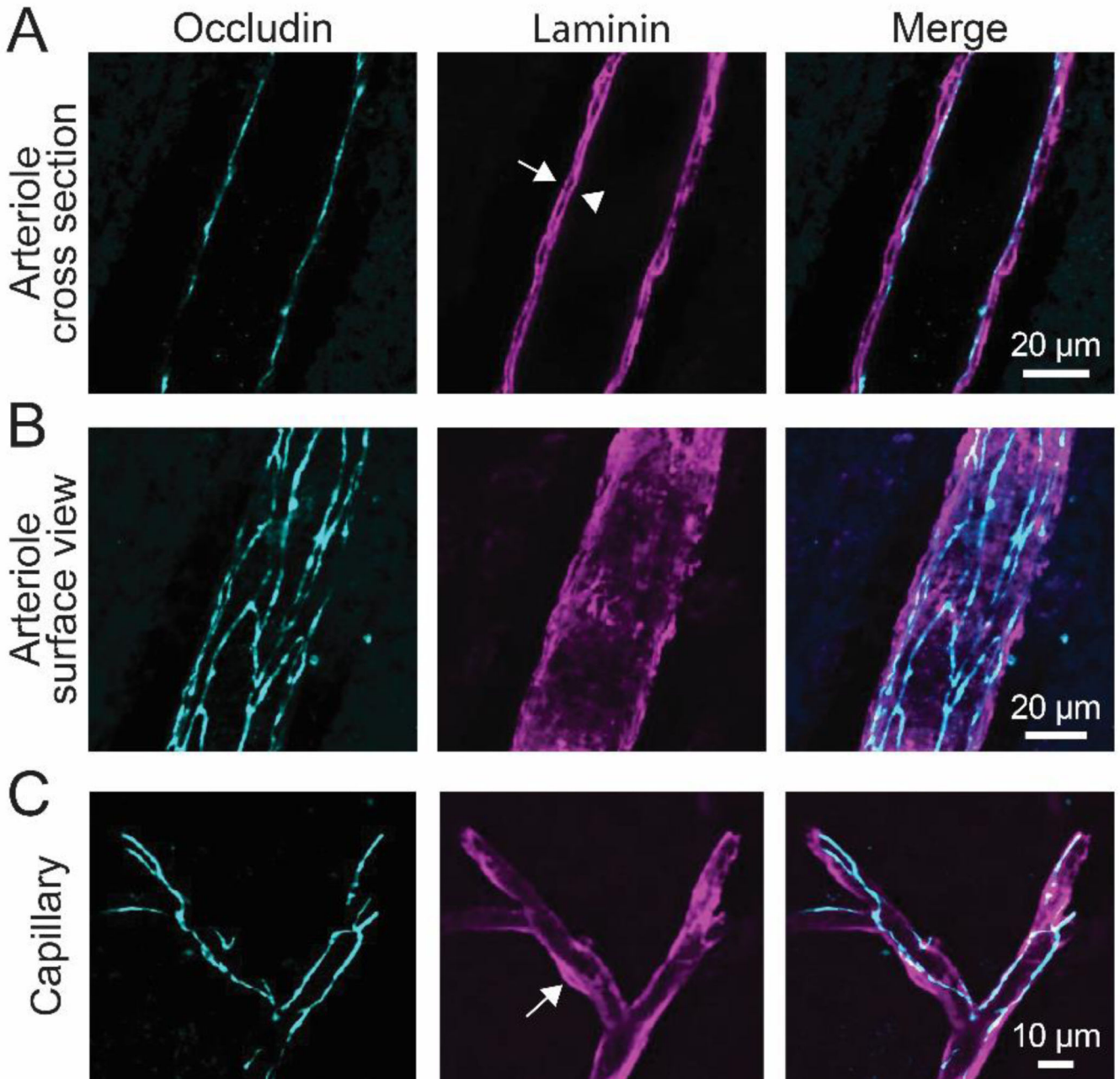
Figure 1.

Vascular distributions of occludin and laminin blood-brain barrier proteins. Double immunofluorescence confocal microscopy performed with anti-occludin (blue) and anti-laminin (magenta) antibodies on formalin-fixed 50-μm-thick rat brain sections. (A) Arteriolar cross section showing occludin-positive tight junctions between endothelial cells arranged circumferentially around the vascular lumen. Laminin-containing basement membrane circumscribes the occludin staining and delineates both the endothelial-secreted vascular basement membrane (arrowhead) and the astrocyte-secreted parenchymal basement membrane layer (arrow). Vascular smooth muscle cells occupy the space between the layers of laminin (not labeled). (B) Surface view of an arteriole shows occludin-positive

tight junctions outlining the contiguous and elongated endothelial cells. Laminin staining reveals basement membrane coverage along the entire vessel. (C) View of a capillary branch point shows occludin tight junction staining between adjacent endothelial cells. The laminin-positive vascular and parenchymal basement membranes are fused along most regions along a capillary and indistinguishable except in regions encasing pericytes (arrow). Merged images are shown on the right.
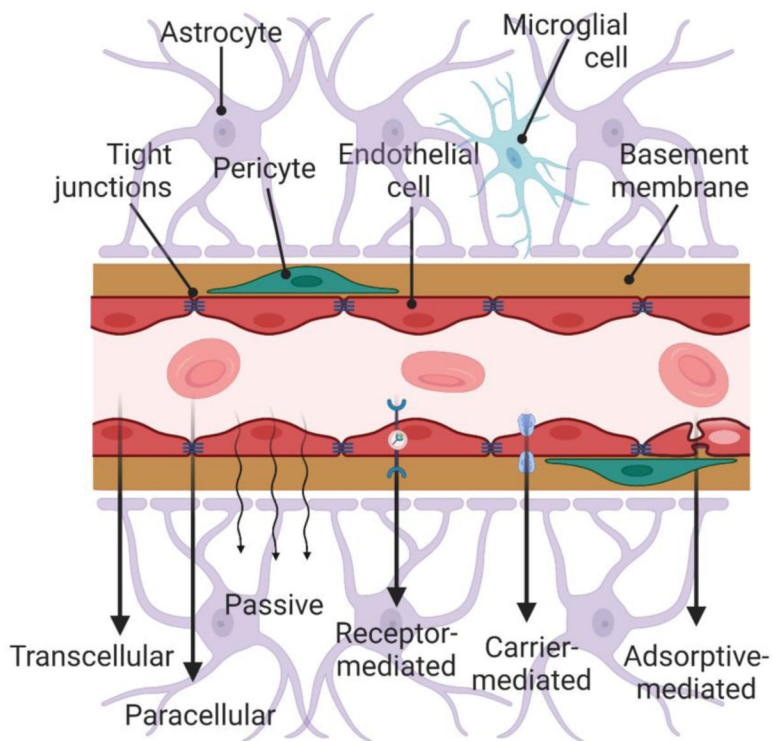
Figure 2.
Specialized brain vascular endothelial cells regulate transport at the blood-brain barrier.
A contiguous monolayer of vascular endothelial cells lines the brain vasculature.
These cells are interconnected by tight junctions, creating a physical barrier with a
remarkably high transendothelial electrical resistance. Transport across the blood-brain
barrier is mediated by three energy-independent diffusion mechanisms and three energy-
dependent transport mechanisms. The energy-independent mechanisms include transcellular
diffusion, paracellular diffusion, and passive diffusion. The energy-dependent mechanisms
encompass receptor-mediated transcytosis, carrier-mediated transport, and adsorptive-
mediated transport. Transport is shown only unidirectionally for simplicity. Created with
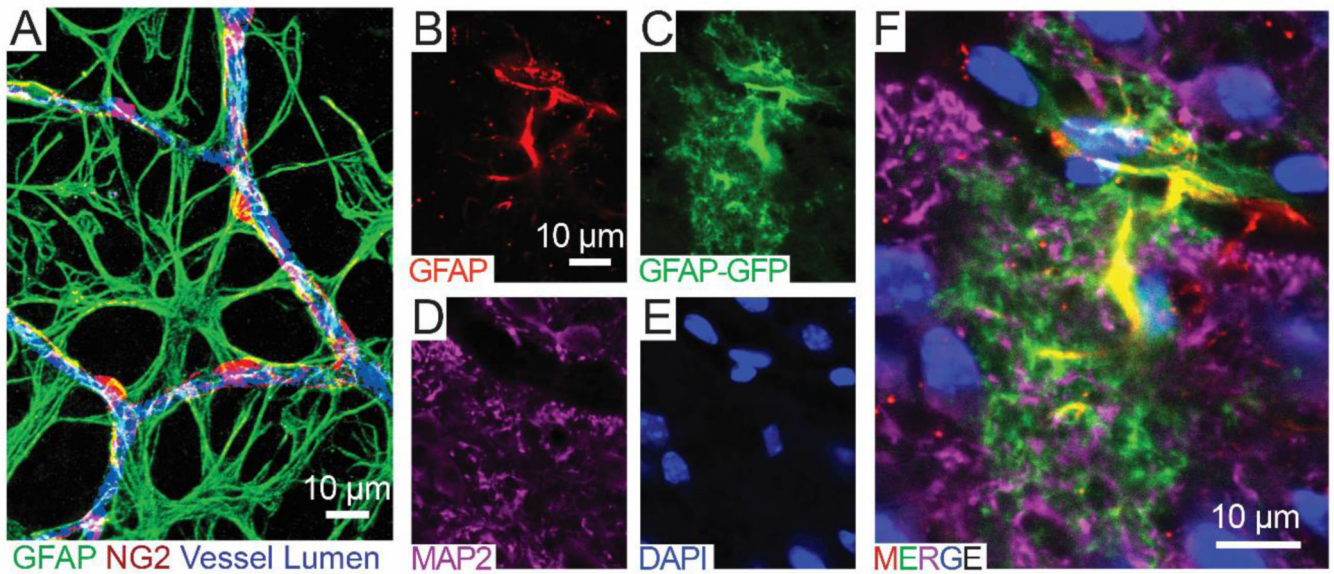BioRender.com.

Figure 3.

Astrocyte ensheathment of the cerebral vasculature. (A) A capillary network in a mouse retina showing the relationship of astrocytes to pericytes. Astrocytes are labeled with an anti-glial fibrillary acidic protein (GFAP) antibody (green) in a retina whole mount from an NG2-dsRed mouse, wherein the red fluorescent protein dsRed is expressed in NG2-positive pericytes. The vascular lumen is labeled with an anti-mouse secondary (blue) that labels natural mouse serum IgGs present in blood vessels (retina post-fixed following live dissection without perfusion). Astrocyte endfeet encapsulate the capillary wall and contact all pericytes. (B-F) Astrocyte endfeet around a cortical capillary with the surrounding neuropil. (B) An astrocyte labeled with anti-GFAP antibody (red). (C) The same astrocyte expressing virally induced cytosolic green fluorescent protein (GFP) under the truncated GFAP promoter, GfaABC$_1$D (green). (D) The surrounding neuropil labeled with the neuronal marker, microtubule-associated protein 2 (MAP2; magenta). (E) Nuclei are labeled with DAPI (blue). (F) Merged image showing GFAP, GFP, MAP2, and DAPI. Though GFAP labeling shows only the cytoskeleton of the astrocyte, including the endfoot process, GFP expression reveals its extensive fine processes permeating the neuropil (where it contacts synapses) and its complete ensheathment of the capillary via its endfoot.
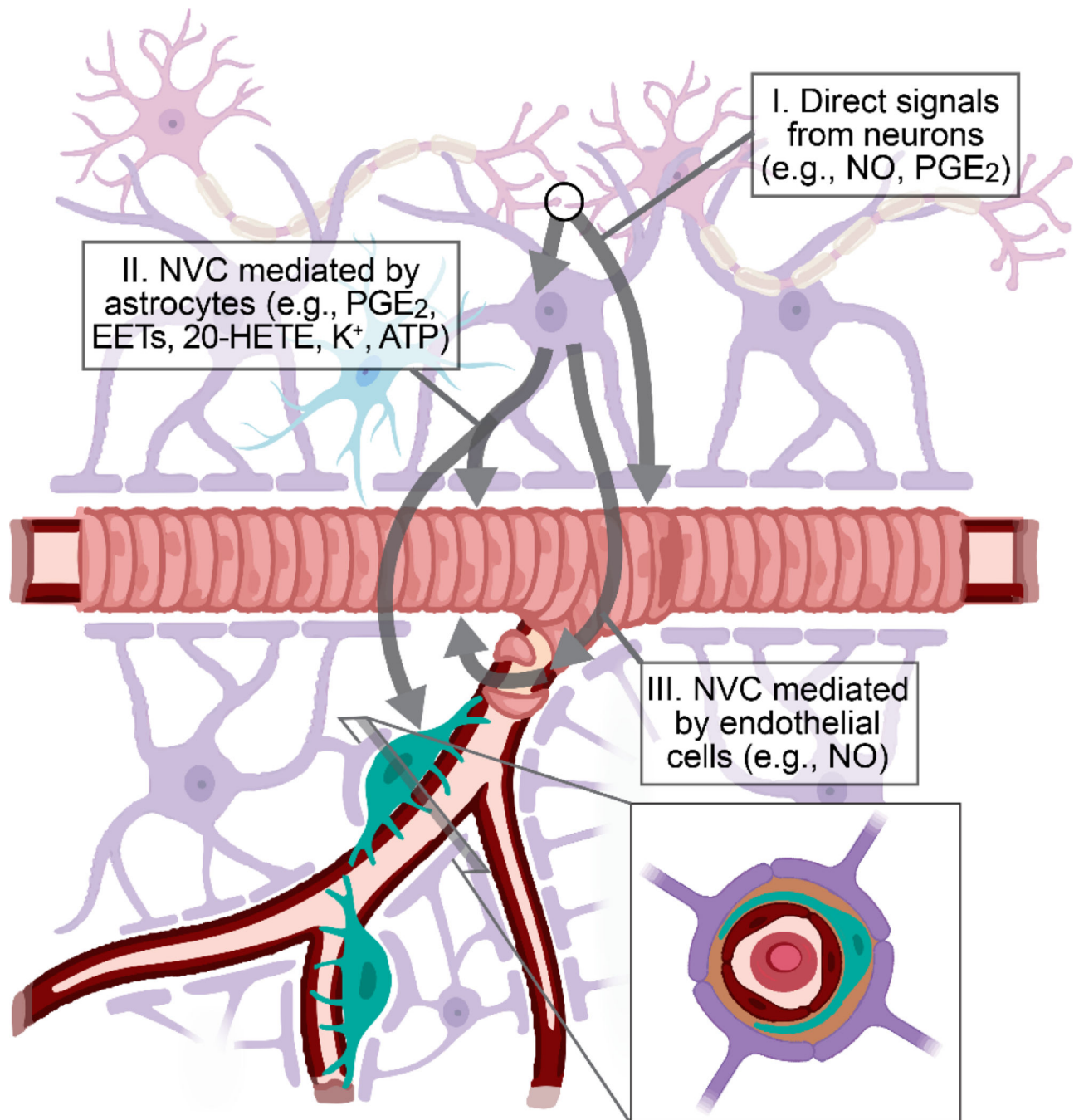
Figure 4.
Neurovascular coupling (NVC) is the phenomenon by which active neurons increase local blood flow to satisfy their energy demands. During synaptic activity (circled), post-synaptic neurons (magenta) can directly release vasoactive substances onto vessels to change the luminal diameter (I). Synaptic activity can also stimulate astrocytes (purple) to release vasoactive substances via their endfeet (II). Vascular endothelial cells (red) may also respond to signals from astrocytes, and perhaps neurons, to release vasoactive substances onto vessels (III). Regulation of lumenal diameter at arterioles occurs via signaling from neurons

and endothelial cells to vascular smooth muscle cells (pink), while astrocytes can mediate NVC by signaling to capillary pericytes (green) and modulate the response of arteriolar smooth muscle cells. Further, endothelial cells, pericytes, and vascular smooth muscle cells can also propagate the spread of vascular responses via gap junctions to mediate regional changes in CBF. Through this coordinated intercellular signaling, NVC components function as a dynamic functional unit to tightly regulate brain blood flow. Inset shows positioning of astrocytes, pericytes, basement membrane (brown), and endothelial cells within a capillary cross-section. Created with BioRender.com.
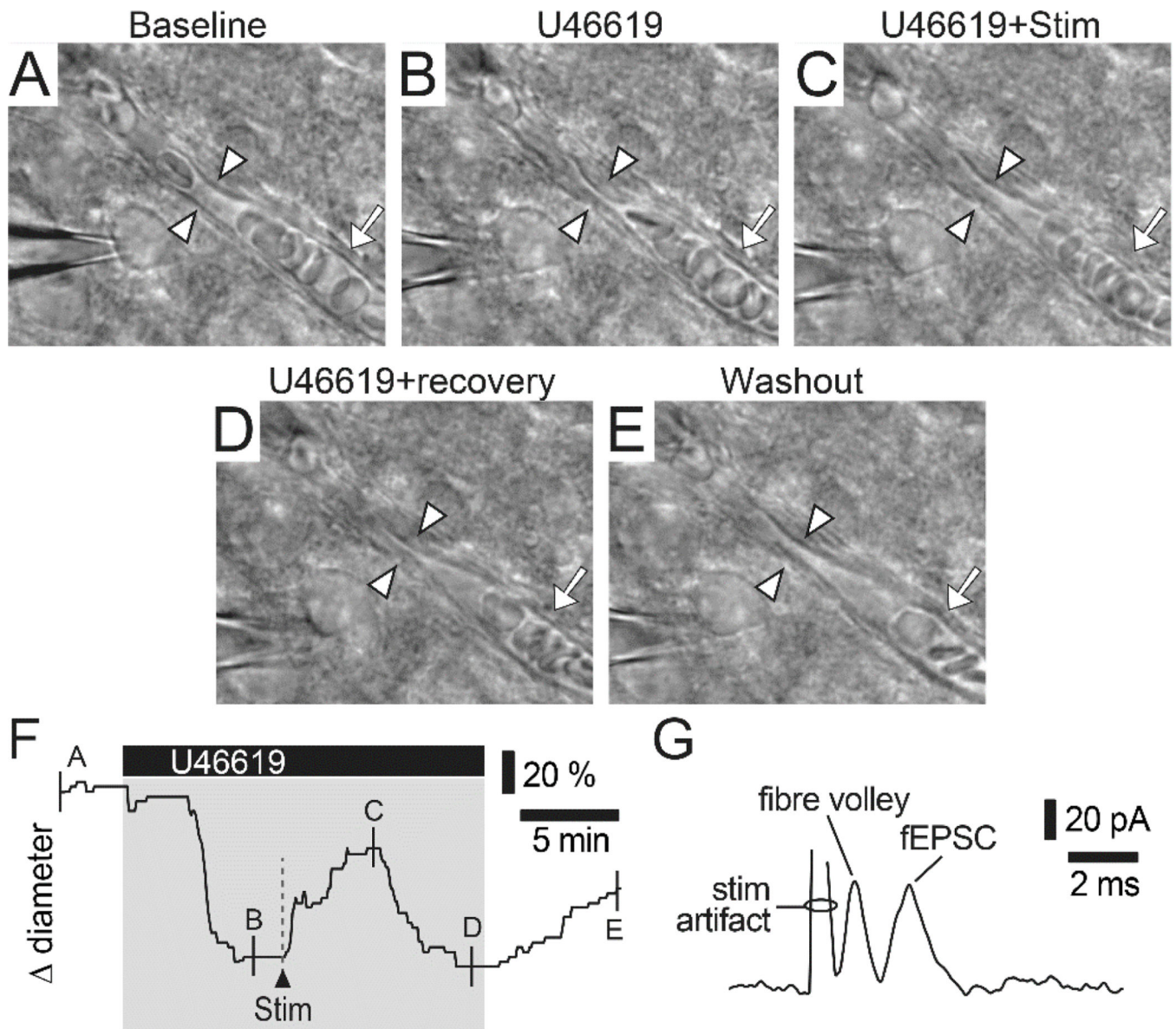
Figure 5.

Neuronal stimulation evokes dilation of capillaries in the cortex. (A-E) A 300-μm-thick acute rat brain slice preparation showing a cortical capillary and the surrounding neuropil. A recording electrode for measuring field potentials is also visible. Successive images show the response of the capillary to the preconstrictor U46619 (B), electrical stimulation of cortical layer I/II axons (C), recovery from stimulation-evoked response (D) and after washout of U46619 (E). (F) The diameter of the capillary region marked by arrowheads in **A-E** is plotted over time, demonstrating a strong constriction in response to the thromboxane analog U46619 (200 nM), followed by a brief dilation in response to neuronal stimulation. Washing out U46619 reverses the constricted tone (end of trace). Notably, the changes in capillary diameter occur only in a restricted segment while the neighboring region remains undisturbed (arrow). Such a response is characteristic of pericyte-mediated diameter changes. This response is blocked by inhibitors of action potentials and post-synaptic

activity, indicating that it is dependent on neuronal activity (data not shown, see (52) for more details). (G) A field recording of neuronal activity evoked by electrical stimulation of layer 1/II in the same experiment (detected by the recording electrode visible in **A**). The fiber volley (compound action potentials) and the field excitatory post-synaptic current are clearly visible. A 3 s, 20 Hz stimulus train was applied to induce the capillary response shown in **F**.