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Review

The Neurovascular Unit Coming of Age: A Journey through Neurovascular Coupling in Health and Disease

Costantino Iadecola[1,*]
[1]Feil Family Brain and Mind Research Institute, Weill Cornell Medicine, New York, NY 10065, USA
*Correspondence: coi2001@med.cornell.edu
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The concept of the neurovascular unit (NVU), formalized at the 2001 Stroke Progress Review Group meeting of the National Institute of Neurological Disorders and Stroke, emphasizes the intimate relationship between the brain and its vessels. Since then, the NVU has attracted the interest of the neuroscience community, resulting in considerable advances in the field. Here the current state of knowledge of the NVU will be assessed, focusing on one of its most vital roles: the coupling between neural activity and blood flow. The evidence supports a conceptual shift in the mechanisms of neurovascular coupling, from a unidimensional process involving neuronal-astrocytic signaling to local blood vessels to a multidimensional one in which mediators released from multiple cells engage distinct signaling pathways and effector systems across the entire cerebrovascular network in a highly orchestrated manner. The recently appreciated NVU dysfunction in neurodegenerative diseases, although still poorly understood, supports emerging concepts that maintaining neurovascular health promotes brain health.

The brain, an organ of unparalleled sophistication, seems to have a fundamental design glitch: it consumes a large amount of energy but lacks a reservoir to store fuel for use when needed. Therefore, the brain receives energy substrates, primarily oxygen and glucose, "on the fly" through its blood supply. Considering the dynamic and regionally diverse energy requirements imposed by brain activity, blood flow needs to reach the brain at the right time and place and in the right amount. Failure to do so has disastrous consequences. Complete interruption of the cerebral blood supply for more than a few minutes (for example, when a cerebral artery is occluded in stroke or after pump failure in cardiac arrest) causes irretrievable brain damage and death. On the other hand, if flow is not completely stopped but is reduced or not well matched to the energy demands of the tissue, then more subtle brain alterations ensue, leading to chronic brain injury in vulnerable areas often associated with cognitive impairment (Iadecola, 2013).

There has been a long-standing interest in how the brain regulates its own blood supply, driven not only by the desire to gain a better understanding of the harmful effects of cerebrovascular insufficiency but also by the early realization that regional changes in cerebral blood flow (CBF) may provide a window on brain function. This large body of work spanning over almost two centuries revealed a remarkable complexity in the interaction between the brain and its vessels that is unmatched by the vasculature in other organs.

In this context, the concept of the neurovascular unit (NVU) emerged from the first Stroke Progress Review Group meeting of the National Institute of Neurological Disorders and Stroke of the NIH (July 2001) to emphasize the unique relationship between brain cells and the cerebral vasculature (https://www.ninds.nih.gov/About-NINDS/Strategic-Plans-Evaluations/Strategic-Plans/Stroke-Progress-Review-Group). Although the vital importance of cerebral blood vessels in brain health had long been appreciated, hard-core neuroscientists considered brain cells and cerebral blood vessels distinct entities. Such a dichotomy led to the tacit assumption that, unless the delivery of blood flow to the brain was critically compromised, neurons had little to do with the vasculature and vice versa. Similarly, a rigid distinction was placed between "neurodegenerative diseases" (e.g., Alzheimer's disease [AD]) and cerebrovascular diseases (e.g., stroke), so that these conditions were considered mutually exclusive and mechanistically unrelated. The NVU concept challenged these assumptions and emphasized the symbiotic relationship between brain cells and cerebral blood vessels, calling attention to their developmental, structural, and functional interdependence in health and disease.

The neuroscience community embraced the concept enthusiastically, and the NVU has drawn increasing interest, as attested by the dramatic rise in the number of yearly citations over the past decade (Figure 1). New technologies have enabled investigators to delve deeper into the functions of the NVU in health and disease. Consequently, the NVU has taken center stage in all facets of normal brain function and in the pathobiology of a wide variety of brain diseases.

Here we will assess the current state of knowledge of the NVU, focusing on one of its prototypical functions: the coupling between neural activity and CBF (neurovascular coupling). To this end, we will review and integrate observations related to the contribution of different NVU cells to neurovascular coupling in an attempt to provide a comprehensive picture of how activation of restricted groups of neurons is able to generate hemodynamic responses engaging the entire cerebrovascular tree, from capillaries deep in the substance of the brain to pial arteries on the

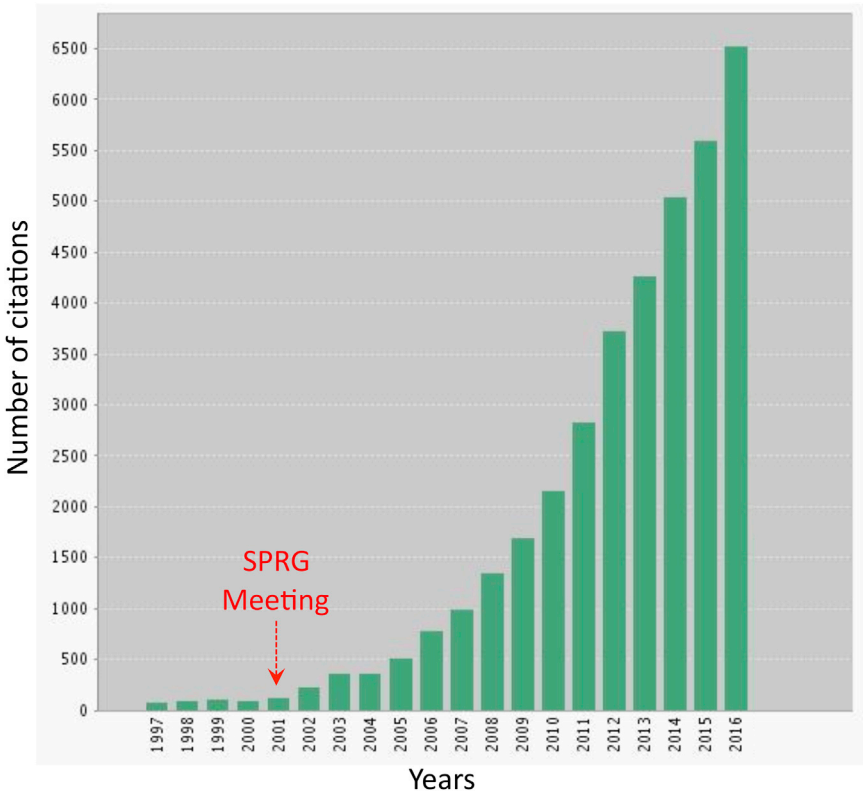

Figure 1. Citations of the NVU in the Literature from 1997 to 2016
The search term "neurovascular unit" was used in the Web of Science database (Thompson Reuters). Since the Stroke Progress Review Group (SPRG) meeting in 2001, a dramatic rise in the number of citations can be observed. Prior to 2001, the term was often used for critical care units combining neurology and neurosurgery.

brain surface. The contribution of NVU dysfunction to brain pathologies will also be examined, focusing on neurodegenerative diseases, highly prevalent conditions previously considered unrelated to vascular factors. Finally, a critical assessment of the evidence will be provided in an effort to flesh out unresolved issues and outstanding questions.

Neurovascular Interactions through the Ages

Throughout the 18th and 19th century, the prevailing view was that the brain was not involved in regulating its own blood supply, thought to be controlled exclusively by the systemic circulation (Friedland and Iadecola, 1991). Hints of change can be appreciated at the end of the 19th century in the work of Roy and Sherrington (1890) in animals and Angelo Mosso in humans (Figure 2; Mosso, 1880). Roy and Sherrington (1890), based on the effect of injecting brain extracts into the carotid arteries of animals equipped with a device to monitor intracranial pressure, proposed that metabolites produced by neuronal activity diffuse to local blood vessels to increase blood flow to the activated brain. Even earlier, Mosso (1880), studying individuals with skull defects, had discovered that somatic, mental, or emotional stimuli induce changes in brain volume attributable to hemodynamic factors. This body of work, albeit based on indirect indices of CBF, not measurable at the time, raised the possibility that brain activity was able to elicit changes in cerebral perfusion. These prescient observations were dismissed and quickly forgotten, and the idea that the brain could regulate its own blood supply did not reemerge until decades later (Friedland and Iadecola, 1991). In the late 1930s, Schmidt and Hendrix (1938) demonstrated in the cat that illuminating the eye increases visual cortex temperature, a proxy for cerebral perfusion (Figure 2). Remarkably, the work of Roy and Sherrington (1890) was not mentioned in this and related publications of this period, resurfacing in the literature only in the 1960s (Friedland and Iadecola, 1991). A separate line of investigations in the early 20th century revealed that vascular density differs across the brain and suggested that the density of vessels correlated with regional levels of energy consumption and functional activity (Craigie, 1945). Consistent with this hypothesis, it was found that sustained increases in neural activity lead to increases in local vascular density (Craigie, 1945). These early observations led to the idea that the vascular changes induced by neural activity were needed to fulfill the increased metabolic needs of active brain regions, providing initial evidence of the critical relationship linking brain function to the cerebral blood supply.

Kety and Schmidt (Kety, 1950) pioneered a method to measure CBF quantitatively using nitrous oxide as a tracer and demonstrated that CBF increases with global brain hyperactivity (for example, in anxiety or hyperthyroidism) and is reduced when brain activity is suppressed (e.g., in coma). However, this method provided an average of CBF for the whole brain and could not assess regional cerebral perfusion and its relationship to local neural activity. The development of autoradiographic techniques using diffusible tracers to measure CBF quantitatively and in multiple brain regions provided evidence that neural activity evokes CBF increases highly restricted to the activated network (Figure 2). Building on this work, Lassen et al. (1978) developed methods to measure regional CBF in the human brain using intracarotid injection of radioactive tracers and external detection by a γ-camera (Figure 2). The subsequent development of positron emission tomography and MRI-based methods allowed investigators to monitor CBF in humans with great spatial resolution (Raichle and Mintun, 2006). In particular, the discovery of the blood oxygenation level-dependent (BOLD) effect, reflecting excess CBF delivery relative to local oxygen consumption, enabled the non-invasive detection of activity-dependent hemodynamic signals across the behaving human brain (Raichle and Mintun, 2006). While advancing our understanding of the neurobiology of human behavior, MRI-based functional brain imaging has also firmly

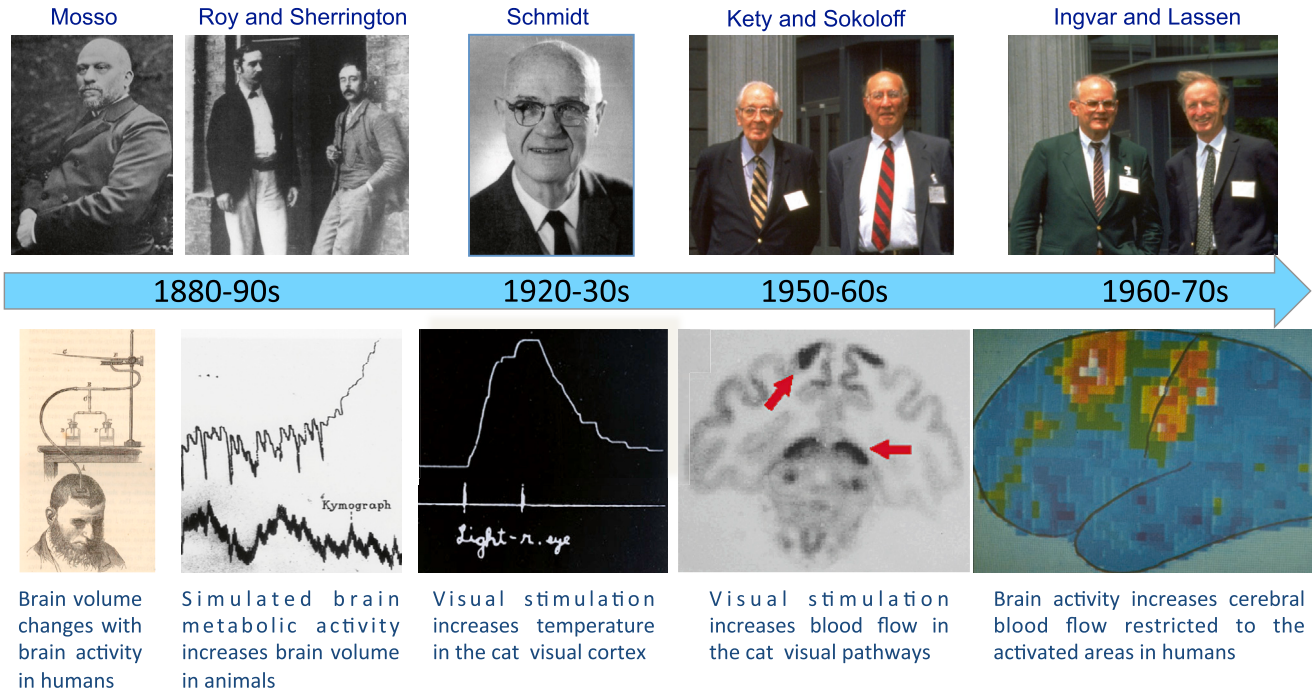

Figure 2. Historical Overview of the Concept of Neurovascular Coupling

Pioneers in establishing the concept of neurovascular coupling are shown at the top, and examples of their work are presented below the timeline. Studies in the late 1800s, by Angelo Mosso and Charles Roy and Charles Sherrington, hinted at the possibility that brain activity increases cerebral blood flow. The bottom images illustrate the apparatus used by Mosso to record changes in brain volume in patients with skull defects (Mosso, 1880) and Roy and Sherrington's recordings of brain expansion in response to intracarotid infusion of a brain extract (Roy and Sherrington, 1890). In the 1930s, Carl Schmidt recorded increases in temperature in the cat visual cortex by shining light into the eye. The original recording is shown at the bottom (Schmidt and Hendrix, 1938). In the 1950–1960s, Seymour Kety and Lou Sokoloff developed autoradiographic methods to image regional CBF during neural activation. The bottom image shows the increase in CBF produced in the calcarine cortex and superior colliculus by visual stimulation (Freygang and Sokoloff, 1958). In the 1960–1970s, David Ingvar and Niels Lassen developed methods to measure regional CBF in the human brain using radioactive tracers and external detection. The bottom image illustrates the increase in CBF produced by hand movement in the contralateral sensory motor cortex and supplementary motor area (Lassen et al., 1978), marking the birth of functional brain imaging.

established the concept that cerebrovascular function is intimately related to brain activity.

Structural Diversity of the NVU along the Cerebrovascular Tree

The association between neurons and vessels varies significantly across the cerebrovascular network. Branching off the circle of Willis at the base of the brain, cerebral vessels run on the brain surface within the subarachnoid space (pial arteries and arterioles), forming a highly collateralized network (Blinder et al., 2013; Cipolla, 2010) Figures 3 and 4). Pial arterioles have multiple layers of smooth muscle cells (SMCs) separated from the endothelium by a prominent elastic lamina (Roggendorf and Cervós-Navarro, 1977; Figure 4). Although not in direct contact with brain cells, pial vessels are richly innervated by nerve fibers originating from peripheral autonomic and sensory ganglia, including the superior cervical, sphenopalatine, and trigeminal ganglia (Hamel, 2006; Figure 4). These nerve fibers, which express a wide variety of neurotransmitters and neuropeptides, run at the adventitia-media border, forming an intricate mesh enveloping the vessels (Iadecola et al., 1993). Pial arteries dive into the substance of the brain surrounded by an extension of the subarachnoid space, the perivascular space, a virtual space delimited by the vascular basement membrane and the glia limitans (Virchow-Robin space) (Jones, 1970; Zhang et al., 1990; Figure 4). Penetrating arterioles are endowed with a thinner SMC layer that eventually becomes a single layer (Dahl, 1973; Roggendorf and Cervós-Navarro, 1977). At this level, perivascular nerves are more sparse, and the elastic lamina becomes less prominent (Roggendorf and Cervós-Navarro, 1977). The perivascular compartment contains several cell types, including perivascular macrophages (PVMs), Mato cells, pial cells, and mast cells, among others, as well as nerve and collagen fibers (Zhang et al., 1990; Figure 4). As arterioles penetrate deeper into the brain (intraparenchymal arterioles), the glial membrane and the vascular basement membrane fuse together, obliterating the perivascular space (Jones, 1970; Zhang et al., 1990; Figure 5). At this level, arterioles have a single or discontinuous layer of SMCs, lack perivascular nerves, and are encased in astrocytic endfeet (Roggendorf and Cervós-Navarro, 1977; Figure 5). Occasionally, axonal terminals or dendrites are seen in close apposition to the vascular basement membrane, often with an intervening glial leaflet (Iadecola et al., 1993; Wang et al., 2005). These neural processes originate from interneurons or from subcortical and brain stem nuclei projecting to the cortex, such as the basal forebrain cholinergic nuclei, locus coeruleus, and raphe magnus (Cohen et al., 1996; Hamel, 2006; Iadecola,

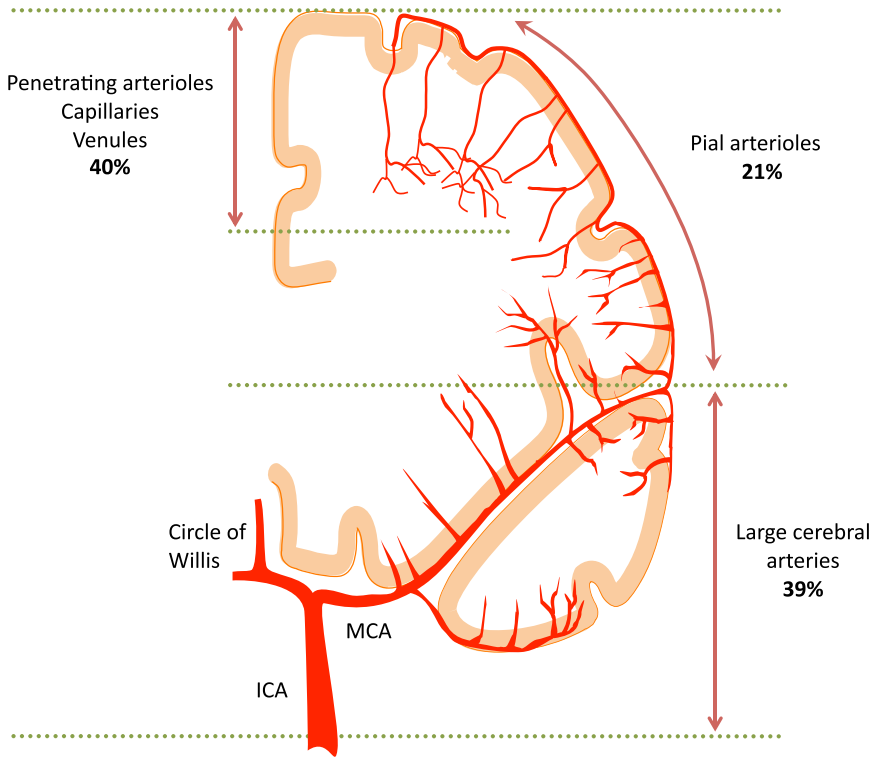

Figure 3. Anatomy of the Cerebrovascular Tree and Segmental Vascular Resistance
The internal carotid artery (ICA) enters the skull and merges with branches of the vertebral arteries to form the circle of Willis at the base of the brain. The middle cerebral artery (MCA) takes off from the circle of Willis and supplies a large territory of the cerebral cortex. The MCA gives rise to pial arteries and arterioles that run on the surface of the brain, forming a heavily interconnected network from which arterioles penetrating into the substance of the brain originate (penetrating arterioles). Penetrating arterioles give rise to the capillary network, which feeds into the venous system returning the blood to the heart. The component (percentage) of the total vascular resistance each cerebrovascular segment offers to blood flow, which reflects their potential for flow control, is indicated. Therefore, vessels outside of the brain are responsible for 60% of the resistance and vessels within the substance of the brain for 40% (based on data from Stromberg and Fox (1972) and De Silva and Faraci (2016).

1993). Endothelial cells extend protrusions through the basal lamina into SMCs (myoendothelial projections) enriched with gap junctions (Dahl, 1973; Longden et al., 2016). As arterioles transition into cerebral capillaries (Figure 5), SMCs are replaced by pericytes, mural cells embedded into the endothelial basement membrane, covering approximately 30% of the vascular surface and occasionally extending "peg and socket" protrusions into endothelial cells (Armulik et al., 2011; Dahl, 1973; Damisah et al., 2017). Like in arterioles, the circumference of the capillaries is covered by astrocytic endfeet, although neural processes can be at times observed next to the capillary basal lamina.

Neurovascular Coupling

One of the better-appreciated and most studied roles of the NVU pertains to the link between neural activity and CBF. In the "resting" brain, CBF varies in proportion to the energy consumption of each brain region. Thus, flow is higher in regions with higher energy utilization (e.g., the inferior colliculus) and lower in regions with lower energy use, like the white matter (Sokoloff, 2013). Furthermore, increases in neural activity lead to increases in CBF highly restricted to the activated areas (functional hyperemia) (Chaigneau et al., 2003; Cox et al., 1993; Freygang and Sokoloff, 1958; Iadecola, 1993; LeDoux et al., 1983). The spatial and temporal correspondence between neural activity and the associated hemodynamic response is sufficiently precise, at least at the regional level, that the flow response can be used to map brain function (functional brain imaging) (Raichle and Mintun, 2006). However, at the microvascular level, the CBF increase in some regions exceeds the area of activation. For example, in the auditory, visual, and cerebellar cortex, the vascular response does not faithfully match the activated area (Harrison et al., 2002; Iadecola et al., 1997; O'Herron et al., 2016), whereas in the olfactory bulb, there is close overlap between the two (Chaigneau et al., 2003). Non-overlapping vascular and neuronal topology in the neocortex (Blinder et al., 2013) and retrograde vasodilatation (discussed later in this review) are likely responsible for such lack of fidelity (Chen et al., 2014; Iadecola et al., 1997; Longden et al., 2017). Because hemodynamic signals are the most powerful tool at our disposal for functional brain mapping, their spatial alignment with neural activity is becoming increasingly relevant as the resolution of fMRI increases.

The close relationship between neuronal and vascular function is also illustrated by the profound changes in neurovascular coupling that take place in the developing brain. In rodents before post-natal day 11, brain activation is not associated with sustained CBF increases, leading to an absent or negative BOLD signal (Colonnese et al., 2008; Kozberg et al., 2013). The lack of a flow response may be important for vascular development because the resulting hypoxia is a critical stimulus for cerebral angiogenesis (Lacoste and Gu, 2015). However, in the second and third week of life, neural activity leads to increasingly larger hemodynamic responses, resulting in progressively larger BOLD signals (Colonnese et al., 2008). The transition between the second and third post-natal week corresponds to dramatic neurovascular and systemic changes, including increases in vascular density, synaptogenesis, myelination and connectivity, energy metabolism, resting CBF, and sensitivity of the cerebral microcirculation to vasoactive stimuli (Colonnese et al., 2008; Engl et al., 2017; Goyal et al., 2014; Nehlig et al., 1989) as well as systemic blood pressure (Kozberg et al., 2013). Although the factors responsible for the developmental shift in neurovascular coupling remain to be elucidated, the findings attest to

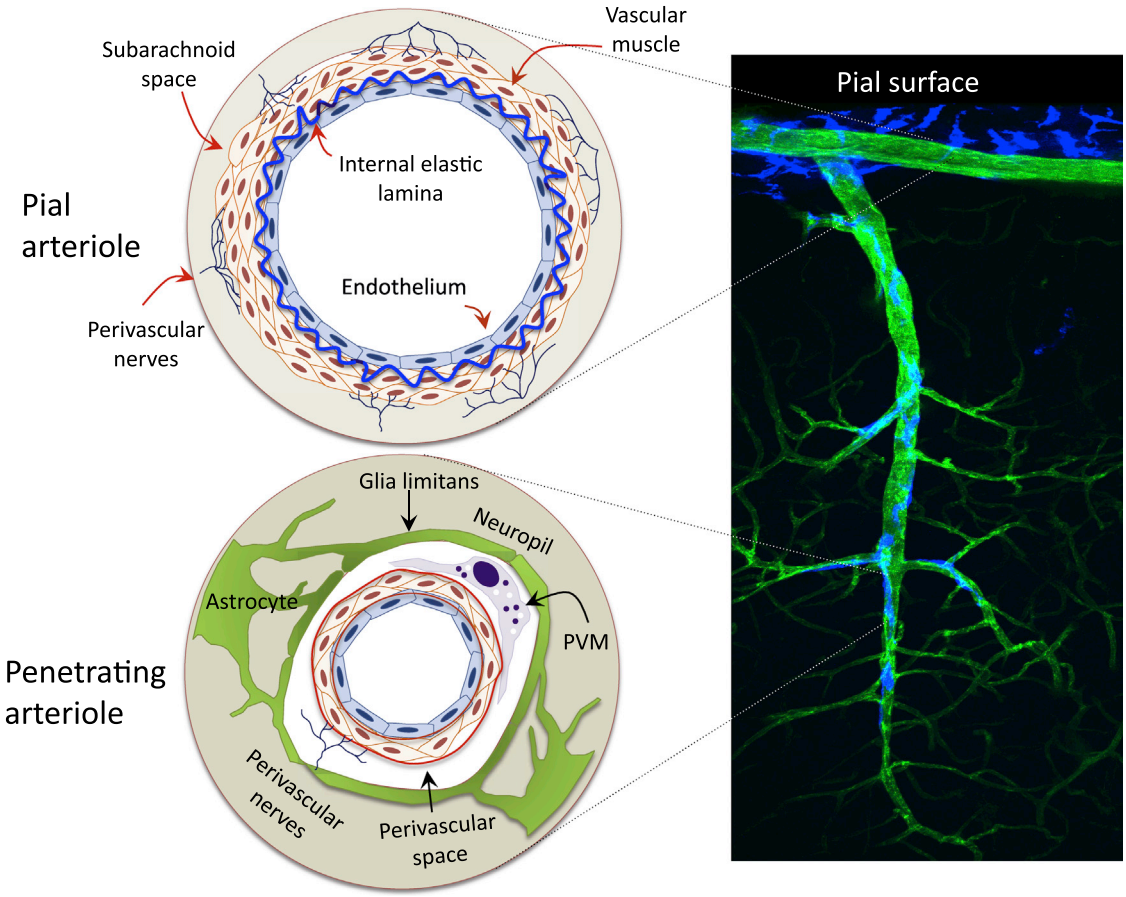

Figure 4. Neurovascular Associations along the Cerebrovascular Tree: Pial Arteries and Penetrating Arterioles
A pial arteriole on the cortical surface giving rise to a penetrating arteriole is shown on the right. Pial arterioles have a thick coat of SMCs, are surrounded by the subarachnoid space, and are densely innervated by nerve fibers originating from cranial autonomic and sensory ganglia, such as the sympathetic, parasympathetic, and trigeminal ganglia. Penetrating arterioles enter the substance of the brain and are surrounded by a perivascular space containing several cell types, including perivascular macrophages (PVMs). In the right panels of this figure and Figure 5 the vasculature (green) was visualized with the lipophilic dye DiO injected into the circulation. PVMs and meningeal macrophages (blue), located in the perivascular and subarachnoid space, respectively, were visualized by CD206 immunocytochemistry.

the profound influence of key structural, metabolic, and functional changes in the developing brain on shaping cerebral perfusion.

Why Does CBF Increase during Brain Activity?

The tight coupling between neural activity and CBF is thought to reflect the lack of energy reserves in the brain requiring a well-timed delivery of oxygen and glucose restricted to the activated areas. The flow increase may also be needed to clear the brain of potentially toxic by-products of brain activity (for example, lactate, CO_2, the amyloid-β peptide [Aβ], and tau) as well as for brain temperature regulation (Tarasoff-Conway et al., 2015; Zhu et al., 2006). These considerations have led to a "feedback" model in which the metabolic and clearance needs of the tissue drive the delivery of blood flow. Indeed, metabolic by-products of brain activity include potent vasodilators such as adenosine, CO_2, H^+, and lactate, which could potentially initiate the flow response (Freeman and Li, 2016; Ko et al., 1990). However, other evidence supports a "feedforward" model in which the increase in CBF is not driven by the tissue metabolic state. As revealed by the transformative work of Fox and Raichle (reviewed in Raichle and Mintun, 2006), the increase in CBF is greater than the need of the tissue oxygen, resulting in excess delivery of O_2. In addition, increases in CBF also occur under conditions of excess oxygen and glucose, suggesting that activation-induced depletion of these energy substrates does not drive the flow increase (reviewed in Attwell and Iadecola, 2002). Based on this evidence, it was suggested that CBF delivery is regulated by a feedforward mechanism driven by neurovascular signaling pathways, resulting in the release of vasoactive by-products of synaptic activity, such as K^+, nitric oxide (NO), and prostanoids (Attwell et al., 2010; Attwell and Iadecola, 2002; Drake and Iadecola, 2007).

In the end, these two models may not be mutually exclusive (Figure 6). Microvascular studies showing that, at the onset of neural activity, there is a reduction in O_2 and/or glucose preceding the CBF increase have rekindled the view that metabolic factors may also play a role in neurovascular coupling (Freeman and Li, 2016), but there might be regional diversity in their

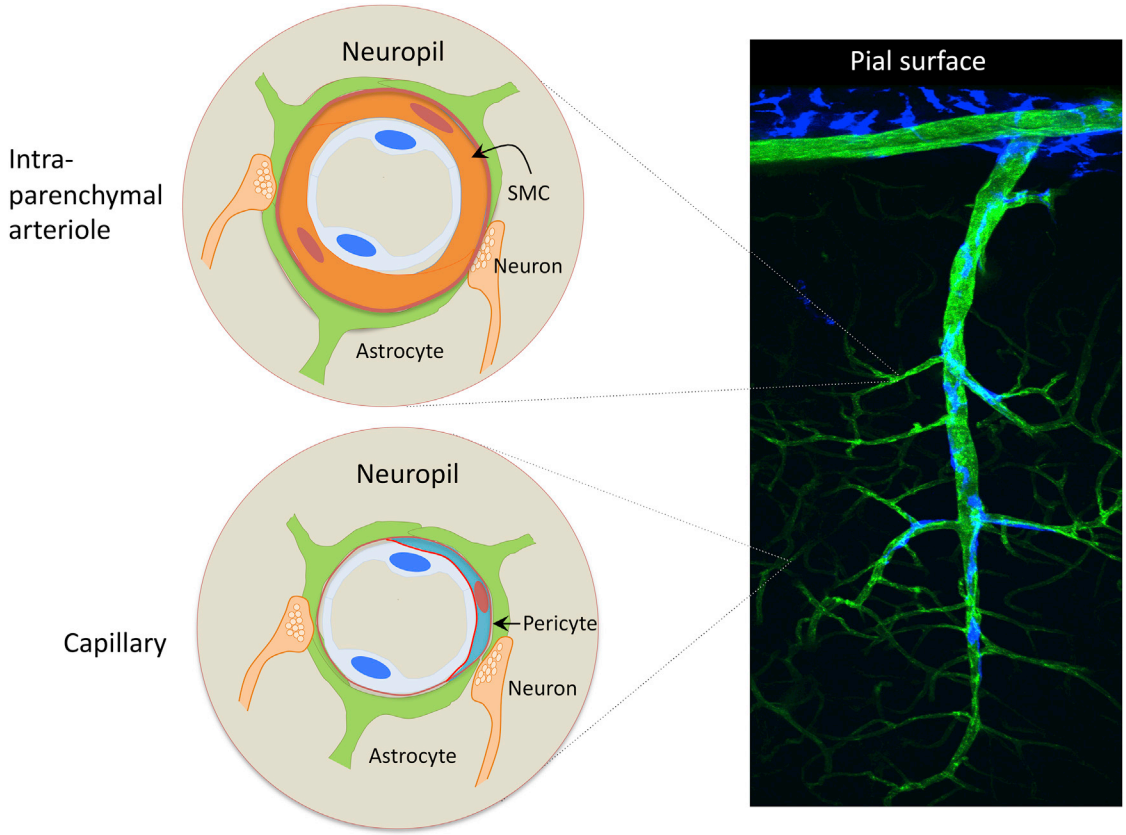

Figure 5. Neurovascular Associations along the Cerebrovascular Tree: Intraparenchymal Arterioles and Capillaries
As the arterioles penetrate deeper into the brain, the perivascular space disappears, and the vessels become encased in astrocytic endfeet (intraparenchymal arterioles). Endowed with a single layer of SMCs, intraparenchymal arterioles lack perivascular nerves. Occasionally, neural processes originating from local neurons or subcortical pathways projecting to the cerebral cortex terminate near the vessel. Capillaries are devoid of SMCs but are endowed with pericytes, which are fully enclosed by the basement membrane of the endothelium. Occasional neurovascular contacts similar to those seen in intraparenchymal arterioles are also observed.

involvement. Basal oxygen levels vary widely in different brain regions (Lyons et al., 2016), and, depending on local vascular topology and the intensity of the activating stimulus, regional hypoxia may develop, promoting vasodilatation of local vessels. Another hint that intravascular hypoxia may contribute to the flow response is provided by experiments demonstrating that, at the beginning of activation, a dip in intravascular O_2 leads to increased deformability of red blood cells, which improves microvascular rheology and increases capillary flow (Wei et al., 2016). These lines of evidence collectively suggest that a feedforward mechanism may trigger an exaggerated flow response driven by neurovascular signaling, but feedback mechanisms may also be in place to adjust CBF delivery more closely to the metabolic needs of the tissue. In support of this hypothesis, the vascular response during sustained neural activation tends to peak at the onset, subsequently readjusting toward a lower level (Drew et al., 2011; Freeman and Li, 2016; Ngai et al., 1988). Therefore, both metabolism-dependent (feedback) and independent (feedforward) mechanisms may be involved in functional hyperemia, depending on the timing, intensity, and duration of the activation as well as the brain region and the brain's developmental stage. Although the mechanistic details remain unclear, the evidence suggests that the main function of neurovascular coupling is to maintain the homeostasis of the cerebral microenvironment by delivering the energy substrates needed to initiate and sustain neural activity while clearing potentially toxic by-products of brain metabolism, including heat.

It has also been suggested that, in a reversal of roles, the hemodynamic response could influence the neural response via mechanical, thermal, or chemical effects on astrocytes (hemo-neural hypothesis or vasculo-neuronal coupling) (Kim et al., 2016; Moore and Cao, 2008). This concept received support from a study of brain slices in which increases or decreases in transmural pressure/flow in penetrating arterioles was found to suppress or enhance, respectively, pyramidal cell activity through mechanosensitive transient receptor potential vanilloid receptor type 4 (TRPV4) channels in astrocytes (Kim et al., 2016). These data suggest an autoregulatory mechanism whereby intravascular pressure, and possibly tissue pressure, would directly modulate resting neural activity via astrocytic mechanosensors. The mechanisms and implications of these intriguing observations need further exploration.

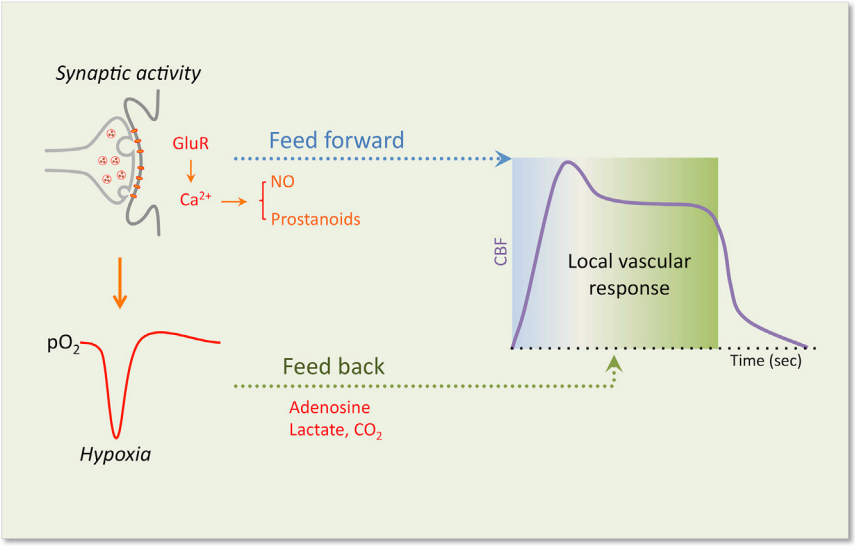

Figure 6. Potential Feedforward and Feedback Mechanisms Driving the Local Vascular Response Evoked by Synaptic Activity

Glutamate released by synaptic activity activates post-synaptic glutamate receptors (GluRs), leading to activation of Ca^{2+}-dependent signaling pathways, resulting in the release of vasoactive factors that may drive the initial feedforward component (metabolism-independent) of the local vascular response in arterioles and capillaries. At the same time, a reduction in tissue O_2 caused by the increased energy consumption induced by activation leads to the accumulation of vasoactive metabolic by-products that may drive a secondary feedback component (metabolism-dependent) to better match the flow response to the metabolic needs of the tissue.

Cellular Bases of Neurovascular Coupling

During the previous decades, most studies focused on the mediators responsible for neurovascular coupling. This work suggested that multiple agents released by neural activity are involved. However, it was also recognized that local release of vasoactive mediators could not account for hemodynamic changes in upstream vascular segments remote from the activated site. The availability of tools to investigate neurovascular interactions at the cellular level has provided greater insight into the cellular processes initiating, transmitting, propagating, and implementing the vascular response (Table 1). These will be examined next.

Neurons: Initiation of the Local Vascular Response. Neurons regulate CBF by generating signals that, either directly or through interposed cells, act on local blood vessels to initiate the vascular response. Glutamatergic synaptic activity activates post-synaptic N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazol epropionic acid (AMPA) receptors, leading to increases in intracellular Ca^{2+} and activation of Ca^{2+}-dependent enzymes such as neuronal NO synthase (nNOS) and cyclooxygenase 2 (COX-2), which produce potent vasodilators (NO and prostanoid, respectively) (Attwell et al., 2010; Lecrux and Hamel, 2016; Figure 6). At the same time, glutamate acts on metabotropic glutamate receptors in astrocytes, triggering Ca^{2+} increases in these cells and production of vasoactive agents (see below). Adenosine, a potent vasodilator produced from ADP by ecto-nucleotidases, also contributes to the increase in CBF (Iadecola, 1993; Ko et al., 1990). In addition, a role for ATP acting on purinergic receptors has been postulated both in the retina and neocortex (Biesecker et al., 2016; Mishra et al., 2016). Finally, ionic changes evoked by neural activity, in particular the increase in extracellular K^+, a vasodilator at concentrations of less than 12 mM (Filosa et al., 2006), could also participate (see Astrocytes: Signal Transmission to the Local Vasculature and Endothelial Cells: Retrograde Propagation of Vasomotor Responses).

Recent studies have begun to shed light on the role of specific neuronal types and their mediators in neurovascular coupling. In whisker barrel cortex hyperemia induced by stimulation of the contralateral facial whiskers, pyramidal cells expressing the prostanoid-synthesizing enzyme COX-2 are activated from thalamic afferents (Lecrux et al., 2011) and contribute to the vascular response through prostaglandin E_2 (PGE_2) acting on EP2 and EP4 receptors (Lecrux and Hamel, 2016). These pharmacological observations are consistent with earlier findings in transgenic mice that COX-2, but not COX1, is required for the full expression of the vascular response (Niwa et al., 2000a; 2001a). Somatosensory stimuli also activate γ-aminobutyric acid (GABA) interneurons (Lecrux et al., 2011). Increasing evidence suggests that interneurons may be critical for neurovascular coupling. Interneurons are enriched with vasoactive neurotransmitters and neuromodulators and have close associations with cerebral microvessels (Cauli and Hamel, 2010). In the cerebral cortex and cerebellum, inhibitory interneurons are able to modify vascular diameter in brain slices through the vasoconstrictor neuropeptide Y (NPY) or the vasodilator NO (Cauli et al., 2004; Rancillac et al., 2006). In vivo, optogenetic stimulation of GABAergic interneurons induces biphasic hemodynamic responses, dilatation followed by constriction, closely resembling those induced by somatosensory activation in this preparation (Uhlirova et al., 2016b). Although the constrictor response was attributed to NPY, the mediator(s) responsible for the dilatation could not be identified, but a role of NO or vasoactive intestinal polypeptide (VIP) was suggested (Uhlirova et al., 2016b). Interestingly, optogenetic activation of GABAergic interneurons increases CBF even when glutamatergic or GABAergic activity is pharmacologically blocked (Anenberg et al., 2015), suggesting that these cells can influence flow independently of the activity of their neuronal network, but the mechanisms of the vasodilatation remain to be established. Although interneurons have the potential to modulate CBF, evidence for their participation in neurovascular coupling to physiological stimuli is limited. In mice deficient in the cell cycle enzyme D2 cyclin, which lack stellate interneurons in the cerebellar molecular layer, the increase in blood flow produced by somatosensory activation of the cerebellar crus II is suppressed (Yang et al., 2000).

Table 1. Key Cellular Steps Underlying Neurovascular Coupling in Neocortex

Step	Cell Type	Mediator/Mechanism	Cellular Targets	Vascular Segment
Initiation	principal neuron	NO	astrocytes	capillaries
	interneuron	neurotransmitters	endothelium	arterioles
		neuropeptides	pericytes	
		prostanoids	SMCs	
		adenosine	red blood cells	
		ATP		
		K^+		
		hypoxia		
Modulation/spatial shaping	subcortical projection neurons (LC, BF, RM)	acetylcholine	neurons	capillaries
	perivascular innervation (?)	serotonin	astrocytes	arterioles
		noradrenalin	endothelium	pial arteries
		neuropeptides	pericytes	
			SMCs	
Neurovascular transmission	astrocytes	K^+	endothelium	capillaries
		prostanoids (?)	pericytes	arterioles
		adenosine (?)	SMCs	
Retrograde propagation	endothelium	gap junctions	endothelium	capillaries
	pericytes (?)	myoendothelial junctions	pericytes (?)	arterioles
	astrocytes (?)	myogenic response/FMV	astrocytes (?)	
		hyperpolarizing factor (?)	SMCs	
Implementation	SMCs	hyperpolarization	–	capillaries (?)
	contractile mural cells (pericytes?)	$\downarrow Ca^{2+}$ or Ca^{2+} sensitivity		arterioles
		contractile proteins		pial arteries

BF, basal forebrain; LC, locus coeruleus; RM, raphe magnus; NO, nitric oxide.

Considering that stellate interneurons are enriched with nNOS and that nNOS-derived NO is the major determinant of functional hyperemia in the cerebellum (Rancillac et al., 2006; Yang et al., 1999, 2003; Yang and Iadecola, 1998), these findings are consistent with the hypothesis that stellate interneurons mediate the response by releasing NO. However, more direct evidence in cerebellum or elsewhere in brain is lacking.

Another aspect of the involvement of neurons in neurovascular coupling pertains to neurovascular projections arising from subcortical nuclei. The locus coeruleus, raphe, and basal forebrain send diffuse adrenergic, serotoninergic, and cholinergic projections, respectively, to the neocortex (Cohen et al., 1996; Hamel, 2006). These projections often terminate on astrocytic endfeet close to cerebral microvessels and are able to powerfully modulate cerebral perfusion (Cohen et al., 1996; Toussay et al., 2013; Zhang et al., 1995). In particular, activation of the basal forebrain cholinergic system increases neocortical CBF diffusely, an effect attributable to acetylcholine release and endothelial NO production and to activation of COX-2-expressing pyramidal cells and GABAergic interneurons (Lecrux and Hamel, 2016; Zhang et al., 1995). Although its lesion does not alter the changes in CBF induced by neural activity (Ibayashi et al., 1991), this pathway may contribute to maintaining resting CBF and modulate the increases in CBF produced by somatosensory activation (Iadecola et al., 1983; Lecrux and Hamel, 2016; Lecrux et al., 2017). On the other hand, cortical norepinephrine levels, which depend in large part on noradrenergic innervation from the locus coeruleus, have been shown to increase vasoconstrictor tone in the cerebral cortex and help focus oxygen delivery to the activated areas (Bekar et al., 2012). Therefore, these neurovascular pathways do not directly mediate vasodilatation in response to activation but, consistent with their broader role in neocortical neuromodulation (Lee and Dan, 2012), may contribute to shaping the spatial and temporal features of the hemodynamic response (Table 1).

Little is known about the participation of perivascular nerves arising from the cranial ganglia in neurovascular coupling, but surgical ablation or pharmacological inactivation of the sympathetic and parasympathetic innervation does not affect the magnitude of the pial arterial dilatation evoked by somatosensory activation (Ibayashi et al., 1991). However, these nerves may protect cerebral blood vessels from forced vasodilatation during seizures or acute hypertension (Bill and Linder, 1976; Mueller et al., 1979). Furthermore, they may also contribute to the development and integrity of the vascular wall (Eichmann and Brunet, 2014), a process requiring molecular signals distinct from those in peripheral arteries (Brunet et al., 2014). Interestingly, the perivascular innervation has been implicated in generating resting-state fMRI connectivity signals in surgically disconnected brain regions in humans (Warren et al., 2017).

This finding, if confirmed, would suggest a role of perivascular nerves in the synchronization of hemodynamic signals across the brain and require a re-evaluation of the significance of fMRI-based assessments of functional neural connectivity.

Astrocytes: Signal Transmission to the Local Vasculature. Because of their intimate association with both synapses and local microvessels, astrocytes are well positioned to link neural activity to microvascular function. Paulson and Newman (1987) proposed that astrocytes could influence blood flow by "siphoning" extracellular K^+ released by neural activity into endfeet abutting cerebral microvessels. However, subsequent studies in the retina of inward rectifier $K_{IR}4.1$ channel-null mice, in which glia K^+ siphoning is suppressed, showed that neurovascular coupling was still present, suggesting that this mechanism may not be involved in retinal arteriolar dilatation (Metea et al., 2007).

Zonta et al. (2003) provided evidence that Ca^{2+} increases in astrocytes, evoked by electrical stimulation, could influence the diameter of adjacent microvessels in brain slices through metabotropic glutamate receptors (mGluR) and COX reaction products (Zonta et al., 2003). Subsequent studies also using pharmacological approaches in brain slices extended these initial observations, demonstrating that increases in astrocytic Ca^{2+}, triggered by neural activity, photolysis of caged Ca^{2+}, or activation of mGluR5, have the potential to modulate microvascular diameter, producing constriction or dilatation depending on the oxygen levels (Gordon et al., 2008), resting vascular tone (Blanco et al., 2008), or extracellular K^+ (Girouard et al., 2010). The postulated sequence of events leading to vasodilation is that glutamate released by synaptic activity activates mGluR on astrocytes, leading to inositol triphosphate (IP_3) mobilization and Ca^{2+} release from intracellular stores, phospholipase A_2 (PLA_2) activation, release of arachidonic acid, and production of PGE_2 and epoxyeicosatrienoic acids (EETs) via the COX and cytochrome p450 epoxygenase pathways, respectively (Attwell et al., 2010; Mishra, 2017). An alternative mechanism is that Ca^{2+} transients in astrocytes activate large-conductance Ca^{2+}-activated K^+ (BK) channels in astrocytic endfeet, leading to K^+ release and vascular SMC relaxation through K_{IR} channels (Filosa et al., 2006). However, the relevance of this mechanism remains unclear because mice lacking a subunit of the BK channel have normal neurovascular coupling (Girouard et al., 2010), probably reflecting intervening compensatory mechanisms. Similarly, experiments in brain slices suggest that activity-induced release of D-serine by astrocytes may induce endothelial NO production (Stobart et al., 2013), a mechanism that also needs further study because neurovascular coupling is preserved in mice lacking endothelial NO synthase (Girouard et al., 2007).

Subsequent in vivo studies challenged the role of astrocytic Ca^{2+} transients and attendant upstream and downstream signaling in neurovascular coupling (reviewed in Mishra, 2017; Petzold and Murthy, 2011; Uhlirova et al., 2016a). The increases in Ca^{2+} may be too slow (Takano et al., 2006), often occurring after the onset of the hemodynamic response (Bonder and McCarthy, 2014; Nizar et al., 2013). Faster responses have been described (Lind et al., 2013), but questions have been raised about spillover of signals from neurons because of bulk labeling with organic Ca^{2+} dyes (Otsu et al., 2015; Petzold and Murthy, 2011; Uhlirova et al., 2016a). In experiments using genetically encoded astrocytic Ca^{2+} sensors in the visual cortex, no arteriolar blood flow changes were observed when astrocytic Ca^{2+} was modulated by chemogenetic approaches (Bonder and McCarthy, 2014). In contrast, in the activated olfactory bulb, rapid increases in astrocytic Ca^{2+} preceding the vascular response were observed, even with genetically encoded sensors (Otsu et al., 2015), suggesting regional differences in the role of astrocytic Ca^{2+} surge in neurovascular coupling. Questions have also been raised about the role of mGluR5 and IP_3 receptors. mGluR5 expression is developmentally regulated and suppressed after the third week of life in mice (Sun et al., 2013), challenging the hypothesized mechanism that the Ca^{2+} increase in astrocytes in adult mice depends on these receptors. Indeed, in the olfactory bulb, mGluR5 were responsible for the Ca^{2+} increase in astrocytes of juvenile but not adult mice (Otsu et al., 2015). In mice lacking IP_3 type 2 receptors, the main regulators of Ca^{2+} release from intracellular stores in astrocytes, neurovascular coupling occurs in the absence of astrocytic Ca^{2+} elevations (Bonder and McCarthy, 2014; Jego et al., 2014; Nizar et al., 2013), although persistence of Ca^{2+} transients in astrocytic processes has been described in these mice (Srinivasan et al., 2015). The role of COX-1, a rate-limiting enzyme for PGE_2 synthesis, in astrocytic neurovascular coupling also remains unclear. The expression of COX-1 in astrocytes has been questioned (Anrather et al., 2011; Lau et al., 2014; Lecrux et al., 2011), and neurovascular coupling is not altered by COX-1 inhibitors or in COX-1-null mice (Liu et al., 2012; Niwa et al., 2001a; Rosenegger et al., 2015). Therefore, whatever the source and timing of the Ca^{2+} signal in astrocytes, the downstream signaling pathways leading to vasodilatation remain unclear.

Recent data may help reconcile, at least in part, these discordant observations. The role of astrocytes in neurovascular coupling may be restricted to capillaries and may not involve arterioles, and the signaling pathways regulating the response of these vascular segments may be distinct (Biesecker et al., 2016; Mishra et al., 2016). In brain slices, electrical stimulation increases the diameter of cerebral capillaries and astrocytic Ca^{2+}, an effect suppressed by AMPA or P2X1 receptor inhibition, suggesting that ATP released from neurons acts on astrocytic P2X1 receptors, leading to the Ca^{2+} rise (Mishra et al., 2016). Contradicting previous evidence implicating PLA_2 and cytochrome p450 epoxygenase pathways (Attwell et al., 2010; Mishra, 2017), pharmacological studies indicated that the Ca^{2+} rise leads to activation of phospholipase D2, arachidonic acid production, COX-1 activation, and PGE_2 release, which, in turn mediates vasodilatation by acting on EP4 receptors, presumably on pericytes (Mishra et al., 2016). In contrast, neurovascular coupling in arterioles does not involve astrocytes but is mediated by NMDA receptors and neuronal NO production (Mishra et al., 2016). Accordingly, the arteriolar response to activation is blocked by NOS inhibition or NMDA receptor antagonism, which does not affect the response of capillaries (Mishra et al., 2016). Findings supporting a dual neurovascular regulation of capillaries and arterioles were also obtained in the retina. Ca^{2+} transients in Müller cells, assessed with genetically encoded

sensors, were linked to dilatation of capillaries, although the timing between Ca^{2+} rise and vascular response could not be consistently resolved (Biesecker et al., 2016). The Ca^{2+} transients were not linked to dilatation of arterioles, and conditional deletion of IP_3 type 2 receptors in Müller cells abolished the dilatation of capillaries but not arterioles. Although there is still considerable controversy about the role of capillaries and pericytes (discussed in subsequent sections) and COX-1 in neurovascular coupling (discussed above), the data may help explain some of the negative studies of the role of Ca^{2+} transients in neurovascular coupling that focused on arteriolar responses. Taken together, these findings support the concept that astrocytic signals may be a critical link between neural activity and the microvasculature, at least in certain segments of the cerebrovascular network.

Endothelial Cells: Retrograde Propagation of Vasomotor Responses. Endowed with powerful vasoactive agents (NO, prostanoids, endothelin, etc.), endothelial cells have long been known to regulate CBF in response to chemical and mechanical signals (Andresen et al., 2006; Seals et al., 2011), but their role in neurovascular coupling has not received attention until recently. Emerging evidence suggests a crucial role of the endothelium in the retrograde propagation of activity-induced neurovascular signals.

Hemodynamic considerations dictate that, in a vascular network, there has to be a coordinated dilatation of downstream and upstream vessels to increase flow while avoiding a "flow steal" from interconnected vascular territories (Segal, 2015). Pial arterioles are an important site of flow control (Figure 3), and signals generated by neural activity deep in the brain tissue have to be conveyed to upstream arterioles remote from the area of activation to increase flow efficiently. Indeed, visualization of pial arterioles in the somatosensory cortex revealed branch-selective vasodilatation supplying the activated site during somatosensory stimuli activating layer IV neurons (e.g., Ngai et al., 1988). Because diffusion of vasoactive agents released by active neurons could not explain these remote vascular adjustments, it was proposed that, in analogy with the retrograde vasodilation observed in the hamster cheek pouch by Segal (2015), the vascular response generated in or near the activated area is conducted retrogradely along blood vessels engaging larger pial arteries upstream (Iadecola, 1993; Woolsey and Rovainen, 1991). Evidence was subsequently provided that isolated cerebral arterioles exhibit conducted vasodilation (Dietrich et al., 1996), and neural activity-induced retrograde propagation of vasodilatation was described in cerebellar cortex pial arterioles in vivo (Iadecola et al., 1997). A role for brain-derived vasoactive factors released in the subarachnoid space and acting on pial arterioles was also excluded (Ngai and Winn, 2002). At the same time, vascular mapping and fMRI studies revealed that, during somatosensory activation, vascular responses are first observed in deep cortical laminae, where the thalamic afferents terminate, and then more superficially, suggesting retrograde propagation of the vascular response (Silva and Koretsky, 2002; Uhlirova et al., 2016b). These observations supported the idea that activation restricted to a specific site generates a vascular response that propagates retrogradely to the upstream vascular segment supplying the activated region, but the cellular bases of the effect remained unclear.

In systemic vessels, the endothelium is well known to participate in the retrograde propagation of vascular signals (Segal, 2015). In the brain, however, damage of the pial vessel endothelium using the light and dye technique did not attenuate the peak pial dilatation produced by somatosensory activation (Xu et al., 2008), but the temporal profile of the vasodilatation was not resolved. More recently, Chen et al. (2014) used a similar approach to produce a highly localized lesion of the endothelium of a single somatosensory cortex pial arteriole and found that the conducted vasodilation induced by somatosensory activation failed to propagate beyond the lesion site. Furthermore, wide-field endothelial lesions markedly altered the amplitude and temporal dynamics of the hemodynamic response, with a slower rise and no initial peak (Chen et al., 2014), implicating endothelial cells in the full expression and temporal coordination of the vasomotor response induced by somatosensory activation.

What drives the endothelial propagation of vasomotor signals? In peripheral vessels, conducted vasomotor responses have two components: a fast component mediated by Ca^{2+}-activated K^+ channels (K_{Ca}) and spreading through electrical coupling between endothelial cells and a slow component mediated by Ca^{2+} waves triggering the endothelial release of NO and prostanoids (Segal, 2015; Tallini et al., 2007). Although much less is known about the mechanisms of retrograde vasodilatation in the cerebral vasculature, a recent study implicates endothelial K_{IR} channels, rather than K_{Ca}, in the mechanisms of the fast component. Enriched with K_{IR} channels, but not K_{Ca} channels, capillary cerebral endothelial cells are highly sensitive to K^+ generated during neural activity, either from the abutting astrocytic endfeet or diffusion from nearby synapses (Longden et al., 2017). Because of a unique capillary-parenchymal arteriole preparation, this study showed that micropipette application of 6–10 mM K^+ to capillaries generated robust hyperpolarization in endothelial cells, transmitted retrogradely to penetrating arterioles at an estimated speed of 2 mm/s and leading to hyperpolarization and relaxation of SMCs (Longden et al., 2017). K^+ application did not induce capillary dilatation, suggesting that the capillary was the K^+ sensor and the upstream penetrating arteriole the effector of the vasodilation. This conclusion was supported by in vivo experiments demonstrating that the increase in capillary flow produced by K^+ application is not due to local effects on capillary diameter but linked to conducted dilatation of upstream arterioles (Longden et al., 2017). The propagation of the vasodilatation was blocked by K_{IR} inhibition with barium or endothelial deletion of $K_{IR}1.2$ channels, pointing to K_{IR} channels as key mediators of the conducted hyperpolarization (Longden et al., 2017). This rapid propagation mechanism could be mediated by ionic currents traveling through the endothelium via gap junctions and from the endothelium to SMCs via myoendothelial junctions (Segal, 2015; Tallini et al., 2007). Consistent with their role in neurovascular coupling, endothelial deletion of $K_{IR}1.2$ suppressed the increase in CBF produced by somatosensory activation (Longden et al., 2017). However, in contrast with the endothelial injury model, in which the time course of the hemodynamic response was altered (Chen et al., 2014), in $K_{IR}1.2$-null mice, the CBF increase evoked by whisker

stimulation was uniformly reduced by ≈50% without major effects on its temporal profile (Longden et al., 2017). Although methodological differences cannot be excluded, the suppression of the flow response may suggest functions of endothelial $K_{IR}1.2$ channels beyond conducted vasodilatation. Conducted vasomotor responses, both vasodilation and vasoconstriction, can also be generated by other agents; for example, acetylcholine and catecholamines, respectively (Bagher and Segal, 2011). In brain arterioles, ATP and prostaglandin F2α generate constriction followed by conducted vasodilatation (Dietrich et al., 1996), but their role in neurovascular coupling has not been explored.

SMCs and Pericytes: the Vasomotor Apparatus. Signals generated by neurons, astrocytes, and endothelial cells ultimately engage the vasomotor apparatus to induce vasodilatation, reduce vascular resistance, and increase blood flow. In the brain as in other organs, SMCs wrapping around resistance vessels are considered the major effectors of vasomotor responses and flow regulation (Cipolla, 2010), and neural activity leads to SMC relaxation in pial and penetrating arterioles (Drew et al., 2011; Ngai et al., 1988; Uhlirova et al., 2016b). SMCs constrict and relax in response to a wide variety of vasoactive agents and conducted vasodilatatory stimuli originating from downstream vessels (see Endothelial Cells: Retrograde Propagation of Vasomotor Responses). Another key physiological characteristic of SMCs is their ability to constrict or relax in response to increases or decreases in intravascular pressure, a phenomenon termed myogenic response (Cipolla, 2010; Longden et al., 2016). Cerebral arteries, especially penetrating arterioles, have a strong propensity to generate myogenic tone, a property that is essential for cerebral blood vessels to keep CBF stable during changes in arterial pressure within a certain range (cerebrovascular autoregulation) (Koller and Toth, 2012). Thus, blood pressure increases lead to vasoconstriction and decreases in vasodilatation. The resulting changes in vascular resistance keep CBF relatively stable. Cerebrovascular autoregulation and myogenic tone set the level of resting CBF and may contribute to neurovascular coupling (see Which Segment of the Cerebrovascular Network Mediates Neurovascular Coupling?). The changes in the contractile state of SMCs are mediated by the interplay between membrane potential and intracellular Ca^{2+} and the Ca^{2+} sensitivity of the contractile apparatus, which ultimately controls the assembly of contractile proteins (Cipolla, 2010; Longden et al., 2016).

In capillaries, SMCs are replaced by pericytes (Figure 5). Pericytes have an important role in establishing and maintaining vascular structure and the blood-brain barrier (BBB) (Armulik et al., 2010, 2011; Daneman et al., 2010) but have long been implicated in flow regulation (Krueger and Bechmann, 2010). Some studies have shown that a proportion of pericytes are contractile, respond to brain-generated vasoactive signals, and are able to influence capillary diameter both in vitro and in vivo (Hall et al., 2014; Mishra et al., 2016; Peppiatt et al., 2006). In addition, a 30% pericyte loss in mice with haploinsufficiency of platelet-derived growth factor receptor β (Pdgfr-β) is associated with reduced neurovascular coupling and brain oxygenation levels (Kisler et al., 2017b). However, other studies have failed to demonstrate a role of pericytes in flow regulation (Cudmore et al., 2016; Fernández-Klett et al., 2010; Hill et al., 2015; Wei et al., 2016). A major problem is that, because of the lack of specific markers, it has been difficult to distinguish pericytes from SMCs at the arteriolar-capillary transition. Vessel size is not a reliable indicator of capillaries, and the commonly used Pdgfr-β and proteoglycan NG2 are not specific pericyte markers (Armulik et al., 2011). Consequently, because of different criteria for pericyte identification, there has been considerable confusion about their functional roles (Attwell et al., 2016; Krueger and Bechmann, 2010). Pericytes are morphologically heterogeneous, but the functional significance of such diversity remains unclear (Armulik et al., 2011). One interpretation is that mural cells next to feeding arterioles, which encircle the vessel like SMCs and contain SMC actin, are "contractile pericytes," whereas cells running along the major axis of the capillary with short stubby processes projecting sideways are "non-contractile pericytes" (Attwell et al., 2016; Hartmann et al., 2015). Another point of view is that contractile pericytes are SMCs located at the arteriole-capillary transition and that "true" pericytes have no contractile function (Hill et al., 2015). A limitation of these studies is that the ultrastructural features of these cells (for example, their relationship to the endothelial basement membrane, which envelops the pericyte, a key feature for their identification; Armulik et al., 2011; Park et al., 2013) was not determined. A recently introduced tracer may provide a new tool to study pericytes (Damisah et al., 2017), but, as discussed in Knowledge Gaps in Neurovascular Coupling, other approaches would also be needed to reliably phenotype mural cells. Irrespective of whether these contractile cells are pericytes or SMCs, the larger issue concerns the role of cerebral capillaries in the regulation of CBF during neural activity, which will be addressed next.

Which Segment of the Cerebrovascular Network Mediates Neurovascular Coupling?

Neural activity evokes coordinated vasomotor responses distributed over the vascular network supplying the activated area. The structural diversity of the NVU along the cerebrovascular tree (Figures 4 and 5) suggests differences in the role of each vascular segment in CBF regulation. In the cat neocortex, pooled measurements of pressure gradients between vascular segments indicated that 39% of the total resistance to flow is attributable to vessels upstream of pial arteries 300 μm in diameter, 21% across the pial microcirculation (300- to 50-μm pial arterioles), and 40% downstream of these vessels (De Silva and Faraci, 2016; Stromberg and Fox, 1972; Figure 3). This distribution of vascular resistance seems to be conserved across species (De Silva and Faraci, 2016). Therefore, although the pial microcirculation is responsible for a sizable fraction of the total resistance, hence the potential for flow control, an even larger component is attributable to downstream intracerebral vessels. However, the breakdown of the vascular resistance among penetrating arterioles, precapillary arterioles, capillaries, and venules has been difficult to assess because intravascular pressure measurements in smaller vessels embedded deep in the brain are not feasible. Capillaries are closer to neurons than arteries, and, because of their large surface area, minimal changes in their diameter could produce large changes in flow (Attwell et al., 2010). Consequently, capillaries are well suited to regulate CBF in response to the metabolic needs of the brain. Computational studies attempting to assess segmental

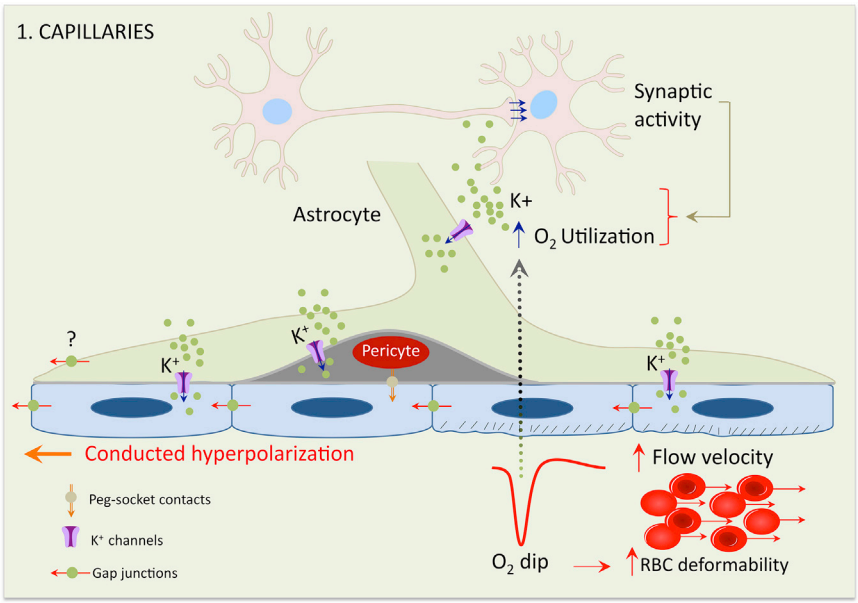

Figure 7. Neurovascular Coupling at the Capillary Level

Synaptic activity leads to release of extracellular K^+ and increased O_2 utilization. In turn, K^+ activates K_{IR} channels on endothelial cells and pericytes, leading to endothelial cell hyperpolarization, which is conducted retrogradely via gap junctions linking adjacent endothelial cells. At the same time, the reduction in O_2 leads to increased deformability of red blood cells (RBCs), reducing their viscosity and increasing capillary flow. The resulting shear stress on the capillary endothelium may also contribute to their hyperpolarization. The role of astrocytes is less clear, but activation of K^+ channels in astrocytes may also contribute to increased K^+ in the vicinity of endothelial cells, and, because of their link to adjacent astrocytes, could also play a role in the retrograde propagation of hyperpolarization. ATP released during neural activity could increase astrocytic Ca^{2+} via P2X1 receptors and relax contractile mural cells via reaction products of the COX pathways (Mishra et al., 2016), but, as discussed in the text, there is no consensus on the ability of capillaries to dilate. Neurovascular contacts from interneurons or subcortical pathways are not depicted (see Figure 5).

cerebrovascular resistance have given varying results because of difficulties in setting boundary parameters in idealized networks (Schmid et al., 2017). In two recent studies, in which realistic vascular networks of the mouse somatosensory cortex were used, one attributed the bulk of resistance to arterioles in deep cortical layers and capillaries in superficial layers (Schmid et al., 2017) and the other to capillaries "adjacent to feeding arterioles" (Gould et al., 2017), highlighting the uncertainty of estimating segmental vascular resistance.

Experiments investigating capillary vasoactivity have also led to inconsistent results. In brain slices, neural activity was found to induce capillary dilatation because of relaxation of pericytes (Hall et al., 2014). Related in vivo experiments demonstrated that the capillary hemodynamic response precedes arteriolar dilatation, suggesting that the increase in capillary flow is not a passive consequence of the increased flow in upstream arterioles (Hall et al., 2014; Kisler et al., 2017b). Based on these findings and on the mouse cerebrovascular tree topology (Blinder et al., 2013), it was estimated that capillaries are responsible for 84% of the flow increase produced by neural activity (Hall et al., 2014). However, other studies demonstrated that somatosensory activation does not change capillary diameter and that relaxation of upstream arterioles accounts for the bulk of the flow response (Drew et al., 2011; Hill et al., 2015; Wei et al., 2016). A lack of capillary vasoactivity was also shown with local application of K^+ (Longden et al., 2017), seizures (Fernández-Klett et al., 2010), ischemia because of carotid artery occlusion or carotid stenosis (Damisah et al., 2017; Hill et al., 2015), and isoflurane anesthesia (Cudmore et al., 2016). One possible explanation for these discordant results is that studies reporting capillary vasoactivity may have focused on transitional microvessels closer to arterioles and endowed with α-actin-containing mural cells, possibly SMC, whereas negative studies of capillary segments with "non-contractile" mural cells, possibly pericytes. Furthermore, considering the small activity-induced increases in capillary diameter (e.g., 1% in Kisler et al., 2017b), the resolution of the imaging approach used could also be a factor (Drew et al., 2011). However, as discussed in the next section, whether capillaries are major contributors to cerebrovascular resistance does not rule out their participation in neurovascular coupling.

Local and Conducted Vasomotor Responses in Neurovascular Coupling

Capillaries are uniquely positioned to detect neuronal and astroglial signals, which led to the hypothesis that neurovascular coupling could be initiated at the microvascular level and conducted upstream (Cox et al., 1993; Iadecola, 1993, 2004; Woolsey and Rovainen, 1991). As reviewed in Which Segment of the Cerebrovascular Network Mediates Neurovascular Coupling?, the evidence indicates that, irrespective of their ability to directly regulate vascular resistance, capillaries are equipped with the signaling mechanisms to detect neural activity and transmit the signal to α-actin-containing mural cells and SMCs in vessels upstream. A likely scenario for the transmission and coordination of the vascular response across the cerebrovascular tree is the following. Activation-induced increases in extracellular K^+ trigger hyperpolarization of capillary endothelial cells and, possibly, pericytes via K_{IR} channels (Longden et al., 2017; Figure 7). The hyperpolarization propagates upstream through inter-endothelial gap junctions and reaches SMCs in penetrating arterioles, most likely through myoendothelial junctions, effecting their relaxation (Longden et al., 2017; Figure 7). At the same time, capillary hypoxia increases the deformability of red blood cells, reducing blood viscosity and increasing capillary flow independent of their diameter (Wei et al., 2016 Figure 7). The resulting shear stress on the endothelium of feeding arterioles produces SMC relaxation by releasing endothelial vasorelaxing factors (flow-mediated vasodilation) (Koller and Toth, 2012; Figure 8). Because of their strong myogenic response (Longden et al., 2016), penetrating

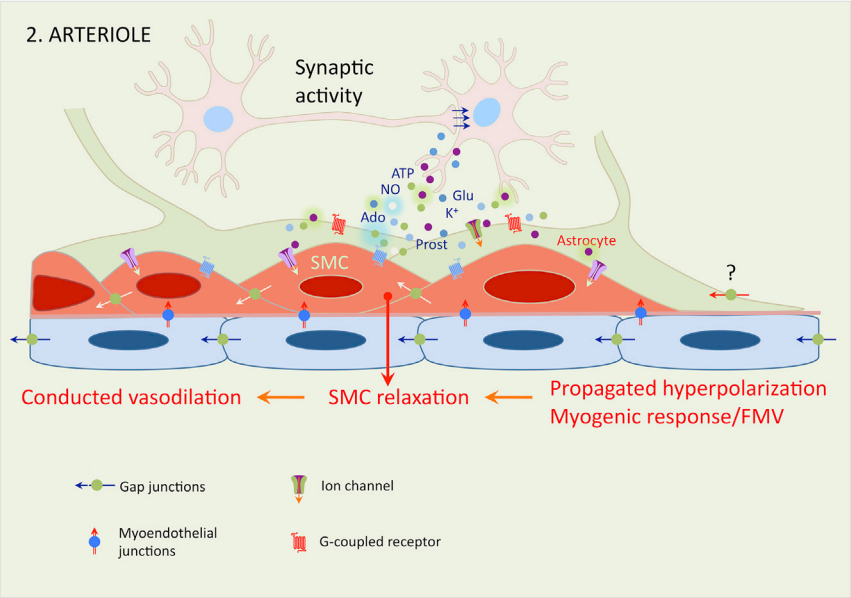

Figure 8. Neurovascular Coupling at the Level of Intraparenchymal Arterioles

The propagated endothelial hyperpolarization arising from capillaries is transferred to smooth muscle cells (SMCs) via myoendothelial junctions, resulting in their relaxation and vasodilatation. The signal continues to be transferred retrogradely to vessels upstream through gap junctions linking SMCs as well as endothelial cells. Vasodilatation is complemented by vasoactive factors released by nearby activated neurons and astrocytes, which contributes to sustain vasodilatation in its propagation to upstream vessels. In addition, the drop in intravascular pressure and increased flow velocity caused by vasodilatation downstream may contribute to smooth muscle hyperpolarization and relaxation by activating myogenic response/flow-mediated vasodilatation (FMV). Therefore, at the arteriolar level, vasodilatation has two components: propagated response from capillaries and local response from activated neurons and astrocytes. Neurovascular contacts from interneurons or subcortical pathways are not depicted (see Figure 5). Ado, adenosine; Glu, glutamate; Prost, prostanoids.

arterioles may also relax further in response to the pressure drop produced by the retrograde vasodilatation. The SMC relaxation produced by conducted hyperpolarization and myogenic tone could be complemented by the direct action on SMCs of vasoactive agents released by neurons and glia during neural activity (NO, adenosine, ATP, prostanoids, etc.) (Table 1; Figure 8). Finally, in upstream pial arterioles remote from the site of activation, vasodilatation is likely to result from two mechanisms: conducted vasodilatation propagating from arterioles downstream and flow-mediated vasodilatation/myogenic response locally (Figure 9). Accordingly, the pial arteriolar relaxation observed on the brain surface may not be driven directly by neural signals, but by intrinsic vascular signals traveling retrogradely from capillaries and arterioles at the activated site deep in the substance of the brain. It must be noted that lesion of the glia limitans near pial arterioles with L-2-aminoadipidic acid attenuates the vasodilatation produced by neural activity, implicating astrocytes in transmission of the vascular response (Xu et al., 2008). However, this observation has been difficult to interpret because damaging effects of this harsh treatment on other cell types cannot be ruled out. Concerning the role of conducted vasodilatation, a caveat is that there are likely to be regional variations in this mechanism related to vascular topology, modality of activation, and location of the site of activation relative to resistance vessels. Furthermore, as mentioned above, there are regional differences in the cellular bases' neurovascular response (e.g., the olfactory bulb, neocortex, cerebellum, etc.), which may also play a role. Regional variations notwithstanding, the available evidence suggests that neurovascular coupling reflects a coordinated multicellular response acting at different levels of the cerebrovascular network through segment-specific mechanisms.

The NVU: Beyond Blood Flow

Neurovascular coupling provides a striking example of the close interaction between the brain and its vessels. However, the NVU has also other structural components and functional attributes not directly related to cerebral perfusion that are critically important for brain health. These are briefly reviewed in the following sections.

Brain Development

Despite their different embryonic origin, the proliferation, fate determination, migration, and terminal differentiation of neural progenitors is intertwined closely with that of vascular cells (Wälchli et al., 2015). A remarkably common array of signaling molecules is involved in both brain and vascular development (Raab and Plate, 2007). Early in brain development, chemoattractant signals (e.g., vascular endothelial growth factor-A [VEGF-A]) secreted by neural progenitors in the subventricular zone guide the in-growth of blood vessels from the perineural vascular plexus, the primitive vascular network surrounding the brain (Raab et al., 2004). In turn, endothelial cells in the subventricular zone and hippocampal subgranular zone provide instructive cues critical for adult neurogenesis, which continues throughout life (Raab and Plate, 2007). The vasculature acts as scaffold for the migration of neuronal progenitors through brain-derived neurotrophic factor (BDNF) produced by endothelial cells (Snapyan et al., 2009), a process that also depends on astrocytic VEGF signaling (Bozoyan et al., 2012). Blood vessels also guide the migration of oligodendrocyte precursors (Tsai et al., 2016), and, in turn, oligodendrocyte precursors are essential for postnatal white matter vascularization through HIF1-dependent transcription (Yuen et al., 2014). The vital trophic and metabolic interaction between brain cells and vessels continues into adulthood, when endothelial metabolism and growth factors contribute to the survival of neurons, astrocytes, and oligodendrocytes (Brix et al., 2012; Carmeliet and Ruiz de Almodovar, 2013) and repair processes following acute brain injury (Hayakawa et al., 2012).

BBB

Neurovascular interactions are also critical for the formation and maintenance of the BBB (Andreone et al., 2015; Zhao et al.,

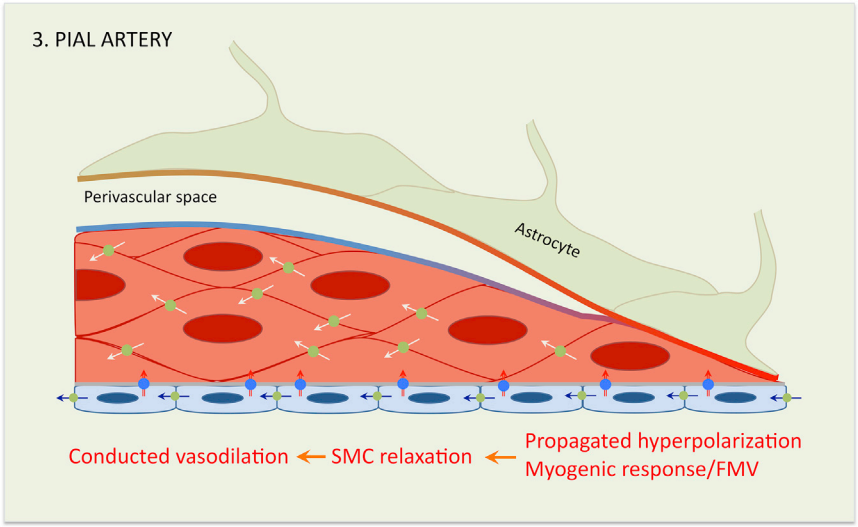

Figure 9. Neurovascular Coupling at the Level of Pial Arterioles
At this level, vasodilation depends on propagated endothelial and SMC hyperpolarization and, possibly, activation of myogenic response/FMV. Therefore, at the level of pial arterioles, the hemodynamic response is not directly related to synaptic activity but depends on the retrograde propagation of vasodilatation arising from smaller arterioles and capillaries surrounding the activated area. Perivascular nerves are not depicted (see Figure 4).

2015a). Cerebral endothelial cells are joined by impermeable tight junctions, express unique molecular transporters, and are endowed with endocytotic systems for transcytosis, the transfer of molecules through cells in membrane-bound vesicles (Zhao et al., 2015a). Although Wnt/β-catenin signaling from neuronal precursors is critical for vascular patterning and BBB development prenatally (Andreone et al., 2015), after birth, astrocytes contribute to the maintenance of the BBB through sonic hedgehog as well as β-catenin, which promote tight junction expression and integrity (Alvarez et al., 2011; Tran et al., 2016). Pericytes are also required for normal BBB development, and pericyte deficiency increases BBB permeability by promoting endothelial transcytosis (Armulik et al., 2010; Daneman et al., 2010). Suppression of transcytosis may depend on the endothelial expression of the omega-3 fatty acid transporter mfsd2a, a member of the major facilitator superfamily of secondarily active transporters (Andreone et al., 2015). Mfsd2a establishes a unique lipid environment in cerebral endothelial cells that suppresses caveola-mediated transcytosis (Andreone et al., 2017). These data suggest that the two key features of the BBB, transcytosis and tight junctions, are regulated independently, which may have disease relevance. For example, in ischemic stroke, a condition association with BBB disruption, the early opening of the BBB, which is pathogenically relevant, is mediated by an increase in transcytosis and followed by tight junction disruption days later, after tissue damage sets in (Knowland et al., 2014).

Cerebrovascular Matrix: From Structural Support to Signal Transduction

An integral part of the NVU, the matrix embeds the neurovascular cellular assembly and is comprised of collagen subunits, constituting the structural framework; proteoglycans, which bind water and secreted regulatory factors, such as growth factors; and glycoproteins, which subserve a wide variety of functions (Haffner et al., 2016; Joutel et al., 2016). Matrix proteins are involved in signal transduction by binding to cell surface receptors, such as integrins, and regulating the distribution of secreted factors with growth-promoting and regulatory function. Initial studies suggest that the matrix of cerebral vessels varies in composition depending on the specific segment of the cerebrovascular tree. For example, collagen and glycoproteins are more abundant in pial arteries, possibly reflecting their ability to withstand higher intravascular pressures, whereas proteoglycans, rich in regulatory factors, predominate in microvessels (Badhwar et al., 2014; Chun et al., 2011). A significant proportion of matrix proteins is located in the basal lamina that separates the different cellular compartments of the NVU (endothelial, smooth muscle, and astrocytes), collagen IV being most abundant (Joutel et al., 2016). Highlighting the critical role of the matrix in cerebrovascular structure and function, mutations of matrix and matrix-related proteins are associated with hereditary cerebral arteriopathies affecting small vessels, including collagen IV-related small vessel diseases, cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL), and cerebral autosomal recessive arteriopathy with subcortical infarcts and leukoencephalopathy (CARASIL) (Haffner et al., 2016; Joutel et al., 2016). These conditions, although uncommon, have provided valuable insight into sporadic small vessel disease, a major cause of cognitive impairment on vascular basis and a contributor to AD (Iadecola, 2013).

Perivascular Compartment: Clearance Pathways and Immune Surveillance

The perivascular compartment has attracted much interest as a major avenue for the removal of potentially toxic by-products of brain activity released in the extracellular space, including Aβ and tau (Tarasoff-Conway et al., 2015; Figure 10). Several vascular clearance pathways have been proposed, with particular reference to factors involved in neurodegenerative diseases. A transvascular pathway has been described through which BBB transporters transfer Aβ from the perivascular space to the circulating blood and vice versa, involving the low-density lipoprotein receptor-related protein 1 (LRP1) and the receptor for advanced glycation end products (RAGE), respectively (Deane et al., 2004, 2012; Zhao et al., 2015b; Figure 10). Pathological levels of Aβ lead to LRP1 degradation via the proteasome, promoting vascular Aβ accumulation (Deane et al., 2004; Park et al., 2013). Pericytes may also be involved because brain Aβ clearance is markedly reduced in Pdgfr-β-deficient mice crossed with mice overexpressing amyloid precursor protein (APP), resulting in Aβ accumulation in the brain and blood vessels (Sagare

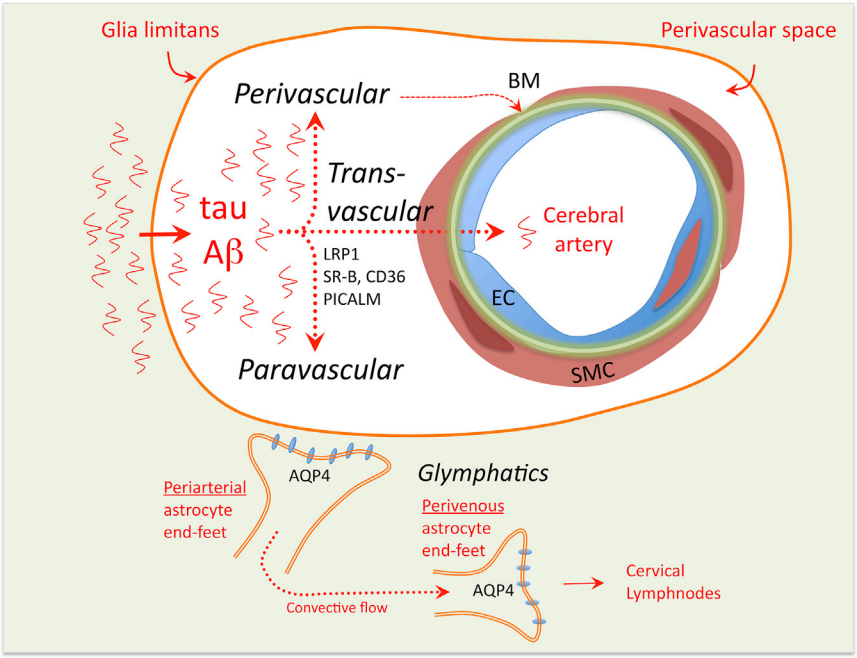

Figure 10. The Perivascular Space and Its Clearance Pathways

The perivascular space surrounding penetrating arterioles is involved in the clearance of unwanted molecules from the brain, including, but not limited to, Aβ and tau. Products of cerebral activity and metabolism reach the perivascular space by diffusion and can be disposed of through different pathways. A transvascular pathway is thought to rely on scavenger receptors and molecular transporters that carry the unwanted molecules across the vascular wall. A perivascular pathway has long been proposed in which the molecules are transported retrogradely along vascular basement membranes, reaching the subarachnoid space and eventually discharging into the cervical nodes. A paravascular (glymphatic) pathway relies on aquaporin-4 channels (AQP4) on astrocytic endfeet to allow the CSF to enter the interstitial space, creating a convective flow that clears unwanted molecules from the brain and feeds into the perivascular venous side, eventually draining into dural lymphatics or the cribriform plate. CD36, cluster of differentiation 36; LRP1, low-density lipoprotein receptor-related protein 1; PICALM, phosphatidylinositol binding clathrin assembly protein; SR-B, scavenger receptor B.

et al., 2013). Interestingly, tight junction proteins at the BBB may hinder Aβ clearance because their disruption enhances the disposal of brain Aβ into the blood (Keaney et al., 2015).

A perivascular pathway has long been proposed in which solutes leave the brain parenchyma by traveling retrogradely along the vascular basal lamina, eventually reaching cerebral arteries on the brain surface and draining into cervical lymph nodes (Hladky and Barrand, 2014; Morris et al., 2016). The routes linking the arterial wall to cervical lymph nodes have not been clearly defined, but they may involve the dural lymphatic system and the cerebrospinal fluid (CSF) outflow pathway through the nasal cribriform plate (Hladky and Barrand, 2014; Louveau et al., 2016).

A paravascular pathway (glymphatic system) runs in the opposite direction to the perivascular pathway and involves the aquaporin-4 water channel enriched in astrocytic endfeet. By imaging the movement of fluorescent dyes injected into the CSF of the cisterna magna, an expansion of the subarachnoid space between the cerebellum and the dorsal medulla, the dye is seen first around arteries on the brain surface, then in the parenchyma, and, later, in draining veins (Iliff et al., 2012). Lack of aquaporin-4 channels slows the movement of the dye dramatically (Iliff et al., 2012). On these bases, it was proposed that the CSF penetrates into the brain substance by tracking around arteries, enters the interstitial space through aquaporin-4 channels in perivascular astrocytic endfeet, and, by exchanging with the interstitial fluid, carries the waste away from the brain parenchyma, exiting the interstitial space through aquaporin-4 channels surrounding the venous perivascular space (Nedergaard, 2013). Eventually, the CSF loaded with waste collected from the interstitial space drains into the dural lymphatics or other CSF exit pathways (Nedergaard, 2013). This system may be involved in the clearance of brain Aβ and tau (Iliff et al., 2012) and is modulated by the brain's physiological state, particularly sleep (Iliff et al., 2014). Aging, brain trauma, and brain microinfarcts impede this clearance pathway (Kress et al., 2014; Plog et al., 2015; Wang et al., 2017), raising the possibility that failure of the glymphatic system is involved in the brain dysfunction associated with these conditions (but see Smith et al., 2017).

These studies raise several questions. In the paravascular pathway, it is unclear how protein waste enters the perivenous compartment and how it reaches the lymphatics. The perivascular and paravascular clearance models are based on different methodological approaches (i.e., injection of markers either into the brain parenchyma [perivascular] or CSF [paravascular], which may introduce artifacts (Hladky and Barrand, 2014). Furthermore, the anatomical relationships between the perivascular and paravascular pathways, which run in opposite directions, and how they share the periarterial compartment need further exploration (Bedussi et al., 2017; Morris et al., 2016). Finally, the relative contribution of these pathways versus endogenous clearance mechanisms (e.g., enzymatic degradation) in the human brain remains to be established (Miners et al., 2014a).

The perivascular space also houses innate immune cells, which are involved in immune homeostasis and have a profound influence on the response of the brain to infectious or immune challenges. PVMs are a subgroup of resident brain macrophages located in the perivascular space, juxtaposed to the outer wall of penetrating arteries and veins (Prinz et al., 2017; Figure 4). PVMs originate from erithromyeloid precursors in the yolk sac and, like microglia, enter the brain early in development (Prinz et al., 2017). PVMs promote hypothalamic-adrenal axis activation and autonomic responses in models of cardiac ischemia, stress, and inflammation; may harbor the HIV virus infecting the brain; and participate in tumor angiogenesis and cerebrovascular repair

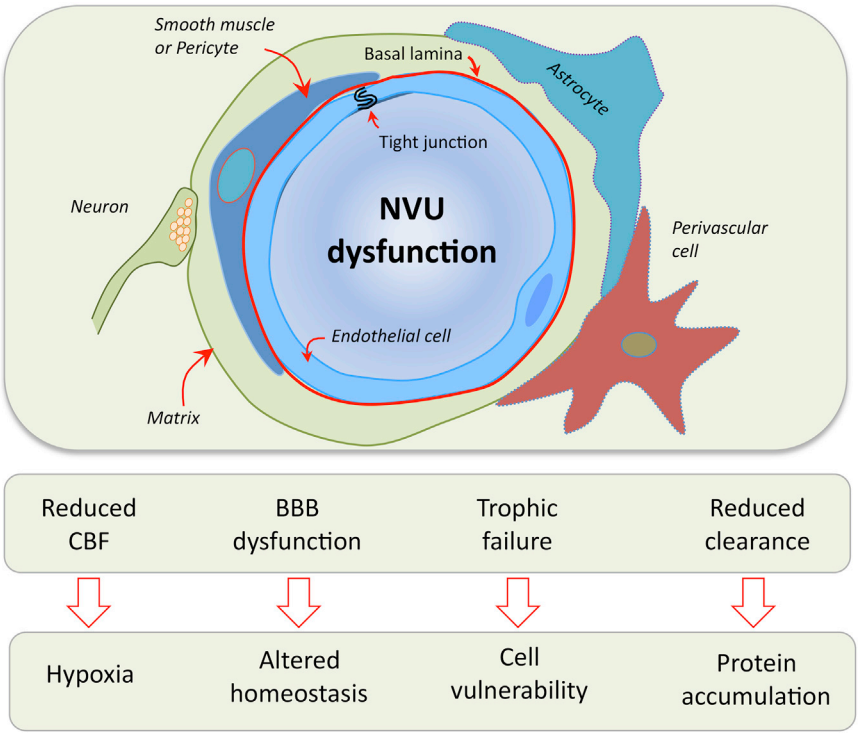

Figure 11. Potential Pathogenic Mechanisms by which Neurovascular Dysfunction Can Cause Brain Dysfunction

Alterations of the NVU may lead to reductions in CBF below the threshold required for normal brain oxygenation, leading to hypoxia. BBB dysfunction may alter the homeostasis of the brain internal milieu by limiting the delivery of glucose and other nutrients and impairing the clearance of unwanted metabolites through efflux transporters. Reduction in trophic factor production by NVU cells may increase neuronal and glial vulnerability and susceptibility to disease. Alterations of clearance pathways may promote the accumulation of molecules, such as Aβ and tau, leading to proteinopathies.

(Prinz et al., 2017). Capable of producing large amounts of free radicals in close proximity to the vessel wall, PVMs are a major source of vascular oxidative stress and key contributors to neurovascular and/or cognitive dysfunction in models of chronic hypertension (Faraco et al., 2016b) and Aβ overproduction (Park et al., 2017). On the other hand, because of their scavenging function, PVMs may prevent cerebrovascular accumulation of amyloid in models of cerebral amyloid angiopathy (CAA) (Hawkes and McLaurin, 2009; Thanopoulou et al., 2010). Overall, PVMs are emerging as a novel constituent of the NVU that awaits further exploration.

The NVU in Neurodegenerative Disease

Owing to their age dependence and lack of effective treatments, neurodegenerative diseases are predicted to grow to epidemic proportions over the next several decades (Gammon, 2014). Because alterations of the NVU are anticipated to lead to brain dysfunction and damage (Figure 11), there has been a growing interest in exploring the potential contribution of neurovascular dysfunction to neurodegeneration (de la Torre, 2017). The resulting body of work has provided evidence for a wide variety of neurovascular alterations in neurodegenerative diseases (Table 2), reviewed in the next sections.

AD

The most common cause of dementia in the elderly, AD is characterized pathologically by accumulation of Aβ in extracellular amyloid plaques and hyperphosphorylated tau in intracellular neurofibrillary tangles, which are thought to induce neuronal dysfunction and damage (De Strooper and Karran, 2016). It has long been known that cerebrovascular alterations are associated with AD (de la Torre, 2017), but only recently has the contribution of vascular factors received direct attention. AD brains have microvascular alterations (increased capillary tortuosity, capillary rarefaction, thickened basement membrane, "string vessels," etc.) (Love and Miners, 2016) and more atherosclerosis in intracranial vessels than age-matched controls (Roher et al., 2004). Community-based pathological studies of clinically diagnosed AD patients have revealed that AD pathology (plaques and tangles) and ischemic lesions frequently coexist in the same brain (Schneider et al., 2007; Toledo et al., 2013) and that ischemic lesions lower the threshold for clinical dementia (Kapasi et al., 2017). Resting CBF is reduced, and hemodynamic responses to neural activation are attenuated in the presymptomatic phase of AD (reviewed in Kisler et al., 2017a), suggesting early involvement in the disease process. Similarly, neurovascular dysfunction is observed in patients with CAA (Charidimou et al., 2017). BBB permeability is increased in patients with prodromal AD, an effect associated with increased soluble Pdgfr-β in the CSF, suggesting pericyte degeneration (Montagne et al., 2015). A pathogenic role for vascular factors in AD has also been suggested by epidemiological studies indicating that cerebrovascular diseases and AD have similar risk factors (hypertension, diabetes, obesity, etc.) and that improved cardiovascular health may have contributed to the recently reported reduction in AD incidence (Pase et al., 2017). Indeed, vascular risk factors, such as hypertension, promote amyloid deposition (Gottesman et al., 2017; Petrovitch et al., 2000; Rodrigue et al., 2013), attributable to reduced Aβ clearance and/or enhanced APP processing (Carnevale et al., 2012; Faraco et al., 2016a).

Most studies of the mechanisms of neurovascular dysfunction in AD have focused on the role of Aβ (Kisler et al., 2017a). Thomas et al. (1996) first reported that Aβ, in addition to causing neuronal dysfunction, also impairs the ability of endothelial cells to relax systemic vessels in vitro. Using mice overexpressing mutated APP (Tg2576 mice), it was subsequently established that Aβ impairs all major factors regulating the cerebral circulation: neurovascular coupling, endothelial function, and cerebrovascular autoregulation (Iadecola et al., 1999; Niwa et al., 2002a,

Table 2. Evidence of Neurovascular Dysfunction in Major Neurodegenerative Diseases

Disease	Vascular Morphology	Blood-Brain Barrier	Cerebral Perfusion	Selected References
Alzheimer's disease	microvascular damage microbleeds WM lesions ATS in cerebral arteries	early increase in BBB perm. to Gd late small increase in Qalb	reduced CBF before symptoms reduced neurovascular coupling	Janelidze et al., 2017; Toledo et al., 2013; Roher et al., 2004; Montagne et al., 2015; Kisler et al., 2017a
Frontotemporal dementia	microvascular damage microbleeds WM lesions perivascular astrocyte damage	increase in Qalb	reduced CBF before symptoms	Sudre et al., 2017; Janelidze et al., 2017; Dopper et al., 2016; Martin et al., 2001; Thal et al., 2015
Amyotrophic lateral sclerosis	microvascular damage RBC extravasation in some cases	increase in Qalb reduced TJ proteins plasma protein extravasation	reduced CBF correlates with disease progression reduced neurovascular coupling	Murphy et al., 2012; Rule et al., 2010; Abrahams et al., 1996; Henkel et al., 2009; Winkler et al., 2013
Idiopathic Parkinson disease	microvascular damage late in disease increased endothelial cell nuclei in SN	increase in Qalb in late phase BBB Pgp dysfunction increase in VEGF in CSF	early CBF reductions preserved neurovascular coupling	Janelidze et al., 2015; Kortekaas et al., 2005; Pisani et al., 2012; Rosengarten et al., 2010; Al-Bachari et al., 2014; Guan et al., 2013; Faucheux et al., 1999
Dementia with Lewy bodies	microvascular damage ATS in cerebral arteries	increase in Qalb	early CBF reductions	Janelidze et al., 2017; Roquet et al., 2016; Ghebremedhin et al., 2010; Miners et al., 2014b

ATS, atherosclerosis; Qalb, CSF/plasma albumin ratio (index of BBB permeability); Gd, gadolinium; Pgp, P-glycoprotein efflux transporter; SN, substantia nigra; TJ, tight junctions; WM, white matter; perm., permeability.

2000b). Aβ was also found to reduce resting CBF and to promote vasoconstriction (Niwa et al., 2002b; 2001b), resulting in increased susceptibility to ischemic brain injury (Zhang et al., 1997). These neurovascular alterations were observed before amyloid plaques and behavioral deficits, suggesting an early pathogenic role, and were subsequently confirmed in other APP overexpression models (Beckmann et al., 2003; Tong et al., 2005). Mechanistically, Aβ may act on the innate immunity receptor CD36 in PVMs, inducing vascular oxidative stress through the superoxide-synthesizing enzyme nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (Han et al., 2008; Park et al., 2008, 2013, 2017; Tong et al., 2005). Radicals, in turn, cause endothelial dysfunction via DNA damage, poly-ADP ribose activation, ADP-ribose-mediated TRPM2 channel opening, and Ca^{2+} overload (Park et al., 2014b). In addition to failure in LRP1-mediated transvascular Aβ clearance (Deane et al., 2004), innate immunity receptors, such as CD36, also promote CAA (Park et al., 2013), in which Aβ accumulation leads to vascular oxidative stress, neurovascular dysfunction, and, with aging, irreversible damage to the vasomotor apparatus (Park et al., 2014a).

Frontotemporal Dementia

Frontotemporal dementia (FTD), the leading cause of dementia in middle age, comprises a heterogeneous group of neurodegenerative conditions characterized by diverse cognitive and behavioral manifestations (Olney et al., 2017). Familial forms of the disease are often caused by mutations of the progranulin, tau, or C9orf72 gene, but sporadic forms are more common (Olney et al., 2017). Resting CBF is reduced in the frontal, parietal, and temporal cortex of presymptomatic patients, an effect independent of atrophy (Dopper et al., 2016). Pathological studies have described microvascular damage, microhemorrhages, and white matter ischemic lesions, more pronounced in older individuals, suggesting that the vascular disease may be independent of FTD pathology and age-related (Sudre et al., 2017; Thal et al., 2015). BBB alterations have also been reported (Janelidze et al., 2017). On the other hand, astrogliosis and astrocytic damage, prominent pathological features of FTD, correlate regionally with reductions in CBF (Martin et al., 2001), implicating astrocyte loss in the flow reduction and, possibly, in the mechanisms of the disease. Studies in mouse models reflecting selected aspects of FTD have confirmed some of these neurovascular alterations. In agreement with pathological studies showing microhemorrhages, progranulin-deficient mice exhibit increased BBB permeability and hemorrhages after stroke, but resting CBF or cerebrovascular reactivity were not reduced (Jackman et al., 2013). The BBB dysfunction was attributed to alterations in the structure of endothelial tight junctions, which appeared shorter and simplified (Jackman et al., 2013). BBB alterations have also been reported in mice overexpressing tau mutations found

in FTD; e.g., P301L (Blair et al., 2015). However, in these mice, the cerebrovascular reactivity to CO_2 was reported to be increased (Wells et al., 2015), ruling out global suppression of vasomotor function.

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is characterized by dysfunction and degeneration of upper and lower motor neurons, resulting in progressive weakness and paralysis (van Es et al., 2017). Up to 50% of cases have cognitive dysfunction of the type observed in FTD, and up to 15% of ALS cases also develop FTD (van Es et al., 2017). Alterations in the BBB and blood-spinal cord barrier were initially suggested to play a role in the disease based on increased plasma protein in the CSF and microvascular alterations in spinal cord microvessels in autopsy specimens; e.g., reduced tight junction proteins, endothelial and pericyte damage, capillary rarefaction, and plasma protein extravasation (Evans et al., 2013; Winkler et al., 2013). Studies in a mouse model of familial ALS caused by mutation of the free radical scavenging enzyme SOD1 have demonstrated uncoupling between spinal cord blood flow and glucose metabolism as well as increased permeability of spinal cord capillaries and microhemorrhages, which precede motor neuron degeneration (Miyazaki et al., 2012; Zhong et al., 2008). Prevention of the barrier alterations with an activated protein C analog delayed the development of disease (Winkler et al., 2014). Although frank microhemorrhages were not observed by high-resolution MRI (Verstraete et al., 2010), BBB dysfunction and red blood cell extravasation may be present in a subset of patients (Winkler et al., 2013). A separate line of investigations also suggests the involvement of neurovascular factors in motor neuron degeneration. In mice, deficiency of VEGF leads to motor neuron dysfunction and degeneration (Poesen et al., 2008), and administration of VEGF rescues motor neuron dysfunction in SOD1 mutant mice (Storkebaum et al., 2005). Initial genetic studies suggested a link of ALS with several VEGF haplotypes resulting in low VEGF production, which were not confirmed except for a minor gender-specific effect (Lambrechts et al., 2009). Protection by VEGF is likely to result from its neurotrophic effects, although a microvascular contribution cannot be ruled out (Quaegebeur et al., 2011). However, vasoprotective effects are difficult to reconcile with the observation that increased vascular permeability may be damaging in ALS because VEGF increases vascular permeability (Quaegebeur et al., 2011). Decreases in cortical perfusion, including non-motor areas, are well described early in the disease and correlate with disease progression (Murphy et al., 2012; Rule et al., 2010). In ALS patients with frontal cognitive dysfunction, the increase in CBF evoked by activation tasks in frontal association areas is reduced, an effect that is more widespread in patients with more severe cognitive deficits (Abrahams et al., 1996). These findings point to a more global alteration in neurovascular regulation, which could play a role in the cognitive manifestation of the disease.

Parkinson Disease

Idiopathic Parkinson disease (PD) is a neurodegenerative disorder characterized by motor dysfunction and cognitive deficits in which the pathology starts in the brain stem, particularly the nigrostriatal pathway, and spreads to the cortex (Przedborski, 2017). Postmortem studies of advanced cases have shown capillary damage and fragmentation in the frontal cortex and brain stem nuclei (Guan et al., 2013) as well as increased endothelial cell nuclei in the substantia nigra (Faucheux et al., 1999). Increases in VEGF in the CSF have been reported (Janelidze et al., 2015), together with evidence of increased BBB permeability in the late stages of the disease (Kortekaas et al., 2005; Pisani et al., 2012). On the other hand, CBF reductions have been observed early in the disease course in neocortical regions without overt pathology (Borghammer et al., 2010; Fernández-Seara et al., 2012). In PD with cognitive impairment, CBF reductions in parietal regions correlate with cognitive dysfunction (Al-Bachari et al., 2014), suggesting a link between cognitive deficits and CBF reductions. However, cerebrovascular reactivity seems to be preserved because the hemodynamic response to visual stimulation or to increases in arterial partial pressure of CO_2 (pCO_2) (hypercapnia) is not attenuated (Al-Bachari et al., 2014; Rosengarten et al., 2010). An interesting hypothesis is that the CBF reductions are due alterations of the cholinergic, serotoninergic, and noradrenergic neurovascular innervation of the neocortex (Al-Bachari et al., 2014; Fernández-Seara et al., 2012). The experimental observation that these subcortical pathways may influence resting CBF and not neurovascular coupling (see Cellular Bases of Neurovascular Coupling and Neurons: Initiation of the Local Vascular Response) would be consistent with this hypothesis.

Dementia with Lewy Bodies

CBF is reduced in the frontal, insular, temporal, and occipital cortex in dementia with Lewy bodies (DLB), a condition characterized by fluctuations in cognition, hallucinations, and parkinsonism (Galasko, 2017). The changes in CBF occur in the prodromal stage of the disease (Roquet et al., 2016) and may be due to a reduction in energy metabolism (Ishii et al., 2015). Although vascular alterations such as reduced VEGF, small vessel disease, and atherosclerosis have been described in the occipital cortex (Ghebremedhin et al., 2010; Miners et al., 2014b), there might be an inverse relationship between Lewy body pathology and vascular pathology (Ghebremedhin et al., 2010). Therefore, Lewy body pathology may stave off vascular pathology and vice versa.

Where Do We Go from Here?

The concept of NVU has come a long way since 2001. Central to brain health, neurovascular interactions have emerged as essential contributors to brain development and vital for maintaining the homeostasis of the brain internal milieu. Neurovascular coupling has evolved from a unidimensional process driven by the direct effect of neuronal mediators on local blood vessels to a multi-dimensional one in which a multitude of mediators released from multiple cell types engages unique signaling pathways and effector systems on different cerebrovascular segments in a coordinated fashion (Table 1). The resulting carefully orchestrated vascular response involves the entire cerebrovascular tree and leads to spatially and temporally focused increases in CBF. The evidence implicates neurons and astrocytes in the generation, modulation, and transmission of the signal to local microvessels, capillary endothelial cells in the retrograde propagation of vasomotor signals, and contractile mural cells and SMCs of progressively larger vascular segments as the

main effectors of the CBF increase (Table 1). Therefore, neural activity initiates the process, but implementation of the hemodynamic response requires the coordinated interaction of other NVU cells across the whole cerebrovascular network. Significant progress has also been made in the pathobiology of the NVU. Increasing evidence implicates NVU dysfunction in major neurodegenerative diseases in which the role of vascular factors was traditionally not considered. This new knowledge has raised a number of questions regarding the inner workings of the NVU and its role in brain diseases.

Knowledge Gaps in Neurovascular Coupling

The contribution of feedforward and feedback mechanisms to the flow increase remains unclear. In particular, the region-specific factors determining the recruitment of one mechanism versus the other and the cells and mediators driving the attendant hemodynamic response have not been identified. The cellular bases of neurovascular interactions at the different levels of the cerebrovascular tree have not been established. Initial studies have highlighted the role of endothelial K_{IR} channel-induced hyperpolarization at the capillary level and of neuronal NO in arterioles, but how these responses are generated, transmitted, and appropriately timed requires further study. Furthermore, whether astrocytes contribute to the K^+ surge at the capillary level and their potential contribution to the release of vasoactive factors, metabolic adaptation, and retrograde vasodilatation at the arteriolar level has not been fully elucidated.

The role of capillaries in neurovascular coupling remains controversial. Are capillaries neural activity sensors or also effectors of the initial hemodynamic response? Are the small changes in capillary diameter observed in some studies physiologically relevant? Answering these questions would require a re-examination of what a capillary is and a better characterization of the cells associated with them and their hemodynamic effect. Using vessel size and isolated cell markers as the sole identification tools for mural cells has generated considerable confusion. A different approach is needed based on establishing the ontogeny of mural cells, defining the transcription and growth factors driving their development and localization, and identifying their molecular signature. This effort would require a comprehensive re-evaluation of the brain's vascular development, focusing specifically on mural cells (e.g., Jung et al., 2017).

Relatively little is known about the upstream propagation of vasoactive signals from capillaries to pial arterioles. Are gap junctions and myoendothelial junctions the sole mode of inter-endothelial and endothelial-SMC transmission or are diffusible factors also involved? Do mural cells (e.g., pericytes) play a role? The mechanisms focusing the hemodynamic response by selectively engaging arteriolar branches supplying the activated region (e.g., Ngai et al., 1988) and the possible role of glial limitans astrocytes require further study. In systemic arteries, perivascular sympathetic (noradrenergic) nerves control propagated responses (Segal, 2015), but it is unclear whether they play a similar role in cerebral arteries. In this regard, it is of interest that the noradrenergic innervation of the neocortex may play a role in focusing the vascular response (Bekar et al., 2012), a concept that requires further exploration.

Answering these questions will be facilitated by the use of new models and approaches to study the cerebral vasculature. For example, optical coherence tomography (Baran and Wang, 2016), three-photon microscopy (Ouzounov et al., 2017), and ultrafast ultrasound localization microscopy (Errico et al., 2015) may offer an unprecedented look at the cerebral microcirculatory network at a depth, spatial extent, and/or resolution previously unattainable. Whenever feasible, coupling these imaging tools with genetically encoded activity sensors and cell-specific reporters (Bindocci et al., 2017) as well as chemo- and opto-genetic approaches to activate specific neural pathways may provide novel insights into the spatiotemporal relationships linking neural activity and segmental vascular responses. Using conscious animals will eliminate anesthesia as a major confounder and will allow investigators to probe neurovascular coupling beyond the realm of olfactory, somatosensory, or visual stimuli and toward complex behaviors in multiple brain regions (Gao et al., 2017). The introduction of more realistic multicellular in vitro models of the NVU, such as the 3D microfluidics organ-on-chip technology for the BBB (Adriani et al., 2017) and cerebrovascular organoids derived from human induced pluripotent stem cells (iPSCs) (Appelt-Menzel et al., 2017) provide the opportunity to explore the molecular interactions among NVU cells beyond what can be achieved with conventional co-culture systems. Large-scale single-cell RNA sequencing applied to the cerebral vasculature (He et al., 2016; Tasic et al., 2016) will provide a glimpse into the molecular signature of specific cerebrovascular cells and insight into the molecular bases of the functional differences of vascular and perivascular cells in the different segments of the cerebrovascular tree.

NVU Dysfunction and Neurodegeneration

Our understanding of the role of neurovascular dysfunction in neurodegenerative diseases is still rudimentary. Although there is overwhelming evidence that neurodegeneration is associated with structural and functional alterations of the NVU, often early in the disease course (Table 2), several questions remain to be addressed. Is NVU dysfunction an epiphenomenon of the degenerative process or a pathogenic contributor? In most cases, the reductions in CBF are not severe enough to induce ischemic injury. However, it cannot be excluded that reduced cerebral perfusion, in concert with neurovascular uncoupling, may have deleterious effects on the brain in the long run. BBB dysfunction, neurotrophic failure, and impaired clearance associated with NVU dysfunction may also contribute (Figure 11), but their pathogenic effect remains to the established, which brings us to a related question. Does neurovascular dysfunction promote the neurodegenerative process? In AD, experimental and clinical studies indicate that vascular dysfunction is an early manifestation of the disease and may promote AD pathology. Is this also the case in other neurodegenerative diseases? Considering that oxidative stress and inflammation, major causes of neurovascular dysfunction, have been implicated in virtually all neurodegenerative conditions (Ransohoff, 2016), these factors could be driving both the vascular and neurodegenerative pathologies independently, amplifying their respective pathogenic effect. In diseases like AD, in which the coexistence of neurodegeneration with cerebrovascular lesions is well documented, what is the role of cerebrovascular pathology in the clinical expression of the disease? Vascular lesions could lower the threshold for cognitive deficits by increasing the overall

lesion burden on the brain. Alternatively, the two processes could be synergistic, amplifying each other, as suggested by evidence that vascular dysfunction promotes AD pathology (see above). Although ischemic injury is not inconsequential to the clinical expression of AD (Kapasi et al., 2017), there is no definitive evidence of a synergistic interaction (e.g., Vemuri et al., 2015). However, the reduction in dementia incidence attributed to better cardiovascular health (Pase et al., 2017) suggests a contribution of vascular pathology to neurodegeneration, an attractive hypothesis that requires further testing in randomized clinical trials.

The factors by which neurodegenerative pathology leads to neurovascular dysfunction remain to be defined. Are selected NVU cells and specific cerebrovascular segments particularly vulnerable, and if so, why? Are there common signaling pathways and mediators responsible for the dysfunction in different diseases? What is the contribution of neuronal, glial, and vascular cell metabolism to vascular dysfunction? The difficulty of performing mechanistic studies in human patients, the long interval between onset of pathology and clinical diagnosis, lack of reliable biomarkers, and lack of animal models recapitulating full disease phenotypes have been major impediments to progress. However, developments in both the clinical and experimental arenas may help overcome some of these barriers. Large-scale "omic" studies, advanced imaging with markers of disease, new molecular biomarkers, and iPSCs from patients may provide mechanistic clues to be explored further in experimental models. In turn, new animal models incorporating neurodegenerative pathology with vascular co-morbidities and new cell-specific approaches to probe the NVU promise a dramatic expansion of knowledge that can then be verified in clinical studies. Therefore, there is a strong rationale for bolstering efforts to clarify the role of NVU dysfunction in neurodegenerative diseases (Corriveau et al., 2016). New knowledge in this field may open new avenues in the prevention, early diagnosis, and treatment of some of the most devastating illnesses affecting humankind.

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REFERENCES

Abrahams, S., Goldstein, L.H., Kew, J.J., Brooks, D.J., Lloyd, C.M., Frith, C.D., and Leigh, P.N. (1996). Frontal lobe dysfunction in amyotrophic lateral sclerosis. A PET study. Brain *119*, 2105–2120.

Adriani, G., Ma, D., Pavesi, A., Kamm, R.D., and Goh, E.L.K. (2017). A 3D neurovascular microfluidic model consisting of neurons, astrocytes and cerebral endothelial cells as a blood-brain barrier. Lab Chip *17*, 448–459.

Al-Bachari, S., Parkes, L.M., Vidyasagar, R., Hanby, M.F., Tharaken, V., Leroi, I., and Emsley, H.C.A. (2014). Arterial spin labelling reveals prolonged arterial arrival time in idiopathic Parkinson's disease. Neuroimage Clin. *6*, 1–8.

Alvarez, J.I., Dodelet-Devillers, A., Kebir, H., Ifergan, I., Fabre, P.J., Terouz, S., Sabbagh, M., Wosik, K., Bourbonnière, L., Bernard, M., et al. (2011). The Hedgehog pathway promotes blood-brain barrier integrity and CNS immune quiescence. Science *334*, 1727–1731.

Andreone, B.J., Lacoste, B., and Gu, C. (2015). Neuronal and vascular interactions. Annu. Rev. Neurosci. *38*, 25–46.

Andreone, B.J., Chow, B.W., Tata, A., Lacoste, B., Ben-Zvi, A., Bullock, K., Deik, A.A., Ginty, D.D., Clish, C.B., and Gu, C. (2017). Blood-Brain Barrier Permeability Is Regulated by Lipid Transport-Dependent Suppression of Caveolae-Mediated Transcytosis. Neuron *94*, 581–594.e5.

Andresen, J., Shafi, N.I., and Bryan, R.M., Jr. (2006). Endothelial influences on cerebrovascular tone. J. Appl. Physiol. *100*, 318–327.

Anenberg, E., Chan, A.W., Xie, Y., LeDue, J.M., and Murphy, T.H. (2015). Optogenetic stimulation of GABA neurons can decrease local neuronal activity while increasing cortical blood flow. J. Cereb. Blood Flow Metab. *35*, 1579–1586.

Anrather, J., Gallo, E.F., Kawano, T., Orio, M., Abe, T., Gooden, C., Zhou, P., and Iadecola, C. (2011). Purinergic signaling induces cyclooxygenase-1-dependent prostanoid synthesis in microglia: roles in the outcome of excitotoxic brain injury. PLoS ONE *6*, e25916.

Appelt-Menzel, A., Cubukova, A., Günther, K., Edenhofer, F., Piontek, J., Krause, G., Stüber, T., Walles, H., Neuhaus, W., and Metzger, M. (2017). Establishment of a Human Blood-Brain Barrier Co-culture Model Mimicking the Neurovascular Unit Using Induced Pluri- and Multipotent Stem Cells. Stem Cell Reports *8*, 894–906.

Armulik, A., Genové, G., Mäe, M., Nisancioglu, M.H., Wallgard, E., Niaudet, C., He, L., Norlin, J., Lindblom, P., Strittmatter, K., et al. (2010). Pericytes regulate the blood-brain barrier. Nature *468*, 557–561.

Armulik, A., Genové, G., and Betsholtz, C. (2011). Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. Dev. Cell *21*, 193–215.

Attwell, D., and Iadecola, C. (2002). The neural basis of functional brain imaging signals. Trends Neurosci. *25*, 621–625.

Attwell, D., Buchan, A.M., Charpak, S., Lauritzen, M., Macvicar, B.A., and Newman, E.A. (2010). Glial and neuronal control of brain blood flow. Nature *468*, 232–243.

Attwell, D., Mishra, A., Hall, C.N., O'Farrell, F.M., and Dalkara, T. (2016). What is a pericyte? J. Cereb. Blood Flow Metab. *36*, 451–455.

Badhwar, A., Stanimirovic, D.B., Hamel, E., and Haqqani, A.S. (2014). The proteome of mouse cerebral arteries. J. Cereb. Blood Flow Metab. *34*, 1033–1046.

Bagher, P., and Segal, S.S. (2011). Regulation of blood flow in the microcirculation: role of conducted vasodilation. Acta Physiol. (Oxf.) *202*, 271–284.

Baran, U., and Wang, R.K. (2016). Review of optical coherence tomography based angiography in neuroscience. Neurophotonics *3*, 010902–010913.

Beckmann, N., Schuler, A., Mueggler, T., Meyer, E.P., Wiederhold, K.H., Staufenbiel, M., and Krucker, T. (2003). Age-dependent cerebrovascular abnormalities and blood flow disturbances in APP23 mice modeling Alzheimer's disease. J. Neurosci. *23*, 8453–8459.

Bedussi, B., van der Wel, N.N., de Vos, J., van Veen, H., Siebes, M., VanBavel, E., and Bakker, E.N. (2017). Paravascular channels, cisterns, and the subarachnoid space in the rat brain: A single compartment with preferential pathways. J. Cereb. Blood Flow Metab. *37*, 1374–1385.

Bekar, L.K., Wei, H.S., and Nedergaard, M. (2012). The locus coeruleus-norepinephrine network optimizes coupling of cerebral blood volume with oxygen demand. J. Cereb. Blood Flow Metab. *32*, 2135–2145.

Biesecker, K.R., Srienc, A.I., Shimoda, A.M., Agarwal, A., Bergles, D.E., Kofuji, P., and Newman, E.A. (2016). Glial Cell Calcium Signaling Mediates Capillary Regulation of Blood Flow in the Retina. J. Neurosci. *36*, 9435–9445.

Bill, A., and Linder, J. (1976). Sympathetic control of cerebral blood flow in acute arterial hypertension. Acta Physiol. Scand. *96*, 114–121.

Bindocci, E., Savtchouk, I., Liaudet, N., Becker, D., Carriero, G., and Volterra, A. (2017). Three-dimensional Ca(2+) imaging advances understanding of astrocyte biology. Science *356*, eaai8185.

Blair, L.J., Frauen, H.D., Zhang, B., Nordhues, B.A., Bijan, S., Lin, Y.-C., Zamudio, F., Hernandez, L.D., Sabbagh, J.J., Selenica, M.-L.B., and Dickey, C.A.

(2015). Tau depletion prevents progressive blood-brain barrier damage in a mouse model of tauopathy. Acta Neuropathol. Commun. *3*, 8.

Blanco, V.M., Stern, J.E., and Filosa, J.A. (2008). Tone-dependent vascular responses to astrocyte-derived signals. Am. J. Physiol. Heart Circ. Physiol. *294*, H2855–H2863.

Blinder, P., Tsai, P.S., Kaufhold, J.P., Knutsen, P.M., Suhl, H., and Kleinfeld, D. (2013). The cortical angiome: an interconnected vascular network with noncolumnar patterns of blood flow. Nat. Neurosci. *16*, 889–897.

Bonder, D.E., and McCarthy, K.D. (2014). Astrocytic Gq-GPCR-linked IP3R-dependent Ca2+ signaling does not mediate neurovascular coupling in mouse visual cortex in vivo. J. Neurosci. *34*, 13139–13150.

Borghammer, P., Chakravarty, M., Jonsdottir, K.Y., Sato, N., Matsuda, H., Ito, K., Arahata, Y., Kato, T., and Gjedde, A. (2010). Cortical hypometabolism and hypoperfusion in Parkinson's disease is extensive: probably even at early disease stages. Brain Struct. Funct. *214*, 303–317.

Bozoyan, L., Khlghatyan, J., and Saghatelyan, A. (2012). Astrocytes control the development of the migration-promoting vasculature scaffold in the postnatal brain via VEGF signaling. J. Neurosci. *32*, 1687–1704.

Brix, B., Mesters, J.R., Pellerin, L., and Jöhren, O. (2012). Endothelial cell-derived nitric oxide enhances aerobic glycolysis in astrocytes via HIF-1α-mediated target gene activation. J. Neurosci. *32*, 9727–9735.

Brunet, I., Gordon, E., Han, J., Cristofaro, B., Broquères-You, D., Liu, C., Bouvrée, K., Zhang, J., del Toro, R., Mathivet, T., et al. (2014). Netrin-1 controls sympathetic arterial innervation. J. Clin. Invest. *124*, 3230–3240.

Carmeliet, P., and Ruiz de Almodovar, C. (2013). VEGF ligands and receptors: implications in neurodevelopment and neurodegeneration. Cell. Mol. Life Sci. *70*, 1763–1778.

Carnevale, D., Mascio, G., D'Andrea, I., Fardella, V., Bell, R.D., Branchi, I., Pallante, F., Zlokovic, B., Yan, S.S., and Lembo, G. (2012). Hypertension induces brain β-amyloid accumulation, cognitive impairment, and memory deterioration through activation of receptor for advanced glycation end products in brain vasculature. Hypertension *60*, 188–197.

Cauli, B., and Hamel, E. (2010). Revisiting the role of neurons in neurovascular coupling. Front. Neuroenergetics *2*, 9.

Cauli, B., Tong, X.-K., Rancillac, A., Serluca, N., Lambolez, B., Rossier, J., and Hamel, E. (2004). Cortical GABA interneurons in neurovascular coupling: relays for subcortical vasoactive pathways. J. Neurosci. *24*, 8940–8949.

Chaigneau, E., Oheim, M., Audinat, E., and Charpak, S. (2003). Two-photon imaging of capillary blood flow in olfactory bulb glomeruli. Proc. Natl. Acad. Sci. USA *100*, 13081–13086.

Charidimou, A., Boulouis, G., Gurol, M.E., Ayata, C., Bacskai, B.J., Frosch, M.P., Viswanathan, A., and Greenberg, S.M. (2017). Emerging concepts in sporadic cerebral amyloid angiopathy. Brain *140*, 1829–1850.

Chen, B.R., Kozberg, M.G., Bouchard, M.B., Shaik, M.A., and Hillman, E.M.C. (2014). A critical role for the vascular endothelium in functional neurovascular coupling in the brain. J. Am. Heart Assoc. *3*, e000787.

Chun, H.B., Scott, M., Niessen, S., Hoover, H., Baird, A., Yates, J., 3rd, Torbett, B.E., and Eliceiri, B.P. (2011). The proteome of mouse brain microvessel membranes and basal lamina. J. Cereb. Blood Flow Metab. *31*, 2267–2281.

Cipolla, M.J. (2010).). Integrated Systems Physiology: From Molecule to Function (Morgan & Claypool Life Sciences).

Cohen, Z., Bonvento, G., Lacombe, P., and Hamel, E. (1996). Serotonin in the regulation of brain microcirculation. Prog. Neurobiol. *50*, 335–362.

Colonnese, M.T., Phillips, M.A., Constantine-Paton, M., Kaila, K., and Jasanoff, A. (2008). Development of hemodynamic responses and functional connectivity in rat somatosensory cortex. Nat. Neurosci. *11*, 72–79.

Corriveau, R.A., Bosetti, F., Emr, M., Gladman, J.T., Koenig, J.I., Moy, C.S., Pahigiannis, K., Waddy, S.P., and Koroshetz, W. (2016). The Science of Vascular Contributions to Cognitive Impairment and Dementia (VCID): A Framework for Advancing Research Priorities in the Cerebrovascular Biology of Cognitive Decline. Cell. Mol. Neurobiol. *36*, 281–288.

Cox, S.B., Woolsey, T.A., and Rovainen, C.M. (1993). Localized dynamic changes in cortical blood flow with whisker stimulation corresponds to matched vascular and neuronal architecture of rat barrels. J. Cereb. Blood Flow Metab. *13*, 899–913.

Craigie, E.H. (1945). The architecture of the cerebral capillary bed. Biol. Rev. Camb. Philos. Soc. *20*, 133–146.

Cudmore, R.H., Dougherty, S.E., and Linden, D.J. (2016). Cerebral vascular structure in the motor cortex of adult mice is stable and is not altered by voluntary exercise. J. Cereb. Blood Flow Metab. Published online January 6, 2017. http://dx.doi.org/10.1177/0271678X16682508.

Dahl, E. (1973). The fine structure of intracerebral vessels. Z. Zellforsch. Mikrosk. Anat. *145*, 577–586.

Damisah, E.C., Hill, R.A., Tong, L., Murray, K.N., and Grutzendler, J. (2017). A fluoro-Nissl dye identifies pericytes as distinct vascular mural cells during in vivo brain imaging. Nat. Neurosci. *20*, 1023–1032.

Daneman, R., Zhou, L., Kebede, A.A., and Barres, B.A. (2010). Pericytes are required for blood-brain barrier integrity during embryogenesis. Nature *468*, 562–566.

de la Torre, J.C. (2017). Are major dementias triggered by poor blood flow to the brain? Theoretical considerations. J. Alzheimers Dis. *57*, 353–371.

De Silva, T.M., and Faraci, F.M. (2016). Microvascular dysfunction and cognitive impairment. Cell. Mol. Neurobiol. *36*, 241–258.

De Strooper, B., and Karran, E. (2016). The cellular phase of Alzheimer's disease. Cell *164*, 603–615.

Deane, R., Wu, Z., Sagare, A., Davis, J., Du Yan, S., Hamm, K., Xu, F., Parisi, M., LaRue, B., Hu, H.W., et al. (2004). LRP/amyloid beta-peptide interaction mediates differential brain efflux of Abeta isoforms. Neuron *43*, 333–344.

Deane, R., Singh, I., Sagare, A.P., Bell, R.D., Ross, N.T., LaRue, B., Love, R., Perry, S., Paquette, N., Deane, R.J., et al. (2012). A multimodal RAGE-specific inhibitor reduces amyloid β-mediated brain disorder in a mouse model of Alzheimer disease. J. Clin. Invest. *122*, 1377–1392.

Dietrich, H.H., Kajita, Y., and Dacey, R.G., Jr. (1996). Local and conducted vasomotor responses in isolated rat cerebral arterioles. Am. J. Physiol. *271*, H1109–H1116.

Dopper, E.G.P., Chalos, V., Ghariq, E., den Heijer, T., Hafkemeijer, A., Jiskoot, L.C., de Koning, I., Seelaar, H., van Minkelen, R., van Osch, M.J., et al. (2016). Cerebral blood flow in presymptomatic MAPT and GRN mutation carriers: A longitudinal arterial spin labeling study. Neuroimage Clin. *12*, 460–465.

Drake, C.T., and Iadecola, C. (2007). The role of neuronal signaling in controlling cerebral blood flow. Brain Lang. *102*, 141–152.

Drew, P.J., Shih, A.Y., and Kleinfeld, D. (2011). Fluctuating and sensory-induced vasodynamics in rodent cortex extend arteriole capacity. Proc. Natl. Acad. Sci. USA *108*, 8473–8478.

Eichmann, A., and Brunet, I. (2014). Arterial innervation in development and disease. Sci. Transl. Med. *6*, 252ps9.

Engl, E., Jolivet, R., Hall, C.N., and Attwell, D. (2017). Non-signalling energy use in the developing rat brain. J. Cereb. Blood Flow Metab. *37*, 951–966.

Errico, C., Pierre, J., Pezet, S., Desailly, Y., Lenkei, Z., Couture, O., and Tanter, M. (2015). Ultrafast ultrasound localization microscopy for deep super-resolution vascular imaging. Nature *527*, 499–502.

Evans, M.C., Couch, Y., Sibson, N., and Turner, M.R. (2013). Inflammation and neurovascular changes in amyotrophic lateral sclerosis. Mol. Cell. Neurosci. *53*, 34–41.

Faraco, G., Park, L., Zhou, P., Luo, W., Paul, S.M., Anrather, J., and Iadecola, C. (2016a). Hypertension enhances Aβ-induced neurovascular dysfunction, promotes β-secretase activity, and leads to amyloidogenic processing of APP. J. Cereb. Blood Flow Metab. *36*, 241–252.

Faraco, G., Sugiyama, Y., Lane, D., Garcia-Bonilla, L., Chang, H., Santisteban, M.M., Racchumi, G., Murphy, M., Van Rooijen, N., Anrather, J., and Iadecola, C. (2016b). Perivascular macrophages mediate the neurovascular and cognitive dysfunction associated with hypertension. J. Clin. Invest. *126*, 4674–4689.

Faucheux, B.A., Bonnet, A.M., Agid, Y., and Hirsch, E.C. (1999). Blood vessels change in the mesencephalon of patients with Parkinson's disease. Lancet *353*, 981–982.

Fernández-Klett, F., Offenhauser, N., Dirnagl, U., Priller, J., and Lindauer, U. (2010). Pericytes in capillaries are contractile in vivo, but arterioles mediate functional hyperemia in the mouse brain. Proc. Natl. Acad. Sci. USA *107*, 22290–22295.

Fernández-Seara, M.A., Mengual, E., Vidorreta, M., Aznárez-Sanado, M., Loayza, F.R., Villagra, F., Irigoyen, J., and Pastor, M.A. (2012). Cortical hypoperfusion in Parkinson's disease assessed using arterial spin labeled perfusion MRI. Neuroimage *59*, 2743–2750.

Filosa, J.A., Bonev, A.D., Straub, S.V., Meredith, A.L., Wilkerson, M.K., Aldrich, R.W., and Nelson, M.T. (2006). Local potassium signaling couples neuronal activity to vasodilation in the brain. Nat. Neurosci. *9*, 1397–1403.

Freeman, R.D., and Li, B. (2016). Neural-metabolic coupling in the central visual pathway. Philos. Trans. R. Soc. Lond. B Biol. Sci. *371*, 20150357.

Freygang, W.H., Jr., and Sokoloff, L. (1958). Quantitative measurement of regional circulation in the central nervous system by the use of radioactive inert gas. Adv. Biol. Med. Phys. *6*, 263–279.

Friedland, R.P., and Iadecola, C. (1991). Roy and Sherrington (1890): a centennial reexamination of "On the regulation of the blood-supply of the brain". Neurology *41*, 10–14.

Galasko, D. (2017). Lewy Body Disorders. Neurol. Clin. *35*, 325–338.

Gammon, K. (2014). Neurodegenerative disease: brain windfall. Nature *515*, 299–300.

Gao, Y.-R., Ma, Y., Zhang, Q., Winder, A.T., Liang, Z., Antinori, L., Drew, P.J., and Zhang, N. (2017). Time to wake up: Studying neurovascular coupling and brain-wide circuit function in the un-anesthetized animal. Neuroimage *153*, 382–398.

Ghebremedhin, E., Rosenberger, A., Rüb, U., Vuksic, M., Berhe, T., Bickeböller, H., de Vos, R.A.I., Thal, D.R., and Deller, T. (2010). Inverse relationship between cerebrovascular lesions and severity of lewy body pathology in patients with lewy body diseases. J. Neuropathol. Exp. Neurol. *69*, 442–448.

Girouard, H., Park, L., Anrather, J., Zhou, P., and Iadecola, C. (2007). Cerebrovascular nitrosative stress mediates neurovascular and endothelial dysfunction induced by angiotensin II. Arterioscler. Thromb. Vasc. Biol. *27*, 303–309.

Girouard, H., Bonev, A.D., Hannah, R.M., Meredith, A., Aldrich, R.W., and Nelson, M.T. (2010). Astrocytic endfoot Ca2+ and BK channels determine both arteriolar dilation and constriction. Proc. Natl. Acad. Sci. USA *107*, 3811–3816.

Gordon, G.R.J., Choi, H.B., Rungta, R.L., Ellis-Davies, G.C.R., and MacVicar, B.A. (2008). Brain metabolism dictates the polarity of astrocyte control over arterioles. Nature *456*, 745–749.

Gottesman, R.F., Schneider, A.L.C., Zhou, Y., Coresh, J., Green, E., Gupta, N., Knopman, D.S., Mintz, A., Rahmim, A., Sharrett, A.R., et al. (2017). Association Between Midlife Vascular Risk Factors and Estimated Brain Amyloid Deposition. JAMA *317*, 1443–1450.

Gould, I.G., Tsai, P., Kleinfeld, D., and Linninger, A. (2017). The capillary bed offers the largest hemodynamic resistance to the cortical blood supply. J. Cereb. Blood Flow Metab. *37*, 52–68.

Goyal, M.S., Hawrylycz, M., Miller, J.A., Snyder, A.Z., and Raichle, M.E. (2014). Aerobic glycolysis in the human brain is associated with development and neotenous gene expression. Cell Metab. *19*, 49–57.

Guan, J., Pavlovic, D., Dalkie, N., Waldvogel, H.J., O'Carroll, S.J., Green, C.R., and Nicholson, L.F.B. (2013). Vascular degeneration in Parkinson's disease. Brain Pathol. *23*, 154–164.

Haffner, C., Malik, R., and Dichgans, M. (2016). Genetic factors in cerebral small vessel disease and their impact on stroke and dementia. J. Cereb. Blood Flow Metab. *36*, 158–171.

Hall, C.N., Reynell, C., Gesslein, B., Hamilton, N.B., Mishra, A., Sutherland, B.A., O'Farrell, F.M., Buchan, A.M., Lauritzen, M., and Attwell, D. (2014). Capillary pericytes regulate cerebral blood flow in health and disease. Nature *508*, 55–60.

Hamel, E. (2006). Perivascular nerves and the regulation of cerebrovascular tone. J. Appl. Physiol. *100*, 1059–1064.

Han, B.H., Zhou, M.-L., Abousaleh, F., Brendza, R.P., Dietrich, H.H., Koenigsknecht-Talboo, J., Cirrito, J.R., Milner, E., Holtzman, D.M., and Zipfel, G.J. (2008). Cerebrovascular dysfunction in amyloid precursor protein transgenic mice: contribution of soluble and insoluble amyloid-beta peptide, partial restoration via gamma-secretase inhibition. J. Neurosci. *28*, 13542–13550.

Harrison, R.V., Harel, N., Panesar, J., and Mount, R.J. (2002). Blood capillary distribution correlates with hemodynamic-based functional imaging in cerebral cortex. Cereb. Cortex *12*, 225–233.

Hartmann, D.A., Underly, R.G., Grant, R.I., Watson, A.N., Lindner, V., and Shih, A.Y. (2015). Pericyte structure and distribution in the cerebral cortex revealed by high-resolution imaging of transgenic mice. Neurophotonics *2*, 041402.

Hawkes, C.A., and McLaurin, J. (2009). Selective targeting of perivascular macrophages for clearance of beta-amyloid in cerebral amyloid angiopathy. Proc. Natl. Acad. Sci. USA *106*, 1261–1266.

Hayakawa, K., Pham, L.-D.D., Katusic, Z.S., Arai, K., and Lo, E.H. (2012). Astrocytic high-mobility group box 1 promotes endothelial progenitor cell-mediated neurovascular remodeling during stroke recovery. Proc. Natl. Acad. Sci. USA *109*, 7505–7510.

He, L., Vanlandewijck, M., Raschperger, E., Andaloussi Mäe, M., Jung, B., Lebouvier, T., Ando, K., Hofmann, J., Keller, A., and Betsholtz, C. (2016). Analysis of the brain mural cell transcriptome. Sci. Rep. *6*, 35108.

Henkel, J.S., Beers, D.R., Wen, S., Bowser, R., and Appel, S.H. (2009). Decreased mRNA expression of tight junction proteins in lumbar spinal cords of patients with ALS. Neurology *72*, 1614–1616.

Hill, R.A., Tong, L., Yuan, P., Murikinati, S., Gupta, S., and Grutzendler, J. (2015). Regional Blood Flow in the Normal and Ischemic Brain Is Controlled by Arteriolar Smooth Muscle Cell Contractility and Not by Capillary Pericytes. Neuron *87*, 95–110.

Hladky, S.B., and Barrand, M.A. (2014). Mechanisms of fluid movement into, through and out of the brain: evaluation of the evidence. Fluids Barriers CNS *11*, 26.

Iadecola, C. (1993). Regulation of the cerebral microcirculation during neural activity: is nitric oxide the missing link? Trends Neurosci. *16*, 206–214.

Iadecola, C. (2004). Neurovascular regulation in the normal brain and in Alzheimer's disease. Nat. Rev. Neurosci. *5*, 347–360.

Iadecola, C. (2013). The pathobiology of vascular dementia. Neuron *80*, 844–866.

Iadecola, C., Mraovitch, S., Meeley, M.P., and Reis, D.J. (1983). Lesions of the basal forebrain in rat selectively impair the cortical vasodilation elicited from cerebellar fastigial nucleus. Brain Res. *279*, 41–52.

Iadecola, C., Beitz, A.J., Renno, W., Xu, X., Mayer, B., and Zhang, F. (1993). Nitric oxide synthase-containing neural processes on large cerebral arteries and cerebral microvessels. Brain Res. *606*, 148–155.

Iadecola, C., Yang, G., Ebner, T.J., and Chen, G. (1997). Local and propagated vascular responses evoked by focal synaptic activity in cerebellar cortex. J. Neurophysiol. *78*, 651–659.

Iadecola, C., Zhang, F., Niwa, K., Eckman, C., Turner, S.K., Fischer, E., Younkin, S., Borchelt, D.R., Hsiao, K.K., and Carlson, G.A. (1999). SOD1 rescues cerebral endothelial dysfunction in mice overexpressing amyloid precursor protein. Nat. Neurosci. *2*, 157–161.

Ibayashi, S., Ngai, A.C., Howard, M.A., 3rd, Meno, J.R., Mayberg, M.R., and Winn, H.R. (1991). Lack of sympathetic and cholinergic influences on cerebral vasodilation caused by sciatic nerve stimulation in the rat. J. Cereb. Blood Flow Metab. *11*, 678–683.

Iliff, J.J., Wang, M., Liao, Y., Plogg, B.A., Peng, W., Gundersen, G.A., Benveniste, H., Vates, G.E., Deane, R., Goldman, S.A., et al. (2012). A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. Sci. Transl. Med. *4*, 147ra111.

Iliff, J.J., Chen, M.J., Plog, B.A., Zeppenfeld, D.M., Soltero, M., Yang, L., Singh, I., Deane, R., and Nedergaard, M. (2014). Impairment of glymphatic pathway function promotes tau pathology after traumatic brain injury. J. Neurosci. *34*, 16180–16193.

Ishii, K., Hosokawa, C., Hyodo, T., Sakaguchi, K., Usami, K., Shimamoto, K., Hosono, M., Yamazoe, Y., and Murakami, T. (2015). Regional glucose metabolic reduction in dementia with Lewy bodies is independent of amyloid deposition. Ann. Nucl. Med. *29*, 78–83.

Jackman, K., Kahles, T., Lane, D., Garcia-Bonilla, L., Abe, T., Capone, C., Hochrainer, K., Voss, H., Zhou, P., Ding, A., et al. (2013). Progranulin deficiency promotes post-ischemic blood-brain barrier disruption. J. Neurosci. *33*, 19579–19589.

Janelidze, S., Lindqvist, D., Francardo, V., Hall, S., Zetterberg, H., Blennow, K., Adler, C.H., Beach, T.G., Serrano, G.E., van Westen, D., et al. (2015). Increased CSF biomarkers of angiogenesis in Parkinson disease. Neurology *85*, 1834–1842.

Janelidze, S., Hertze, J., Nägga, K., Nilsson, K., Nilsson, C., Wennström, M., van Westen, D., Blennow, K., Zetterberg, H., and Hansson, O.; Swedish BioFINDER Study Group (2017). Increased blood-brain barrier permeability is associated with dementia and diabetes but not amyloid pathology or APOE genotype. Neurobiol. Aging *51*, 104–112.

Jego, P., Pacheco-Torres, J., Araque, A., and Canals, S. (2014). Functional MRI in mice lacking IP3-dependent calcium signaling in astrocytes. J. Cereb. Blood Flow Metab. *34*, 1599–1603.

Jones, E.G. (1970). On the mode of entry of blood vessels into the cerebral cortex. J. Anat. *106*, 507–520.

Joutel, A., Haddad, I., Ratelade, J., and Nelson, M.T. (2016). Perturbations of the cerebrovascular matrisome: a convergent mechanism in small vessel disease of the brain? J. Cereb. Blood Flow Metab. *36*, 143–157.

Jung, B., Arnold, T.D., Raschperger, E., Gaengel, K., and Betsholtz, C. (2017). Visualization of vascular mural cells in developing brain using genetically labeled transgenic reporter mice. J. Cereb. Blood Flow Metab. *126*, 271678X17697720.

Kapasi, A., DeCarli, C., and Schneider, J.A. (2017). Impact of multiple pathologies on the threshold for clinically overt dementia. Acta Neuropathol. *134*, 171–186.

Keaney, J., Walsh, D.M., O'Malley, T., Hudson, N., Crosbie, D.E., Loftus, T., Sheehan, F., McDaid, J., Humphries, M.M., Callanan, J.J., et al. (2015). Autoregulated paracellular clearance of amyloid-β across the blood-brain barrier. Sci. Adv. *1*, e1500472.

Kety, S.S. (1950). Circulation and metabolism of the human brain in health and disease. Am. J. Med. *8*, 205–217.

Kim, K.J., Ramiro Diaz, J., Iddings, J.A., and Filosa, J.A. (2016). Vasculo-Neuronal Coupling: Retrograde Vascular Communication to Brain Neurons. J. Neurosci. *36*, 12624–12639.

Kisler, K., Nelson, A.R., Montagne, A., and Zlokovic, B.V. (2017a). Cerebral blood flow regulation and neurovascular dysfunction in Alzheimer disease. Nat. Rev. Neurosci. *18*, 419–434.

Kisler, K., Nelson, A.R., Rege, S.V., Ramanathan, A., Wang, Y., Ahuja, A., Lazic, D., Tsai, P.S., Zhao, Z., Zhou, Y., et al. (2017b). Pericyte degeneration leads to neurovascular uncoupling and limits oxygen supply to brain. Nat. Neurosci. *20*, 406–416.

Knowland, D., Arac, A., Sekiguchi, K.J., Hsu, M., Lutz, S.E., Perrino, J., Steinberg, G.K., Barres, B.A., Nimmerjahn, A., and Agalliu, D. (2014). Stepwise recruitment of transcellular and paracellular pathways underlies blood-brain barrier breakdown in stroke. Neuron *82*, 603–617.

Ko, K.R., Ngai, A.C., and Winn, H.R. (1990). Role of adenosine in regulation of regional cerebral blood flow in sensory cortex. Am. J. Physiol. *259*, H1703–H1708.

Koller, A., and Toth, P. (2012). Contribution of flow-dependent vasomotor mechanisms to the autoregulation of cerebral blood flow. J. Vasc. Res. *49*, 375–389.

Kortekaas, R., Leenders, K.L., van Oostrom, J.C.H., Vaalburg, W., Bart, J., Willemsen, A.T.M., and Hendrikse, N.H. (2005). Blood-brain barrier dysfunction in parkinsonian midbrain in vivo. Ann. Neurol. *57*, 176–179.

Kozberg, M.G., Chen, B.R., DeLeo, S.E., Bouchard, M.B., and Hillman, E.M.C. (2013). Resolving the transition from negative to positive blood oxygen level-dependent responses in the developing brain. Proc. Natl. Acad. Sci. USA *110*, 4380–4385.

Kress, B.T., Iliff, J.J., Xia, M., Wang, M., Wei, H.S., Zeppenfeld, D., Xie, L., Kang, H., Xu, Q., Liew, J.A., et al. (2014). Impairment of paravascular clearance pathways in the aging brain. Ann. Neurol. *76*, 845–861.

Krueger, M., and Bechmann, I. (2010). CNS pericytes: concepts, misconceptions, and a way out. Glia *58*, 1–10.

Lacoste, B., and Gu, C. (2015). Control of cerebrovascular patterning by neural activity during postnatal development. Mech. Dev. *138*, 43–49.

Lambrechts, D., Poesen, K., Fernández-Santiago, R., Al-Chalabi, A., Del Bo, R., Van Vught, P.W.J., Khan, S., Marklund, S.L., Brockington, A., van Marion, I., et al. (2009). Meta-analysis of vascular endothelial growth factor variations in amyotrophic lateral sclerosis: increased susceptibility in male carriers of the -2578AA genotype. J. Med. Genet. *46*, 840–846.

Lassen, N.A., Ingvar, D.H., and Skinhøj, E. (1978). Brain function and blood flow. Sci. Am. *239*, 62–71.

Lau, Y.M., Wong, S.C., Tsang, S.W., Lau, W.K., Lu, A.P., and Zhang, H. (2014). Cellular sources of cyclooxygenase-1 and -2 up-regulation in the spinal dorsal horn after spinal nerve ligation. Neuropathol. Appl. Neurobiol. *40*, 452–463.

Lecrux, C., and Hamel, E. (2016). Neuronal networks and mediators of cortical neurovascular coupling responses in normal and altered brain states. Philos. Trans. R. Soc. Lond. B Biol. Sci. *371*, 20150350.

Lecrux, C., Toussay, X., Kocharyan, A., Fernandes, P., Neupane, S., Lévesque, M., Plaisier, F., Shmuel, A., Cauli, B., and Hamel, E. (2011). Pyramidal neurons are "neurogenic hubs" in the neurovascular coupling response to whisker stimulation. J. Neurosci. *31*, 9836–9847.

Lecrux, C., Sandoe, C.H., Neupane, S., Kropf, P., Toussay, X., Tong, X.-K., Lacalle-Aurioles, M., Shmuel, A., and Hamel, E. (2017). Impact of Altered Cholinergic Tones on the Neurovascular Coupling Response to Whisker Stimulation. J. Neurosci. *37*, 1518–1531.

LeDoux, J.E., Thompson, M.E., Iadecola, C., Tucker, L.W., and Reis, D.J. (1983). Local cerebral blood flow increases during auditory and emotional processing in the conscious rat. Science *221*, 576–578.

Lee, S.-H., and Dan, Y. (2012). Neuromodulation of brain states. Neuron *76*, 209–222.

Lind, B.L., Brazhe, A.R., Jessen, S.B., Tan, F.C.C., and Lauritzen, M.J. (2013). Rapid stimulus-evoked astrocyte Ca2+ elevations and hemodynamic responses in mouse somatosensory cortex in vivo. Proc. Natl. Acad. Sci. USA *110*, E4678–E4687.

Liu, X., Li, C., Falck, J.R., Harder, D.R., and Koehler, R.C. (2012). Relative contribution of cyclooxygenases, epoxyeicosatrienoic acids, and pH to the cerebral blood flow response to vibrissal stimulation. Am. J. Physiol. Heart Circ. Physiol. *302*, H1075–H1085.

Longden, T.A., Hill-Eubanks, D.C., and Nelson, M.T. (2016). Ion channel networks in the control of cerebral blood flow. J. Cereb. Blood Flow Metab. *36*, 492–512.

Longden, T.A., Dabertrand, F., Koide, M., Gonzales, A.L., Tykocki, N.R., Brayden, J.E., Hill-Eubanks, D., and Nelson, M.T. (2017). Capillary K(+)-sensing initiates retrograde hyperpolarization to increase local cerebral blood flow. Nat. Neurosci. *20*, 717–726.

Louveau, A., Da Mesquita, S., and Kipnis, J. (2016). Lymphatics in neurological disorders: a neuro-lympho-vascular component of multiple sclerosis and Alzheimer's disease? Neuron *91*, 957–973.

Love, S., and Miners, J.S. (2016). Cerebrovascular disease in ageing and Alzheimer's disease. Acta Neuropathol. *131*, 645–658.

Lyons, D.G., Parpaleix, A., Roche, M., and Charpak, S. (2016). Mapping oxygen concentration in the awake mouse brain. eLife *5*, e12024.

Martin, J.A., Craft, D.K., Su, J.H., Kim, R.C., and Cotman, C.W. (2001). Astrocytes degenerate in frontotemporal dementia: possible relation to hypoperfusion. Neurobiol. Aging *22*, 195–207.

Metea, M.R., Kofuji, P., and Newman, E.A. (2007). Neurovascular coupling is not mediated by potassium siphoning from glial cells. J. Neurosci. *27*, 2468–2471.

Miners, S., Moulding, H., de Silva, R., and Love, S. (2014a). Reduced vascular endothelial growth factor and capillary density in the occipital cortex in dementia with Lewy bodies. Brain Pathol. *24*, 334–343.

Miners, J.S., Palmer, J.C., Tayler, H., Palmer, L.E., Ashby, E., Kehoe, P.G., and Love, S. (2014b). Aβ degradation or cerebral perfusion? Divergent effects of multifunctional enzymes. Front. Aging Neurosci. *6*, 238.

Mishra, A. (2017). Binaural blood flow control by astrocytes: listening to synapses and the vasculature. J. Physiol. *595*, 1885–1902.

Mishra, A., Reynolds, J.P., Chen, Y., Gourine, A.V., Rusakov, D.A., and Attwell, D. (2016). Astrocytes mediate neurovascular signaling to capillary pericytes but not to arterioles. Nat. Neurosci. *19*, 1619–1627.

Miyazaki, K., Masamoto, K., Morimoto, N., Kurata, T., Mimoto, T., Obata, T., Kanno, I., and Abe, K. (2012). Early and progressive impairment of spinal blood flow-glucose metabolism coupling in motor neuron degeneration of ALS model mice. J. Cereb. Blood Flow Metab. *32*, 456–467.

Montagne, A., Barnes, S.R., Sweeney, M.D., Halliday, M.R., Sagare, A.P., Zhao, Z., Toga, A.W., Jacobs, R.E., Liu, C.Y., Amezcua, L., et al. (2015). Blood-brain barrier breakdown in the aging human hippocampus. Neuron *85*, 296–302.

Moore, C.I., and Cao, R. (2008). The hemo-neural hypothesis: on the role of blood flow in information processing. J. Neurophysiol. *99*, 2035–2047.

Morris, A.W.J., Sharp, M.M., Albargothy, N.J., Fernandes, R., Hawkes, C.A., Verma, A., Weller, R.O., and Carare, R.O. (2016). Vascular basement membranes as pathways for the passage of fluid into and out of the brain. Acta Neuropathol. *131*, 725–736.

Mosso, A. (1880). Sulla circolazione del cervello dell'uomo. Atti della R. Accademia dei Lincei *5*, 237–358.

Mueller, S.M., Heistad, D.D., and Marcus, M.L. (1979). Effect of sympathetic nerves on cerebral vessels during seizures. Am. J. Physiol. *237*, H178–H184.

Murphy, M.J., Grace, G.M., Tartaglia, M.C., Orange, J.B., Chen, X., Rowe, A., Findlater, K., Kozak, R.I., Freedman, M., Lee, T.-Y., and Strong, M.J. (2012). Widespread cerebral haemodynamics disturbances occur early in amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. *13*, 202–209.

Nedergaard, M. (2013). Neuroscience. Garbage truck of the brain. Science *340*, 1529–1530.

Nehlig, A., Pereira de Vasconcelos, A., and Boyet, S. (1989). Postnatal changes in local cerebral blood flow measured by the quantitative autoradiographic [14C]iodoantipyrine technique in freely moving rats. J. Cereb. Blood Flow Metab. *9*, 579–588.

Ngai, A.C., and Winn, H.R. (2002). Pial arteriole dilation during somatosensory stimulation is not mediated by an increase in CSF metabolites. Am. J. Physiol. Heart Circ. Physiol. *282*, H902–H907.

Ngai, A.C., Ko, K.R., Morii, S., and Winn, H.R. (1988). Effect of sciatic nerve stimulation on pial arterioles in rats. Am. J. Physiol. *254*, H133–H139.

Niwa, K., Araki, E., Morham, S.G., Ross, M.E., and Iadecola, C. (2000a). Cyclooxygenase-2 contributes to functional hyperemia in whisker-barrel cortex. J. Neurosci. *20*, 763–770.

Niwa, K., Younkin, L., Ebeling, C., Turner, S.K., Westaway, D., Younkin, S., Ashe, K.H., Carlson, G.A., and Iadecola, C. (2000b). Abeta 1-40-related reduction in functional hyperemia in mouse neocortex during somatosensory activation. Proc. Natl. Acad. Sci. USA *97*, 9735–9740.

Niwa, K., Haensel, C., Ross, M.E., and Iadecola, C. (2001a). Cyclooxygenase-1 participates in selected vasodilator responses of the cerebral circulation. Circ. Res. *88*, 600–608.

Niwa, K., Porter, V.A., Kazama, K., Cornfield, D., Carlson, G.A., and Iadecola, C. (2001b). A beta-peptides enhance vasoconstriction in cerebral circulation. Am J. Physiol. Heart Circ. Physiol. *281*, H2417–H2424.

Niwa, K., Kazama, K., Younkin, L., Younkin, S.G., Carlson, G.A., and Iadecola, C. (2002a). Cerebrovascular autoregulation is profoundly impaired in mice overexpressing amyloid precursor protein. Am J. Physiol. Heart Circ. Physiol. *283*, H315–H3123.

Niwa, K., Kazama, K., Younkin, S.G., Carlson, G.A., and Iadecola, C. (2002b). Alterations in cerebral blood flow and glucose utilization in mice overexpressing the amyloid precursor protein. Neurobiol. Dis. *9*, 61–68.

Nizar, K., Uhlirova, H., Tian, P., Saisan, P.A., Cheng, Q., Reznichenko, L., Weldy, K.L., Steed, T.C., Sridhar, V.B., MacDonald, C.L., et al. (2013). In vivo stimulus-induced vasodilation occurs without IP3 receptor activation and may precede astrocytic calcium increase. J. Neurosci. *33*, 8411–8422.

O'Herron, P., Chhatbar, P.Y., Levy, M., Shen, Z., Schramm, A.E., Lu, Z., and Kara, P. (2016). Neural correlates of single-vessel haemodynamic responses in vivo. Nature *534*, 378–382.

Olney, N.T., Spina, S., and Miller, B.L. (2017). Frontotemporal dementia. Neurol. Clin. *35*, 339–374.

Otsu, Y., Couchman, K., Lyons, D.G., Collot, M., Agarwal, A., Mallet, J.-M., Pfrieger, F.W., Bergles, D.E., and Charpak, S. (2015). Calcium dynamics in astrocyte processes during neurovascular coupling. Nat. Neurosci. *18*, 210–218.

Ouzounov, D.G., Wang, T., Wang, M., Feng, D.D., Horton, N.G., Cruz-Hernández, J.C., Cheng, Y.-T., Reimer, J., Tolias, A.S., Nishimura, N., and Xu, C. (2017). In vivo three-photon imaging of activity of GCaMP6-labeled neurons deep in intact mouse brain. Nat. Methods *14*, 388–390.

Park, L., Zhou, P., Pitstick, R., Capone, C., Anrather, J., Norris, E.H., Younkin, L., Younkin, S., Carlson, G., McEwen, B.S., and Iadecola, C. (2008). Nox2-derived radicals contribute to neurovascular and behavioral dysfunction in mice overexpressing the amyloid precursor protein. Proc. Natl. Acad. Sci. USA *105*, 1347–1352.

Park, L., Zhou, J., Zhou, P., Pistick, R., El Jamal, S., Younkin, L., Pierce, J., Arreguin, A., Anrather, J., Younkin, S.G., et al. (2013). Innate immunity receptor CD36 promotes cerebral amyloid angiopathy. Proc. Natl. Acad. Sci. USA *110*, 3089–3094.

Park, L., Koizumi, K., El Jamal, S., Zhou, P., Previti, M.L., Van Nostrand, W.E., Carlson, G., and Iadecola, C. (2014a). Age-dependent neurovascular dysfunction and damage in a mouse model of cerebral amyloid angiopathy. Stroke *45*, 1815–1821.

Park, L., Wang, G., Moore, J., Girouard, H., Zhou, P., Anrather, J., and Iadecola, C. (2014b). The key role of transient receptor potential melastatin-2 channels in amyloid-β-induced neurovascular dysfunction. Nat. Commun. *5*, 5318.

Park, L., Uekawa, K., Garcia-Bonilla, L., Koizumi, K., Murphy, M., Pitstick, R., Younkin, L.H., Younkin, S.G., Zhou, P., Carlson, G.A., et al. (2017). Brain perivascular macrophages initiate the neurovascular dysfunction of Alzheimer Aβ peptides. Circ. Res. *121*, 258–269.

Pase, M.P., Satizabal, C.L., and Seshadri, S. (2017). Role of improved vascular health in the declining incidence of dementia. Stroke *48*, 2013–2020.

Paulson, O.B., and Newman, E.A. (1987). Does the release of potassium from astrocyte endfeet regulate cerebral blood flow? Science *237*, 896–898.

Peppiatt, C.M., Howarth, C., Mobbs, P., and Attwell, D. (2006). Bidirectional control of CNS capillary diameter by pericytes. Nature *443*, 700–704.

Petrovitch, H., White, L.R., Izmirilian, G., Ross, G.W., Havlik, R.J., Markesbery, W., Nelson, J., Davis, D.G., Hardman, J., Foley, D.J., and Launer, L.J. (2000). Midlife blood pressure and neuritic plaques, neurofibrillary tangles, and brain weight at death: the HAAS. Honolulu-Asia aging Study. Neurobiol. Aging *21*, 57–62.

Petzold, G.C., and Murthy, V.N. (2011). Role of astrocytes in neurovascular coupling. Neuron *71*, 782–797.

Pisani, V., Stefani, A., Pierantozzi, M., Natoli, S., Stanzione, P., Franciotta, D., and Pisani, A. (2012). Increased blood-cerebrospinal fluid transfer of albumin in advanced Parkinson's disease. J. Neuroinflammation *9*, 188.

Plog, B.A., Dashnaw, M.L., Hitomi, E., Peng, W., Liao, Y., Lou, N., Deane, R., and Nedergaard, M. (2015). Biomarkers of traumatic injury are transported from brain to blood via the glymphatic system. J. Neurosci. *35*, 518–526.

Poesen, K., Lambrechts, D., Van Damme, P., Dhondt, J., Bender, F., Frank, N., Bogaert, E., Claes, B., Heylen, L., Verheyen, A., et al. (2008). Novel role for vascular endothelial growth factor (VEGF) receptor-1 and its ligand VEGF-B in motor neuron degeneration. J. Neurosci. *28*, 10451–10459.

Prinz, M., Erny, D., and Hagemeyer, N. (2017). Ontogeny and homeostasis of CNS myeloid cells. Nat. Immunol. *18*, 385–392.

Przedborski, S. (2017). The two-century journey of Parkinson disease research. Nat. Rev. Neurosci. *18*, 251–259.

Quaegebeur, A., Lange, C., and Carmeliet, P. (2011). The neurovascular link in health and disease: molecular mechanisms and therapeutic implications. Neuron *71*, 406–424.

Raab, S., and Plate, K.H. (2007). Different networks, common growth factors: shared growth factors and receptors of the vascular and the nervous system. Acta Neuropathol. *113*, 607–626.

Raab, S., Beck, H., Gaumann, A., Yüce, A., Gerber, H.-P., Plate, K., Hammes, H.-P., Ferrara, N., and Breier, G. (2004). Impaired brain angiogenesis and neuronal apoptosis induced by conditional homozygous inactivation of vascular endothelial growth factor. Thromb. Haemost. *91*, 595–605.

Raichle, M.E., and Mintun, M.A. (2006). Brain work and brain imaging. Annu. Rev. Neurosci. *29*, 449–476.

Rancillac, A., Rossier, J., Guille, M., Tong, X.-K., Geoffroy, H., Amatore, C., Arbault, S., Hamel, E., and Cauli, B. (2006). Glutamatergic Control of Microvascular Tone by Distinct GABA Neurons in the Cerebellum. J. Neurosci. *26*, 6997–7006.

Ransohoff, R.M. (2016). How neuroinflammation contributes to neurodegeneration. Science *353*, 777–783.

Rodrigue, K.M., Rieck, J.R., Kennedy, K.M., Devous, M.D., Sr., Diaz-Arrastia, R., and Park, D.C. (2013). Risk factors for β-amyloid deposition in healthy aging: vascular and genetic effects. JAMA Neurol. *70*, 600–606.

Roggendorf, W., and Cervós-Navarro, J. (1977). Ultrastructure of arterioles in the cat brain. Cell Tissue Res. *178*, 495–515.

Roher, A.E., Esh, C., Rahman, A., Kokjohn, T.A., and Beach, T.G. (2004). Atherosclerosis of cerebral arteries in Alzheimer disease. Stroke *35* (11, Suppl 1), 2623–2627.

Roquet, D., Sourty, M., Botzung, A., Armspach, J.-P., and Blanc, F. (2016). Brain perfusion in dementia with Lewy bodies and Alzheimer's disease: an arterial spin labeling MRI study on prodromal and mild dementia stages. Alzheimers Res. Ther. *8*, 29.

Rosenegger, D.G., Tran, C.H.T., Wamsteeker Cusulin, J.I., and Gordon, G.R. (2015). Tonic Local Brain Blood Flow Control by Astrocytes Independent of Phasic Neurovascular Coupling. J. Neurosci. *35*, 13463–13474.

Rosengarten, B., Dannhardt, V., Burr, O., Pöhler, M., Rosengarten, S., Oechsner, M., and Reuter, I. (2010). Neurovascular coupling in Parkinson's disease patients: effects of dementia and acetylcholinesterase inhibitor treatment. J. Alzheimers Dis. *22*, 415–421.

Roy, C.S., and Sherrington, C.S. (1890). On the regulation of the blood-supply of the brain. J. Physiol. *11*, 85–108, 17.

Rule, R.R., Schuff, N., Miller, R.G., and Weiner, M.W. (2010). Gray matter perfusion correlates with disease severity in ALS. Neurology *74*, 821–827.

Sagare, A.P., Bell, R.D., Zhao, Z., Ma, Q., Winkler, E.A., Ramanathan, A., and Zlokovic, B.V. (2013). Pericyte loss influences Alzheimer-like neurodegeneration in mice. Nat. Commun. *4*, 2932.

Schmid, F., Tsai, P.S., Kleinfeld, D., Jenny, P., and Weber, B. (2017). Depth-dependent flow and pressure characteristics in cortical microvascular networks. PLoS Comput. Biol. *13*, e1005392.

Schmidt, C.F., and Hendrix, J.P. (1938). The action of chemical substances on cerebral blood vessels. Res. Publ. Assoc. Res. Nerv. Ment. Dis. *18*, 229–276.

Schneider, J.A., Arvanitakis, Z., Bang, W., and Bennett, D.A. (2007). Mixed brain pathologies account for most dementia cases in community-dwelling older persons. Neurology *69*, 2197–2204.

Seals, D.R., Jablonski, K.L., and Donato, A.J. (2011). Aging and vascular endothelial function in humans. Clin. Sci. *120*, 357–375.

Segal, S.S. (2015). Integration and Modulation of Intercellular Signaling Underlying Blood Flow Control. J. Vasc. Res. *52*, 136–157.

Silva, A.C., and Koretsky, A.P. (2002). Laminar specificity of functional MRI onset times during somatosensory stimulation in rat. Proc. Natl. Acad. Sci. USA *99*, 15182–15187.

Smith, A.J., Yao, X., Dix, J.A., Jin, B.-J., and Verkman, A.S. (2017). Test of the "glymphatic" hypothesis demonstrates diffusive and aquaporin-4-independent solute transport in rodent brain parenchyma. Elife *6*, e27679.

Snapyan, M., Lemasson, M., Brill, M.S., Blais, M., Massouh, M., Ninkovic, J., Gravel, C., Berthod, F., Götz, M., Barker, P.A., et al. (2009). Vasculature guides migrating neuronal precursors in the adult mammalian forebrain via brain-derived neurotrophic factor signaling. J. Neurosci. *29*, 4172–4188.

Sokoloff, L. (2013). Circulation in the central nervous system. In Springer Science & Business Media, U. Windhorst, ed. (Berlin, Heidelberg), pp. 561–578.

Srinivasan, R., Huang, B.S., Venugopal, S., Johnston, A.D., Chai, H., Zeng, H., Golshani, P., and Khakh, B.S. (2015). Ca(2+) signaling in astrocytes from Ip3r2(-/-) mice in brain slices and during startle responses in vivo. Nat. Neurosci. *18*, 708–717.

Stobart, J.L.L., Lu, L., Anderson, H.D.I., Mori, H., and Anderson, C.M. (2013). Astrocyte-induced cortical vasodilation is mediated by D-serine and endothelial nitric oxide synthase. Proc. Natl. Acad. Sci. USA *110*, 3149–3154.

Storkebaum, E., Lambrechts, D., Dewerchin, M., Moreno-Murciano, M.-P., Appelmans, S., Oh, H., Van Damme, P., Rutten, B., Man, W.Y., De Mol, M., et al. (2005). Treatment of motoneuron degeneration by intracerebroventricular delivery of VEGF in a rat model of ALS. Nat. Neurosci. *8*, 85–92.

Stromberg, D.D., and Fox, J.R. (1972). Pressures in the pial arterial microcirculation of the cat during changes in systemic arterial blood pressure. Circ. Res. *31*, 229–239.

Sudre, C.H., Bocchetta, M., Cash, D., Thomas, D.L., Woollacott, I., Dick, K.M., van Swieten, J., Borroni, B., Galimberti, D., Masellis, M., et al.; Genetic FTD Initiative, GENFI (2017). White matter hyperintensities are seen only in GRN mutation carriers in the GENFI cohort. Neuroimage Clin. *15*, 171–180.

Sun, W., McConnell, E., Pare, J.-F., Xu, Q., Chen, M., Peng, W., Lovatt, D., Han, X., Smith, Y., and Nedergaard, M. (2013). Glutamate-dependent neuroglial calcium signaling differs between young and adult brain. Science *339*, 197–200.

Takano, T., Tian, G.-F., Peng, W., Lou, N., Libionka, W., Han, X., and Nedergaard, M. (2006). Astrocyte-mediated control of cerebral blood flow. Nat. Neurosci. *9*, 260–267.

Tallini, Y.N., Brekke, J.F., Shui, B., Doran, R., Hwang, S.-M., Nakai, J., Salama, G., Segal, S.S., and Kotlikoff, M.I. (2007). Propagated endothelial Ca2+ waves and arteriolar dilation in vivo: measurements in Cx40BAC GCaMP2 transgenic mice. Circ. Res. *101*, 1300–1309.

Tarasoff-Conway, J.M., Carare, R.O., Osorio, R.S., Glodzik, L., Butler, T., Fieremans, E., Axel, L., Rusinek, H., Nicholson, C., Zlokovic, B.V., et al. (2015). Clearance systems in the brain-implications for Alzheimer disease. Nat. Rev. Neurol. *11*, 457–470.

Tasic, B., Menon, V., Nguyen, T.N., Kim, T.K., Jarsky, T., Yao, Z., Levi, B., Gray, L.T., Sorensen, S.A., Dolbeare, T., et al. (2016). Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. Nat. Neurosci. *19*, 335–346.

Thal, D.R., von Arnim, C.A., Griffin, W.S., Mrak, R.E., Walker, L., Attems, J., and Arzberger, T. (2015). Frontotemporal lobar degeneration FTLD-tau: preclinical lesions, vascular, and Alzheimer-related co-pathologies. J Neural Transm (Vienna) *122*, 1007–1018.

Thanopoulou, K., Fragkouli, A., Stylianopoulou, F., and Georgopoulos, S. (2010). Scavenger receptor class B type I (SR-BI) regulates perivascular

macrophages and modifies amyloid pathology in an Alzheimer mouse model. Proc. Natl. Acad. Sci. USA *107*, 20816–20821.

Thomas, T., Thomas, G., McLendon, C., Sutton, T., and Mullan, M. (1996). β-Amyloid-mediated vasoactivity and vascular endothelial damage. Nature *380*, 168–171.

Toledo, J.B., Arnold, S.E., Raible, K., Brettschneider, J., Xie, S.X., Grossman, M., Monsell, S.E., Kukull, W.A., and Trojanowski, J.Q. (2013). Contribution of cerebrovascular disease in autopsy confirmed neurodegenerative disease cases in the National Alzheimer's Coordinating Centre. Brain *136*, 2697–2706.

Tong, X.-K., Nicolakakis, N., Kocharyan, A., and Hamel, E. (2005). Vascular remodeling versus amyloid beta-induced oxidative stress in the cerebrovascular dysfunctions associated with Alzheimer's disease. J. Neurosci. *25*, 11165–11174.

Toussay, X., Basu, K., Lacoste, B., and Hamel, E. (2013). Locus coeruleus stimulation recruits a broad cortical neuronal network and increases cortical perfusion. J. Neurosci. *33*, 3390–3401.

Tran, K.A., Zhang, X., Predescu, D., Huang, X., Machado, R.F., Göthert, J.R., Malik, A.B., Valyi-Nagy, T., and Zhao, Y.-Y. (2016). Endothelial β-Catenin Signaling Is Required for Maintaining Adult Blood-Brain Barrier Integrity and Central Nervous System Homeostasis. Circulation *133*, 177–186.

Tsai, H.-H., Niu, J., Munji, R., Davalos, D., Chang, J., Zhang, H., Tien, A.-C., Kuo, C.J., Chan, J.R., Daneman, R., and Fancy, S.P.J. (2016). Oligodendrocyte precursors migrate along vasculature in the developing nervous system. Science *351*, 379–384.

Uhlirova, H., Kılıç, K., Tian, P., Sakadžić, S., Gagnon, L., Thunemann, M., Desjardins, M., Saisan, P.A., Nizar, K., Yaseen, M.A., et al. (2016a). The roadmap for estimation of cell-type-specific neuronal activity from non-invasive measurements. Philos. Trans. R. Soc. Lond. B Biol. Sci. *371*, 20150356.

Uhlirova, H., Kılıç, K., Tian, P., Thunemann, M., Desjardins, M., Saisan, P.A., Sakadžić, S., Ness, T.V., Mateo, C., Cheng, Q., et al. (2016b). Cell type specificity of neurovascular coupling in cerebral cortex. eLife *5*, 155.

van Es, M.A., Hardiman, O., Chio, A., Al-Chalabi, A., Pasterkamp, R.J., Veldink, J.H., and van den Berg, L.H. (2017). Amyotrophic lateral sclerosis. Lancet. Published online May 25, 2017. http://dx.doi.org/10.1016/S0140-6736(17)31287-4.

Vemuri, P., Lesnick, T.G., Przybelski, S.A., Knopman, D.S., Preboske, G.M., Kantarci, K., Raman, M.R., Machulda, M.M., Mielke, M.M., Lowe, V.J., et al. (2015). Vascular and amyloid pathologies are independent predictors of cognitive decline in normal elderly. Brain *138*, 761–771.

Verstraete, E., Biessels, G.J., van Den Heuvel, M.P., Visser, F., Luijten, P.R., and van Den Berg, L.H. (2010). No evidence of microbleeds in ALS patients at 7 Tesla MRI. Amyotroph. Lateral Scler. *11*, 555–557.

Wälchli, T., Wacker, A., Frei, K., Regli, L., Schwab, M.E., Hoerstrup, S.P., Gerhardt, H., and Engelhardt, B. (2015). Wiring the Vascular Network with Neural Cues: A CNS Perspective. Neuron *87*, 271–296.

Wang, H., Hitron, I.M., Iadecola, C., and Pickel, V.M. (2005). Synaptic and vascular associations of neurons containing cyclooxygenase-2 and nitric oxide synthase in rat somatosensory cortex. Cereb. Cortex *15*, 1250–1260.

Wang, M., Ding, F., Deng, S., Guo, X., Wang, W., Iliff, J.J., and Nedergaard, M. (2017). Focal solute trapping and global glymphatic pathway impairment in a murine model of multiple microinfarcts. J. Neurosci. *37*, 2870–2877.

Warren, D.E., Sutterer, M.J., Bruss, J., Abel, T.J., Jones, A., Kawasaki, H., Voss, M., Cassell, M., Howard, M.A., and Tranel, D. (2017). Surgically disconnected temporal pole exhibits resting functional connectivity with remote brain regions. bioRxiv. http://dx.doi.org/10.1101/127571.

Wei, H.S., Kang, H., Rasheed, I.-Y.D., Zhou, S., Lou, N., Gershteyn, A., McConnell, E.D., Wang, Y., Richardson, K.E., Palmer, A.F., et al. (2016). Erythrocytes Are Oxygen-Sensing Regulators of the Cerebral Microcirculation. Neuron *91*, 851–862.

Wells, J.A., Holmes, H.E., O'Callaghan, J.M., Colgan, N., Ismail, O., Fisher, E.M., Siow, B., Murray, T.K., Schwarz, A.J., O'Neill, M.J., et al. (2015). Increased cerebral vascular reactivity in the tau expressing rTg4510 mouse: evidence against the role of tau pathology to impair vascular health in Alzheimer's disease. J. Cereb. Blood Flow Metab. *35*, 359–362.

Winkler, E.A., Sengillo, J.D., Sullivan, J.S., Henkel, J.S., Appel, S.H., and Zlokovic, B.V. (2013). Blood-spinal cord barrier breakdown and pericyte reductions in amyotrophic lateral sclerosis. Acta Neuropathol. *125*, 111–120.

Winkler, E.A., Sengillo, J.D., Sagare, A.P., Zhao, Z., Ma, Q., Zuniga, E., Wang, Y., Zhong, Z., Sullivan, J.S., Griffin, J.H., et al. (2014). Blood-spinal cord barrier disruption contributes to early motor-neuron degeneration in ALS-model mice. Proc. Natl. Acad. Sci. USA *111*, E1035–E1042.

Woolsey, T.A., and Rovainen, C.M. (1991). Whisker barrels: a model for direct observation of changes in the cerebral microcirculation with neuronal activity. In Brain Work and Mental Activity: Quantitative Studies with Radioactive Tracers: Proceedings of the Alfred Benzon Symposium 31 Held at the Premises of the Royal Danish Academy of Sciences and Letters, Copenhagen, August 12–16, 1990, N.A. Lassen, ed. (John Wiley & Sons), pp. 189–200.

Xu, H.-L., Mao, L., Ye, S., Paisansathan, C., Vetri, F., and Pelligrino, D.A. (2008). Astrocytes are a key conduit for upstream signaling of vasodilation during cerebral cortical neuronal activation in vivo. Am. J. Physiol. Heart Circ. Physiol. *294*, H622–H632.

Yang, G., and Iadecola, C. (1998). Activation of cerebellar climbing fibers increases cerebellar blood flow: role of glutamate receptors, nitric oxide, and cGMP. Stroke *29*, 499–507, discussion 507–508.

Yang, G., Chen, G., Ebner, T., and Iadecola, C. (1999). Nitric oxide is the predominant mediator of cerebellar hyperemia during somatosensory activation in rats. Am. J. Physiol. *277*, R1760–R1770.

Yang, G., Huard, J.M., Beitz, A.J., Ross, M.E., and Iadecola, C. (2000). Stellate neurons mediate functional hyperemia in the cerebellar molecular layer. J. Neurosci. *20*, 6968–6973.

Yang, G., Zhang, Y., Ross, M.E., and Iadecola, C. (2003). Attenuation of activity-induced increases in cerebellar blood flow in mice lacking neuronal nitric oxide synthase. Am. J. Physiol. Heart Circ. Physiol. *285*, H298–H304.

Yuen, T.J., Silbereis, J.C., Griveau, A., Chang, S.M., Daneman, R., Fancy, S.P.J., Zahed, H., Maltepe, E., and Rowitch, D.H. (2014). Oligodendrocyte-encoded HIF function couples postnatal myelination and white matter angiogenesis. Cell *158*, 383–396.

Zhang, E.T., Inman, C.B., and Weller, R.O. (1990). Interrelationships of the pia mater and the perivascular (Virchow-Robin) spaces in the human cerebrum. J. Anat. *170*, 111–123.

Zhang, F., Xu, S., and Iadecola, C. (1995). Role of nitric oxide and acetylcholine in neocortical hyperemia elicited by basal forebrain stimulation: evidence for an involvement of endothelial nitric oxide. Neuroscience *69*, 1195–1204.

Zhang, F., Eckman, C., Younkin, S., Hsiao, K.K., and Iadecola, C. (1997). Increased susceptibility to ischemic brain damage in transgenic mice overexpressing the amyloid precursor protein. J. Neurosci. *17*, 7655–7661.

Zhao, Z., Nelson, A.R., Betsholtz, C., and Zlokovic, B.V. (2015a). Establishment and Dysfunction of the Blood-Brain Barrier. Cell *163*, 1064–1078.

Zhao, Z., Sagare, A.P., Ma, Q., Halliday, M.R., Kong, P., Kisler, K., Winkler, E.A., Ramanathan, A., Kanekiyo, T., Bu, G., et al. (2015b). Central role for PICALM in amyloid-β blood-brain barrier transcytosis and clearance. Nat. Neurosci. *18*, 978–987.

Zhong, Z., Deane, R., Ali, Z., Parisi, M., Shapovalov, Y., O'Banion, M.K., Stojanovic, K., Sagare, A., Boillee, S., Cleveland, D.W., and Zlokovic, B.V. (2008). ALS-causing SOD1 mutants generate vascular changes prior to motor neuron degeneration. Nat. Neurosci. *11*, 420–422.

Zhu, M., Ackerman, J.J.H., Sukstanskii, A.L., and Yablonskiy, D.A. (2006). How the body controls brain temperature: the temperature shielding effect of cerebral blood flow. J. Appl. Physiol. *101*, 1481–1488.

Zonta, M., Angulo, M.C., Gobbo, S., Rosengarten, B., Hossmann, K.-A., Pozzan, T., and Carmignoto, G. (2003). Neuron-to-astrocyte signaling is central to the dynamic control of brain microcirculation. Nat. Neurosci. *6*, 43–50.