

Robust and Fragile Aspects of Cortical Blood Flow in Relation to the Underlying Angioarchitecture

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ABSTRACT

We review the organizational principles of the cortical vasculature and the underlying patterns of blood flow under normal conditions and in response to occlusion of single vessels. The cortex is sourced by a two-dimensional network of pial arterioles that feeds a three-dimensional network of subsurface microvessels in close proximity to neurons and glia. Blood flow within the surface and subsurface networks is largely insensitive to occlusion of a single vessel within either network. However, the penetrating arterioles that connect the pial network to the subsurface network are bottlenecks to flow; occlusion of even a single penetrating arteriole results in the death of a 500 μm diameter cylinder of cortical tissue despite the potential for collateral flow through microvessels. This pattern of flow is consistent with that calculated from a full reconstruction of the angioarchitecture. Conceptually, collateral flow is insufficient to compensate for the occlusion of a penetrating arteriole because

penetrating venules act as shunts of blood that flows through collaterals. Future directions that stem from the analysis of the angioarchitecture concern cellular-level issues, in particular the regulation of blood flow within the subsurface microvascular network, and system-level issues, in particular the role of penetrating arteriole occlusions in human cognitive impairment.

KEY WORDS: pial vessels, penetrating vessels, microvessels, imaging, rodent

Abbreviations used: BAP, broadly active promoter plus a set of cis-regulatory sequences; BOLD, blood oxygenation level dependent; DAPI, 4',6-diamidino-2-phenylindole dilactate; eYFP, enhanced yellow fluorescent protein; FLAIR, fluid attenuation inversion recovery; fMRI, functional magnetic resonance imaging; GFP, green fluorescent protein; MCA, middle cerebral artery; RBCs, red blood cells; TPLSM, two-photon laser scanning microscopy.

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The vasculature of the brain is tasked with the immense role of nurturing some one-hundred thousand energy-hungry neurons for every microliter of cortical tissue. To do so, it relies on a uniquely organized topology that leaves no portion of tissue without perfusion, with every neuron lying at most 15 μm of a brain capillary [86]. Here, we review a body of work on understanding the structure and function of the cortical vasculature, with ideas relating these discoveries on the rodent vasculature to human physiology. Our presentation is based on a lecture at the 2014 Microcirculatory Society President's Symposium. In keeping with the

focused nature of that presentation, there is no pretense of historical perspective nor encyclopedic review. Further, we have stripped the presentation of a discussion of technical developments that fueled these results; these are reviewed elsewhere [44,45,79,81,87].

What are the issues that make the study of brain vasculature both interesting and challenging? First, we suggest, there is the need for dynamic allocation of resources, that is, oxygen and nutrients, to brain cells. Blood is a limited resource, yet neural function depends on adequate flow. In particular, the pioneering work of Fox and Raichle

[26] suggest that there is not enough blood to go around if all areas of the cortex were activated at once. To achieve this dynamic allocation, there is a coupling of blood flow with neuronal energetics and electrical activity [3,38,74]. The correlation is imperfect, with poorly understood relationships between flow, oxygenation, and neural activity [43]. Nonetheless, this coupling permits fMRI [50,68] to be used to study brain function in humans, a necessity for addressing many issues in cognition. Next, neuronal and vascular development appear to follow a similar plan and are linked by common genomic markers that control the fate of both systems [13]. Finally, there is strong if only partially understood coupling between neuronal and vascular dysfunction [28]. In particular, vascular disease leads to neurological decline and diminished cognition and memory [29].

CORTICAL ANGIOARCHITECTURE IS ORGANIZED INTO THREE TIERS

A single microliter of cortex holds nearly 1 m of total vascular length [10]. Though densely packed, the vasculature can be organized into three distinct topological tiers that blood must pass through to supply the brain. These tiers consist of the pial surface arteriole and venule networks, the subsurface microvascular network, and the penetrating arterioles and venules that bridge the surface and subsurface networks (Figure 1).

In the first tier, a plexus of arteries and arterioles that collectively occupy the territory of the MCA overlays the surface of about half of neocortex [55,76]. The MCA originates from the circle of Willis at the base of the brain

and extends over the cortical mantle as a network of two-dimensional loops. These loops are formed when branches of the MCA connect through anastomoses [9,15]. Penetrating arterioles branch from the surface arterial network and plunge perpendicularly into cortex [6,66]. The vast majority of penetrating arterioles are singular offshoots that do not branch further on the pial surface before entering the brain [9]. This is an important geometrical feature that ensures each penetrating arteriole can be sourced by two potential inputs within a vascular loop. As penetrating arterioles descend into the cortex, they gradually ramify into precapillary arterioles that, in turn, feed into the subsurface capillary network.

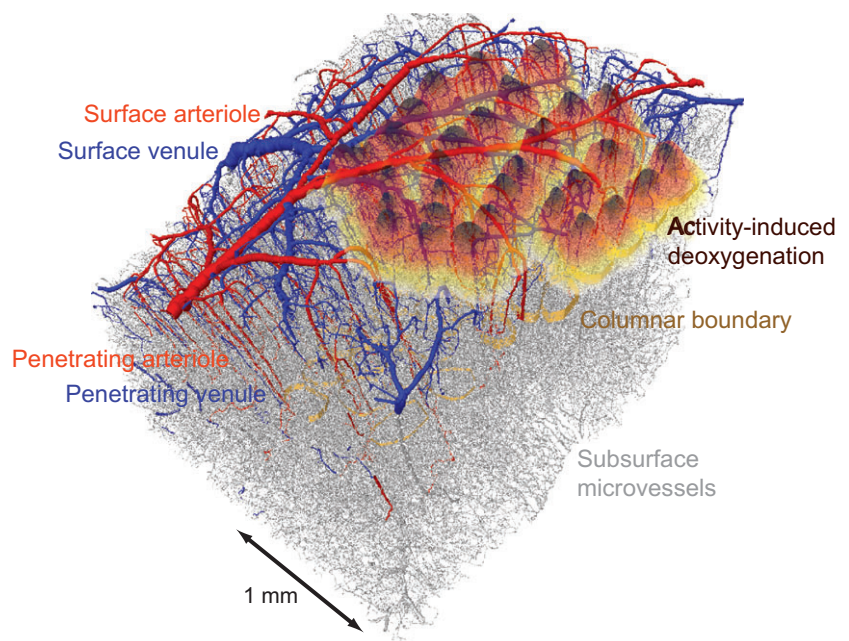
The capillary bed comprises a three-dimensional network [10]. All cerebral blood flow must pass through this network in order to exit the brain, as there are no direct arteriole to venule anastomoses in the healthy brain. The density of the capillaries is largely homogeneous along varying depths of the cortex, even though the density and composition of cells varies greatly over the cortical thickness [86,94].

Finally, blood exits the cortex through a venular system that is similar to the arterial system. Penetrating venules receive blood from postcapillary venules that drain the subsurface microvascular network and run perpendicular to the brain surface [63]. After exiting the parenchyma, blood flows through a tree-like venular surface network and finally drains into the central sinus [55,76].

Robust Blood Flow Through the Pial Network

A detailed analysis of the pial angioarchitecture was created from images of surface vessels that were filled with a gel linked to a fluorescent dye [9]. We mapped the location of all

Figure 1. A vectorized data set of all vasculature in a block of parietal cortex that encompasses the primary vibrissa representation. The block is $2 \times 3 \times 1.2$ mm thick. Surface and penetrating arterioles are colored red, venules blue, and the borders of cortical columns denoted by a golden band. A map of brain activity is superimposed. It was obtained using intrinsic optical signal imaged through a thinned-skull window. The animals were anesthetized with isoflurane so that only a net deoxyhemoglobin signal is observed by reflectance of 625 nm light when individual vibrissae were deflected at 10 Hz for four seconds. The responses from all columns were normalized in amplitude and thresholded to avoid spatial overlap, and are superimposed on the vectorized data set. This new data were obtained using the methodology in Blinder *et al.* [10].



vessels and found that an interconnected backbone of vessels spans the full territory of the MCA (black overlay on green, Figure 2A). This angioarchitecture provides multiple paths for the supply of blood to any penetrating arteriole. This implies that blood flow can be redistributed to areas of need from areas of excess; such a redistribution could not occur with a tree-like branching pattern.

Does the observed architecture support the redistribution of blood over the cortical mantle in response to a “hot spot” of neuronal activation? To test this possibility, we stimulated the forepaw of an anesthetized animal so that the contrast in activity between the core region of somatotopic activation and the surrounding regions is particularly large (left and middle of Figure 2B). Optical imaging with *in vivo* TPLSM [46,85] enables the diameter of the lumen to be observed. We found that there is a local dilation of arterioles at the center of the electrophysiological response [18]. Yet as one moves away from the center, the vascular response is dominated by an average constriction rather than dilation (middle and right of Figure 2B). This center-surround effect suggests that the network of arterioles on the pial surface shunts blood flow from inactive to active brain areas through active dilation and constriction.

These above findings indicate that vascular tone has local control in addition to pial-wide control during functional hyperemia [32]. The mechanism for the spatiotemporal coordination of arteriolar reactivity is incompletely understood, but it is likely to involve conduction of a parenchymal response to the pial network through vascular endothelial gap junctions [4] with an approximately 2 mm electrotonic length along the endothelial cells that form a vessel [77]. The endothelial response results in either dilation or constriction by engaging distinct myoendothelial signaling cascades [19]. Since a multitude of penetrating arterioles exist along a single branch of the MCA, different regions of cortex can be seeded with either dilatory or constrictive signals and, in principle, this can result in a center-surround shift in the spatial pattern of blood flow.

Beyond issues of dynamic reallocation of blood, the high interconnectivity of the surface network should make the distribution of blood relatively insensitive to blockages of a single surface vessel. To test this hypothesis, we used TPLSM to track the speed of RBCs [46] and used focused excitation of a photosensitizer, transiently introduced into the blood stream, to form a clot at a vessel of interest [75] (Figure 2C). Immediately after the clot is formed, we find a reversal in the direction of flow of one of the pair of proximal downstream vessels [75], such that the magnitude of the flow remains roughly unchanged even though the direction of flow has flipped. Thus, the ability of the surface network to source blood to neocortex is unaffected by a single localized defect.

Before moving on, we consider the role of the pial arterioles in distributing blood to the penetrating arterioles

during a transient blockage of the MCA, a model of stroke [56,96]. We find that the available blood is homogeneously distributed to all penetrating arterioles as flow is diminished [80], as opposed to creating patches of cortex with flow versus no-flow. Thus the maintenance of flow homogeneity in penetrating arterioles as a population also relied upon the ability of the pial network to redistribute blood, which also involved reversals of flow in anterior cerebral artery collaterals as well as in smaller collaterals between major MCA branches (Figure 2A). In fact, recent studies show that stroke severity can be predicted by the extent of vascular collateralization from animal to animal [12].

Dynamic Changes in Pial Vessel Diameter are Modest in Extent

We observed changes of 5% in the diameter of pial vessels during sensory stimulation of anesthetized animals [17,18] (Figure 2B). This number is small, suggesting that regulation makes only a modest difference in vasodynamics. Does the fractional change hold up in awake animals? We address this issue in awake, head-fixed mice in which vasodynamics are measured through a transcranial window [23] (Figure 3A). Sensory stimulation, in this case, an air puff to the vibrissae, leads to changes in the diameter of the pial arterioles of at most 40% (Figure 3B). Interestingly, there is also spontaneous vasodilation, referred to as vasomotor events [54], whose maximal dilation is similar in size [23] (Figure 3C). These spontaneous events occur over time scales of seconds to tens of seconds and involve changes in vascular smooth muscle calcium signaling and other components that may control vascular tone [62]. The essential issue is that changes in diameter of pial vessels are modest in extent in comparison with the many-fold increases that are seen in the vasculature of peripheral muscles through capillary recruitment [35].

It is noteworthy that the magnitude of arteriolar dilations measured *in vivo* rarely exceeds a 40% increase from baseline. This apparent limitation exists regardless if an animal is awake or under anesthesia, or if the dilation is evoked by sensory input or part of an ongoing vasomotor oscillation. Exogenous manipulations, such as isoflurane inhalation [41] or shock [42] can drive an increase in the diameter of arterioles in excess of 40%, but under physiological conditions, a homeostatic mechanism may exist to prevent overdistention of the arteriole wall.

Subsurface microvessels show both stimulus-activated and spontaneous changes in the speed and flux of RBCs [46]. Interestingly, the flow of RBCs rarely transitions from moving to stalled [46,91]. All told, since blood in all arterioles and all capillaries is flowing at all times under normal conditions, there is no reserve of vascular volume to be filled by increased flow in upstream arterioles. This may ensure that the brain does not swell beyond the capacity of the intracranial space. Further, the consistency of flow and

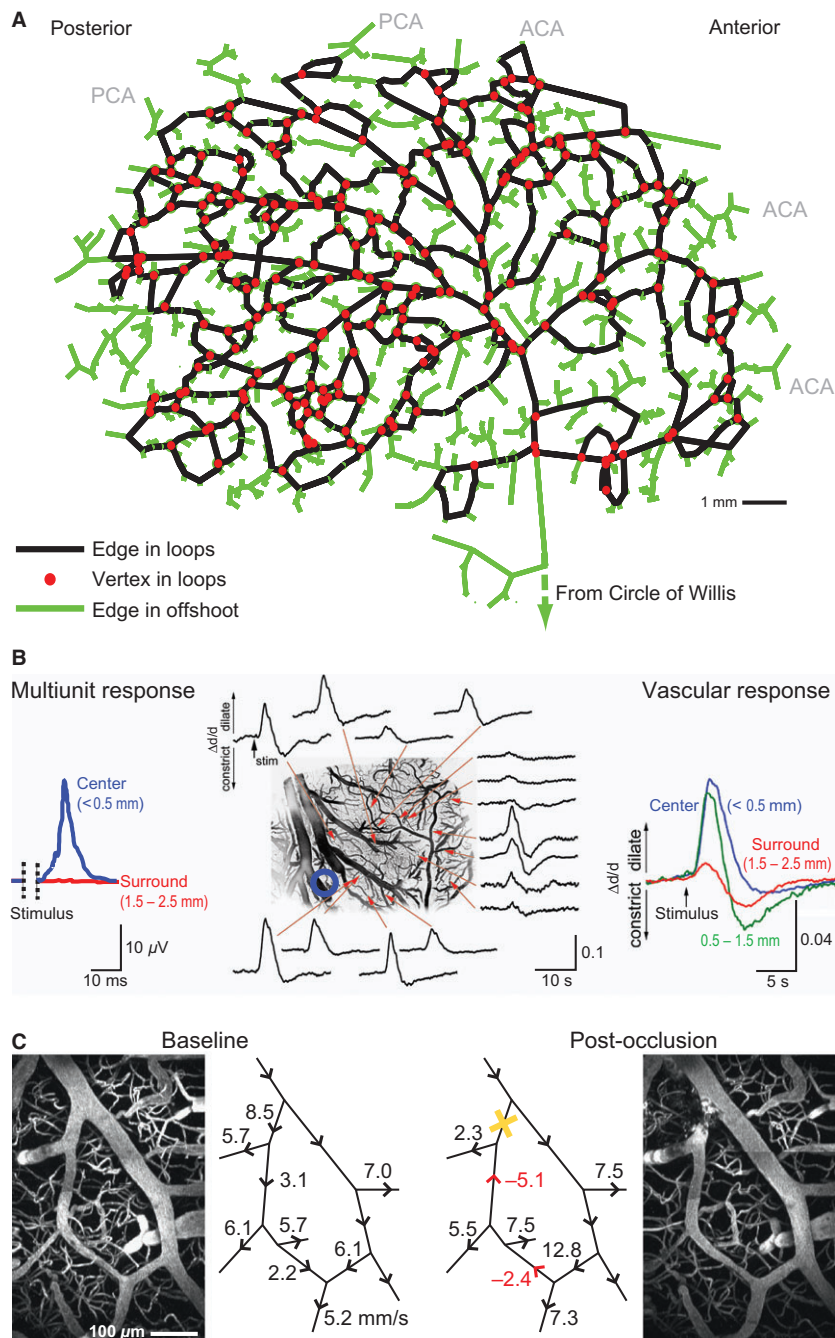


Figure 2. The pial surface forms a two dimensional network. **(A)** Representative example of a complete tracing of the vasculature fed by the MCA. Parts that form loops are highlighted with black edges and red vertices, while nonbackbone offshoots are shown in green. Adapted from Blinder *et al.* [9]. **(B)** Stimulation of the footpad in the alpha-chloralose anesthetized rat leads to local dilation but surrounding constriction of surface arterioles. Data in the left panel show the evoked neuronal response, using ball electrode measurements of surface potentials, compiled from nine different locations. The strongest amplitude and fastest rise time was marked as the center of the receptive field. The middle panel shows a vascular map with the center of the receptive field marked by a blue circle and arteriolar diameter change as a function of time and distance from the center. All data are shown as a fractional change relative to the baseline, that is, $\Delta d/d$, and dilation and constriction are plotted upward and downward, respectively. The right panel is an average of arteriolar diameter changes within 0.5 mm from the center of neuronal response (blue), in a 0.5–1.5 mm annulus around the center (green) and 1.5–2.5 mm ring around the center (red) for all data from this animal. Adapted from Devor *et al.* [18]. **(C)** Occlusion to a single surface arteriole leads to reversal of flow in the immediate downstream vessel and mild adjustment of flow in all branches. Data from rat anesthetized with urethane. On the left and right are *in vivo* two-photon images taken before and after photo-thrombotic clotting of an individual vessel, respectively. Left-center and right-center are diagrams of the surface vasculature with RBC speeds and directions indicated. The yellow “X” indicates the location of the clot, and vessels whose flow direction has reversed are indicated with red arrows. Adapted from Schaffer *et al.* [75].

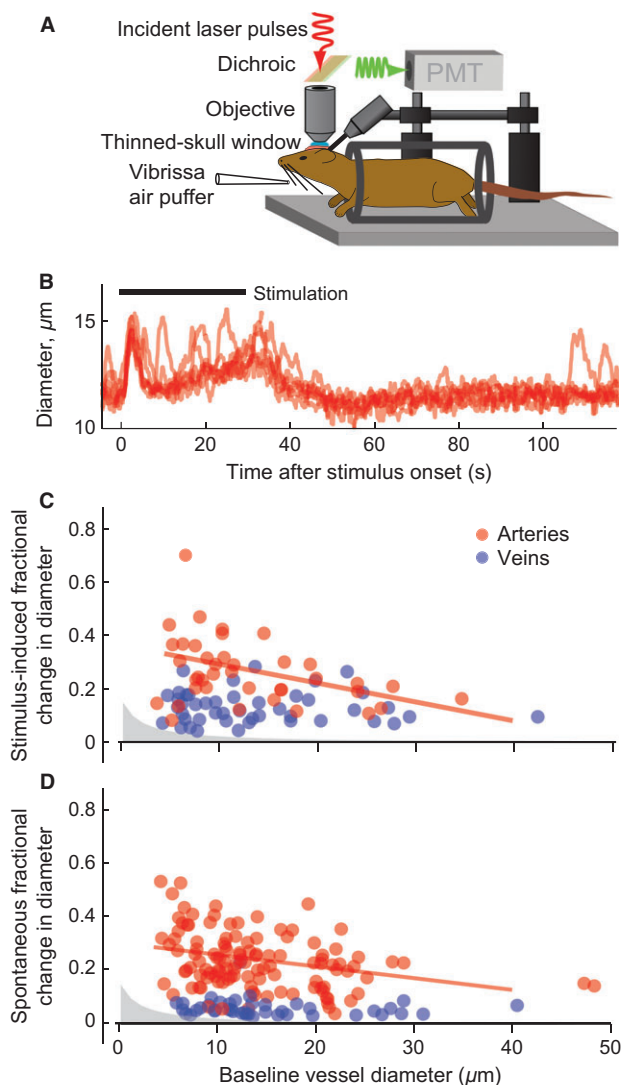


Figure 3. Stimulus driven and spontaneous vascular dynamics in the cortex of awake mice have similar amplitudes. **(A)** Schematic of the experimental setup. The awake mouse is head-fixed and sits passively in an acrylic cylinder beneath the two-photon microscope. Air puffers for sensory stimulation are aimed at the vibrissa and, as a control, at the tail. **(B)** Example of evoked and spontaneous diameter change for a 30 second stimulus. **(C, D)** Relationship between peak value of the dilation and vessel diameter. Data for arteries is in red and for veins is in blue. Grey area shows the 0.2- μm resolution limit of detectable changes. **(C)** shows the peak averaged dilation responses in the first 1–10 seconds of a 30 seconds vibrissae stimulation; the regression line has a statistically significant slope of $-0.007/\mu\text{m}$. **(D)** shows the peak of spontaneous dilation; the regression line has a statistically significant slope of $-0.004/\mu\text{m}$. The regression for veins (not shown) is not significantly different from zero in either case. Adapted from Drew, Shih, and Kleinfeld [23].

the observation that changes in flow are not too great suggests that a linear analysis of flow based on a fixed angioarchitecture should have utility in predicting flow patterns.

Robust Aspects of Subsurface Blood Flow

The reconstruction of the microvasculature across many microliters of tissue (Figure 1) may be analyzed to determine the topology of the subsurface microvasculature (Figure 4A). Like the pial vasculature, we observe loops, albeit composed of a greater number of branches than those on the surface (Figure 4B). This finding suggests that flow in the subsurface vasculature should also be relatively immune to occlusion to a single vessel. We used a sensitizer-free, nonlinear optical technique to injure the endothelium and trigger clotting in a targeted subsurface vessel [67]; nonlinear interactions are required so that light is not absorbed above or below the targeted vessel. We observed highly reduced flow in the proximal downstream vessel, as opposed to the immediate recovery for surface loops (cf., Figures 2C and 4C), yet there was substantial recovery by three vessel branches downstream (Figure 4C). Further, histological analysis of the tissue indicates that there is essentially no neuronal damage in terms of markers of hypoxia or neuronal structure [67,78]. We conclude that clots targeted to single vessels in either surface or subsurface networks cause negligible ischemia or damage to neurons. This occurs as a consequence of redundant flow pathways.

Penetrating Vessels are a Fragile Link in the Sourcing of Blood

Penetrating vessels deliver blood from the surface network to subsurface microvessels. They are interconnected by way of the dense microvasculature (Figures 1 and 4A), but do not connect with each other through direct subsurface collaterals [10] (Figure 5A). Thus, it is not obvious whether the loss of flow to a penetrating vessel would terminate all flow to the tissue volume perfused by the vessel. On the one hand, penetrating vessels deliver, on average, a median flux of blood into the brain that is seventy times the mean flux through an individual capillary [78]. Yet the multitude of capillaries suggests that collateral flow could compensate for the absence of a penetrating vessel. To observe what actually occurs for this critical case, we made use of our previous technique of blocking flow in pial vessels (Figure 2C), but this time directed at the entry point where a penetrating arteriole plunges into cortex (Figure 5A). We measured the change in the speed of RBCs in downstream vessels as a function of lateral distance for the site of occlusion (Figure 5B). We found that the speed, and thus flux of RBCs, goes to zero in downstream vessels that lie distal to the occlusion and does not fully recover until a radius of about 250 μm from the occluded penetrating arteriole [64,66]. Thus, the loss of flow to a single-penetrating arteriole leads to an acute loss of perfusion in a columnar region that spans approximately 500 μm in diameter.

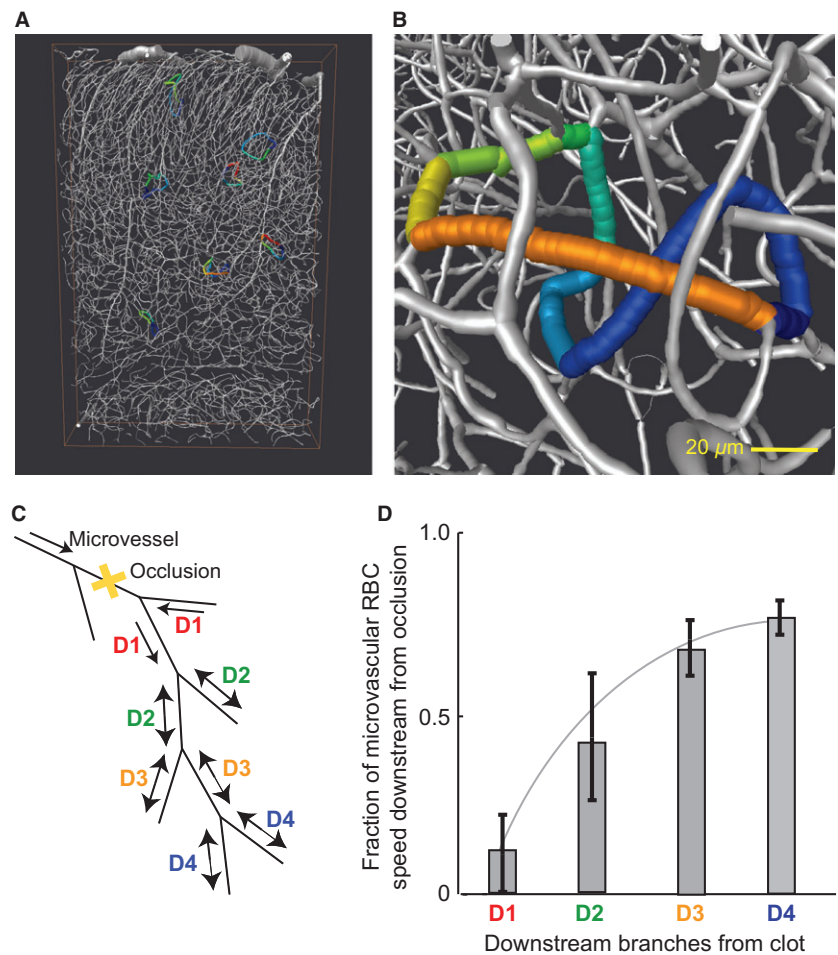


Figure 4. The subsurface microvasculature forms a three dimensional network that is largely insensitive to occlusion in one microvessel. **(A)** Numerical section of a vectorized network from a complete reconstruction (Figure 1). **(B)** Close up of the section in **(A)** showing only the microvasculature. The colored edges highlight a loop that consists of eight branches, each with a distinct color. **(A, B)** adapted from Blinder *et al.* [10]. **(C)** Schematic of the microvasculature labeled with the order of downstream vessels, that is, D1 through D4 in this picture, from a point of occlusion. **(D)** The fraction of speed in downstream microvessels after an occlusion, yellow "X" in **(C)**, relative to that before the occlusion. The data are averaged over twenty networks in terms of downstream branch number. **(C, D)** adapted from Nishimura *et al.* [67].

BIOMEDICAL CONSEQUENCES OF THE LOSS OF ONE PENETRATING ARTERIOLE

What are the chronic consequences of the loss of perfusion from a single penetrating vessel? A simple but telling measurement in the rodent is to probe for ischemic spreading depression, that is, a front of potassium and glutamate that depolarizes neurons and further contributes to stroke damage [59]. These measurements make use of a fluorescent indicator of intracellular calcium as a proxy for electrical depolarization [30] (Figure 6A), yet are qualitative in the sense that the onset time of spreading depression is unpredictable. Nonetheless, in a significant number of cases we see a slowly propagating front of depolarization followed by inactivity (Figure 6B).

A gold standard of neurological damage is the presence of an infarct [7]. In sub-acute stages of stroke, the infarcted region is filled with phagocytic inflammatory cells. As such, we repeated the procedure of occluding a single penetrating vessel in mice that expressed GFP in microglia/monocytes. Further, we used transcranial illumination to obviate the possibility that a chronic cranial window compromise the

data by spuriously increasing cortical inflammation. [22] (Figure 6C). When the animals are sacrificed six days later, we observe a cylindrical region of infarcted tissue, highlighted by microglia and monocytes that have swarmed into the region [22] (Figure 6D). The diameter of the cylinder is approximately 500 μm, the same as the region of acute loss of flow.

The microinfarcts caused by occlusion to a single penetrating arteriole (Figure 6D) are several orders of magnitude smaller than the infarcts seen in experimental strokes caused by occlusion of the MCA [96]. Therefore, it stands to question whether such small lesions could generate a perceptible deficit in brain function. We distilled the behavioral deficit imparted by a single microinfarct [78] with the use of a refined version of the gap-crossing task of Hutson and Masterton [36]. Here, a rat uses a single vibrissa to sense, in the dark, the presence or absence of a platform that is across a gap. The occlusion was strategically generated in a penetrating arteriole that was centered in a cortical column that receives input from that single vibrissa. We found that the microinfarct led to an altered decision on whether to navigate the gap, implying that the rat lost the capability to make a specific sensorimotor decision [78].

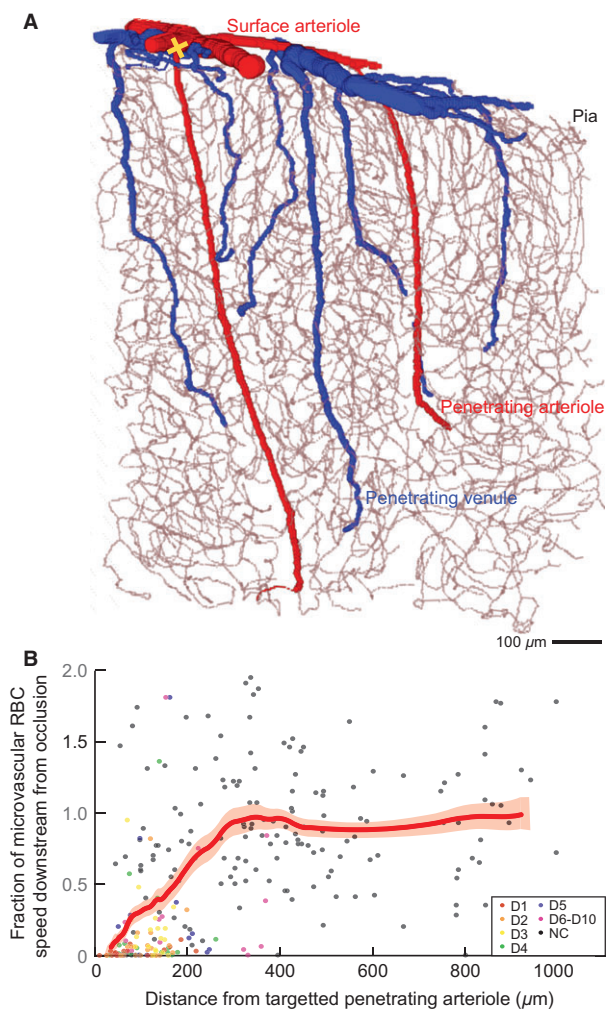


Figure 5. The penetrating arterioles are a fragile link in the delivery of blood from the surface arterioles to the subsurface microvascular network. **(A)** Numerical section of a vectorized network from a complete reconstruction (Figure 1) that highlights the penetrating arterioles (red) and venules (blue) and the continuous microvascular network (gray). Adapted from Blinder *et al.* [10]. **(B)** The fraction of speed in neighboring microvessels that lie in cortical layers two and three after an occlusion to a single microvessel, relative to that before the occlusion. Only some of the data point could be specified in terms of downstream branch number, as shown. The thick red line through the data is the smoothed response averaged over a window that included 50 points, with ± 1 SEM limits indicated by the thin red lines. The data are averaged over 16 networks. Adapted from Nishimura *et al.* [66].

Related studies in mice suggest that microinfarcts exert a diaschisis that impairs neural excitability well outside the region of tissue infarction [2].

CALCULATED VERSUS MEASURED FLOW AFTER AN OCCLUSION

The complete reconstruction of microliter volumes of neocortex (Figure 1) allows us to calculate the expected

change in flow after occlusion of a vessel and compare the calculated change against measured values. This serves as a test of self-consistency between two independent approaches, that is, flow measurements versus computations based on structural measurements. Each vascular junction is connected to three others by vessels of known median radius and length (Figure 7A). A single number, the resistance of the vessel to the flow of RBCs, characterizes the vessel and is based on the formula of Pries and Secomb [72] (Figure 7B; dashed line is the curve expected for water). We then apply Kirchhoff's law to each junction and use known values for the inlet pressure to surface arterioles to calculate the flux through every vessel [10]. Our first numerical experiment is to determine the volume of cortex that receives the major fraction of perfusion from a given penetrating arteriole. We find a linear relation between the calculated volume and the flux of RBCs through the penetrating arteriole over two-orders of magnitude of flux (Figures 7C and 7D). The observed linearity is consistent with the fully connected nature of the subsurface microvasculature, while the scatter in the calculated values reflects the random pattern of subsurface connections (Figure 4A and B). Critically, the volume of a cyst formed by blockage of a single penetrating vessel (Figure 6D) coincides with the volume of perfusion (Figure 7D). This establishes the notion of a territory of perfusion for each penetrating arteriole. These territories are unrelated to the columnar organization of neuronal afferents [10].

Our second numerical experiment addresses the catastrophic loss of perfusion in the vicinity of an occluded penetrating arteriole. We calculate the change in RBC flux, and accompanying speed, in microvessels downstream of the occluded arteriole in terms of branch number (Figure 7E). There are no free parameters in the calculation. As an average over all penetrating vessels in all reconstructions, we observe that the median value of the speed recovers to half of the preocclusion value by six downstream branches [10] (D6 in Figure 7F). These calculated values are compared with measurements of the decrement in preocclusion speed as a function of the branch order away from an occluded penetrating vessel [64] (cf., yellow/green and red diamonds in Figure 7E). The midpoint of the recovery matches and the overall shape of the curves are consistent; the mismatch for branches D1–D4 occurs since the calculated speed cannot go to zero in our linear analysis. We argue that this analysis supports the role of linear analysis for modeling blood flow.

Insight into the Lack of Collateral Flow

The above numerical analysis establishes self-consistency, yet gives little insight into the reason why collateral flow through microvessels does not counter the loss of perfusion through the penetrating arteriole. Motivation for a simplified model comes from the anatomical drawings of retinal vasculature by

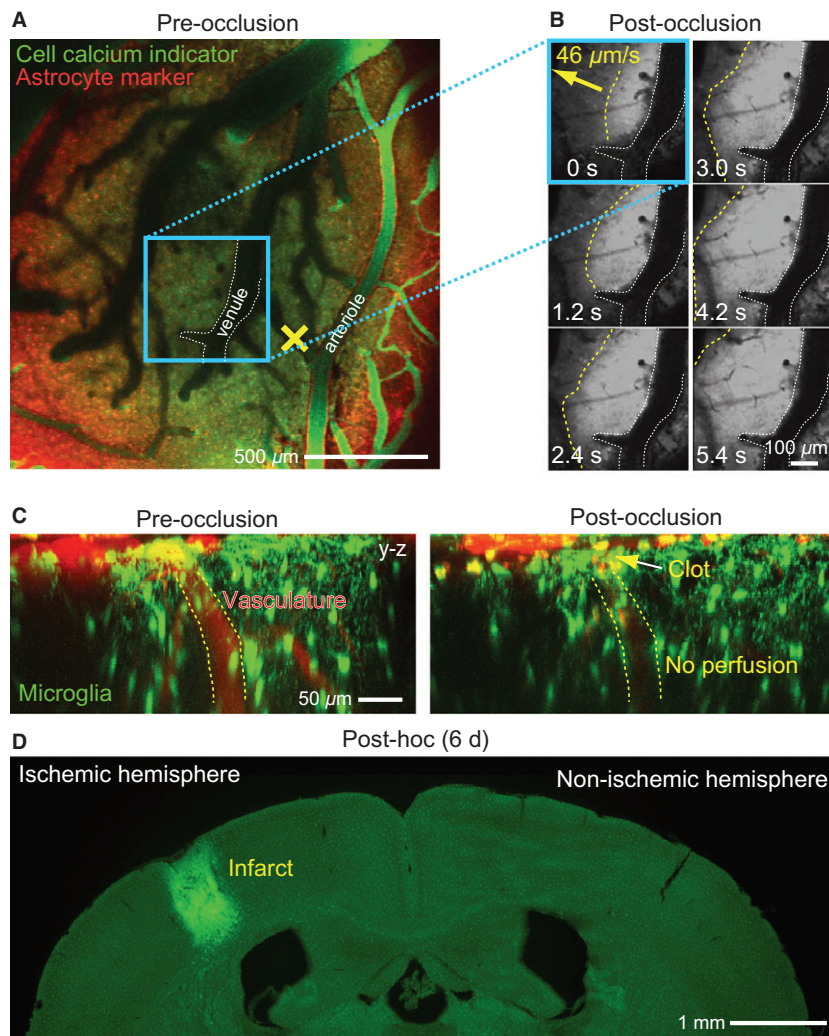


Figure 6. Occlusion to one penetrating arteriole results in neuronal death and formation of a cyst. **(A)** *In vivo* two-photon imaging of neuronal activity in the forelimb region of somatosensory cortex of an α -chloralose anesthetized rat that was loaded with the $[Ca^{2+}]$ indicator OGB1-AM and the astrocyte-specific dye SR101. The single penetrating arteriole marked by a yellow "X" was occluded. **(B)** Wave of cortical spreading depression, propagating at $46 \mu\text{m}/\text{sec}$, observed by *in vivo* $[Ca^{2+}]$ imaging (yellow dashed line) about 30 minutes after occlusion of the penetrating arteriole (yellow arrow, left). **(A, B)** adapted from Shih *et al.* [78]. **(C)** Maximal projection through a $200\text{-}\mu\text{m}$ depth of a $Cx3cr1^{eGFP/+}$ mouse cortex before (left) and 100 minutes after (right) occlusion of a single penetrating arteriole using targeted optical activation of the photothrombotic agent Rose Bengal (arrow) made one day after implantation of a transcranial window [22]. Dashed lines indicate the boundaries of the penetrating artery in which flow was blocked (yellow "X"). **(D)** Extent of the infarct, for the same mouse as in **(C)**, visualized six days after the optically generated stroke. The bright green fluorescence indicates the invasion of eGFP-labeled microglia into a cyst of necrotic tissue. Adapted from Drew *et al.* [22].

Snodderly, Weinhaus, and Choi [83], which shows that the blood supply to a disk of microvasculature is sourced and sunk by radial, alternating penetrating arterioles and penetrating venules. We thus consider a two-dimensional rhombic lattice, with the observed ratio of two penetrating venules to one penetrating arteriole, as a model of neocortical vasculature [10] (Figure 8A). We take penetrating arterioles as ideal pressure sources, penetrating venules as grounds, and replace individual microvessels with the Thévenin resistance between distant junctions; this ignores the resistance of penetrating vessels, which is half that of the Thévenin

resistance. Blockage of a penetrating arteriole leads to an unperfused hexagon of tissue as flow through the microvascular network toward the region of the occlusion is shunted through the penetrating venules (Figure 8B). Similarly, blockage of a penetrating venule leads to an unperfused hexagon of tissue as the removal of a sink raises the impedance of flow into the region (Figure 8C). This model highlights why blockage of any penetrating vessel leads to an infarct through a lack of flow, with good agreement between theory and experiment especially considering the brutal nature of approximations (Figure 8B and C). The inability

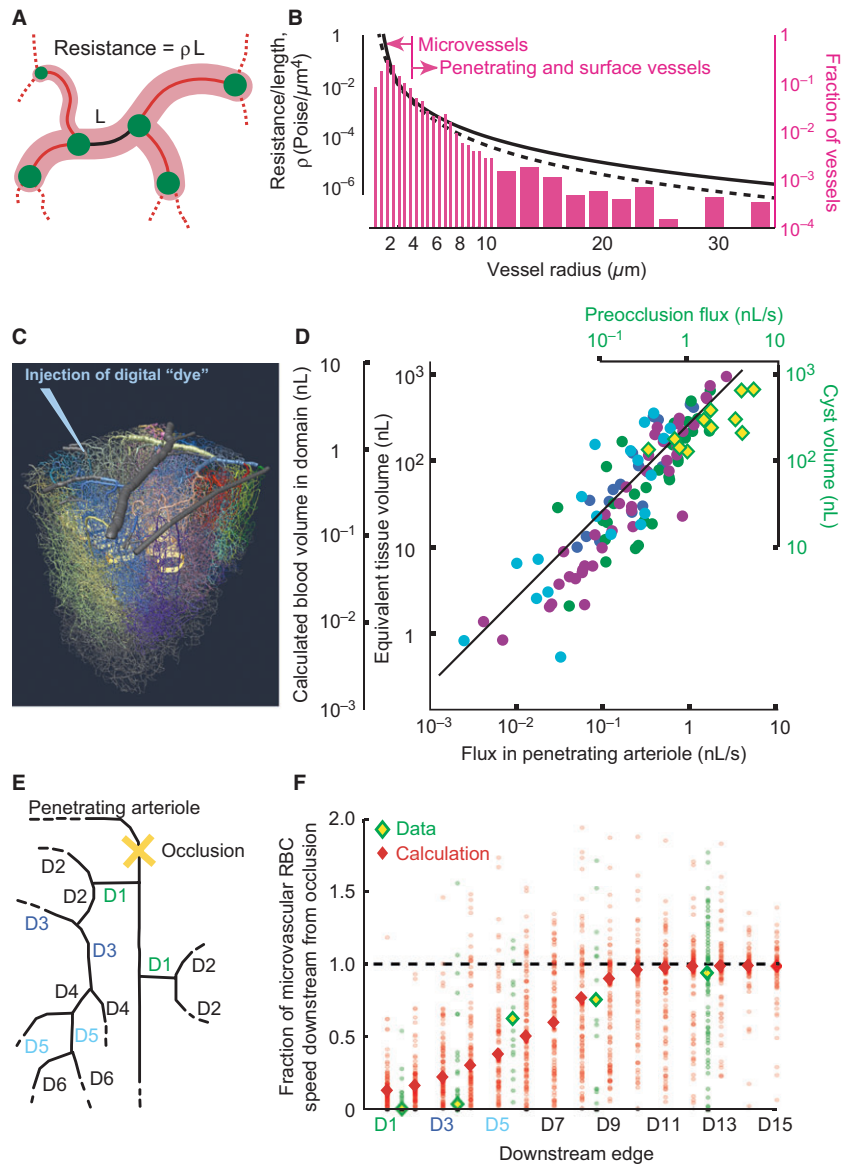
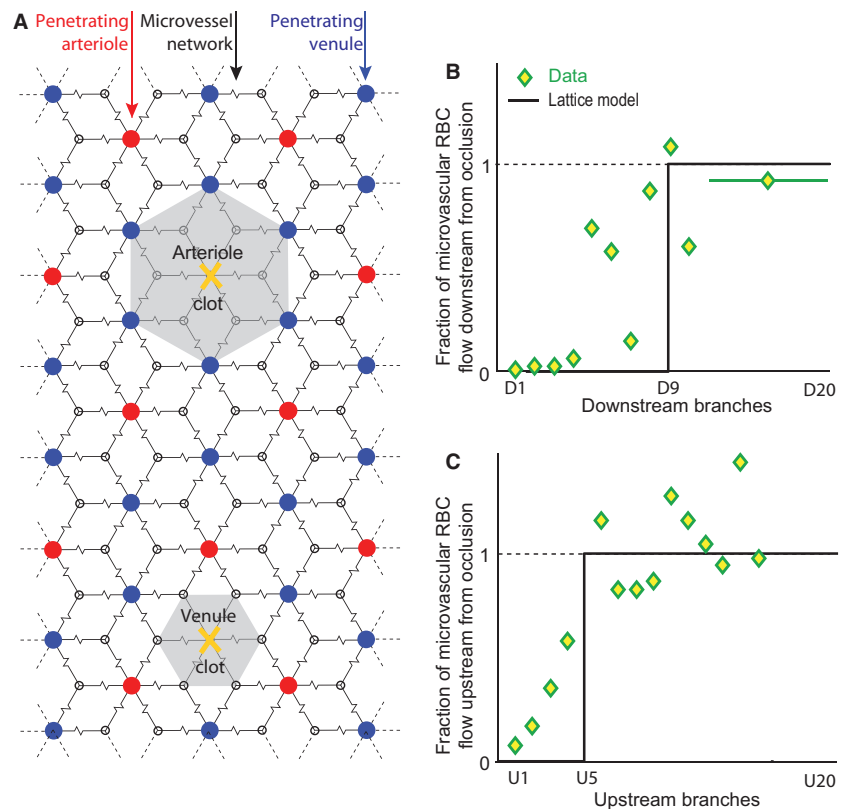


Figure 7. The calculated pattern of blood flow for a full reconstruction of the vasculature (Figure 1) accounts for the measured degradation in flow after blocking a single penetrating arteriole. **(A)** Reduction of the angioarchitecture to vertices (green dots) and branches (red). The resistance of each branch depends on the radius and length, L . **(B)** Plot of the flow resistance, ρ , per unit length as a function of vessel radius (solid curve); the total resistance is found by multiplying ρ by the length, in micrometers. Note the marked increase in resistance for radii below $\sim 5 \mu\text{m}$. The Hagen–Poiseuille law is given by the dashed curve. The concurrent histogram shows the distribution of vessel radii for all vessels. **(C)** Vectorized vasculature in which the blood flow through a single penetrating arteriole is numerically labeled to determine the spatial domain of vessels that receive at least half of their flow from the chosen penetrating arteriole. This exercise is repeated for all penetrating arterioles and each domain is additionally labeled by the flux through the penetrating vessel. **(D)** Plot of the computed vascular perfusion domains versus flux in the penetrating arteriole. The volume of the parenchymal domains (115 domains in four data sets; colored circles) are consistent with measured cyst volumes formed after a single artery occlusion (11 rats). **(E)** Schematic of the microvasculature labeled with the order of downstream vessels, that is, D1 through D6 in this picture, from a point of occlusion of an individual penetrating arteriole. **(F)** The calculated redistribution of flow in microvessels, up to 15 edges downstream from the sites of simulated occlusion of selected, individual penetrating arterioles. The reduction in vascular flux is plotted as a function of the order of the downstream vessel (red circles are the results of 100 simulations per order of the downstream edge and red diamonds are the median reduction in flux). These numerical results are compared with the published *in vivo* data of downstream flux measurement before and after penetrating arteriole occlusion in rat neocortex (green points are data from 175 vessels with median values shown as yellow and green diamonds). All panels adapted from [10].

Figure 8. A two-dimensional lattice model of the vasculature that approximates the measured degradation in flow after blocking a single penetrating arteriole. (A) Planar circuit with a rhombic lattice and two penetrating venules for each penetrating arteriole, consistent with measurements of actual penetrating arteriole versus ascending venule densities. Blockage of a penetrating arteriole leads to a region of no flow with an effective radius of 0.9-times the median spacing between penetrating venules, whereas blockage of a penetrating venule leads to a region of no flow with an effective radius of 0.5-times the median spacing between penetrating venules. Adapted from Blinder *et al.* [10]. (B) Comparison of the prediction from the lattice model and data for flow in downstream microvessels after blockage to a penetrating arteriole. Adapted from Nishimura *et al.* [64] and Blinder *et al.* [10]. (C) Comparison of the prediction from the lattice model and data for flow in upstream microvessels after blockage to a penetrating venule. Adapted from Nguyen *et al.* [63] and Blinder *et al.* [10].



for the vasculature to compensate for flow loss to the regions of an occluded penetrating vessel is a key fragility in an otherwise remarkably robust system [64,66].

SYNOPSIS

All told, the picture that emerges is one of a highly interconnected surface vasculature that forms a two-dimensional network and is a robust source of blood to the penetrating arterioles (Figure 2). The subsurface microvasculature forms a three-dimensional network that is fully connected and forms a robust source of nutriment to the neurons and glia throughout neocortex (Figure 4). However, flow in the penetrating arterioles and venules that traffic between the surface and subsurface networks is fragile (Figure 5A), in the sense that blockage of one vessels leads to a break in the supply chain and neuronal death throughout a cylinder of cortex (Figures 5B and 6D).

NEXT STEPS

Open issues remain at the level of basic physiology and biofluid mechanics as well as in biomedicine. With regard to basic issues, none is greater than the means by which the nervous system controls vascular tone.

Resting-State Fluctuation in Blood Oxygenation

We consider the issue of vasomotion [23,54] (Figure 4) and the potential connection to the resting state signal in BOLD fMRI [27]. The resting state signal is a roughly 0.1 Hz oscillation in the blood volume and/or the oxygenation state of hemoglobin in the blood that, further, is correlated in time between regions of cortex with similar function [8,92]. For example, the areas of motor cortex that control motion of the index finger in each hand would have correlated BOLD signals. There is evidence for callosal inputs that communicate low frequency electrical activity between such areas [48]. Thus, we conjecture that low frequency electrical activity entrains the intrinsic oscillators in smooth muscle that drive vasomotion [1,69], and that tissue oxygenation is slaved to vasomotion, as the origin of the synchronous activity in the resting state. Evidence to support this conjecture represents work in progress [52]. In particular, it remains unclear if vasomotion drives tissue oxygenation or, rather, that vasomotion is slaved to tissue oxygenation and renders our conjecture false.

Pericytes as Control Elements to Flow Through the Subsurface Microvasculature

The flux of blood through the microvessels is a function of the resistance of the network and the pressure difference between pial arterioles and venules. The resistance of a microvessel is an

exquisitely sharp function of its diameter (Figure 7B) since the size of the vessel is close to that of RBCs [72]. This suggests that significant changes in resistance will accompany sub-micrometer changes in diameter. Both smooth muscle and pericytes are cell types that could act as control elements as these cells ensheath vessels and contain contractile filaments although pericytes are unique to subsurface microvessels [21]. Evidence from *in vitro* studies that include demonstrations of pericyte contraction in response to electrical stimulation [70], neurotransmitters [31,40,70], and simulated ischemia [31] support a role for the control of microvessel diameter through the activation of pericytes. However, there is contradictory data obtained in live animals concerning changes in the diameter of microvessels that are ensheathed by a pericyte [25,31]. A key difficulty is distinguishing pericytes from smooth muscle *in vivo*.

What molecular markers are available to highlight pericytes for guidance during *in vivo* measurements? Pericytes express the smooth muscle isoform of actin [5], which makes them difficult to distinguish from the smooth muscle of subsurface arterioles, although pericytes located on microvessels that lie midway between penetrating arterioles and venules may lack contractile elements [60]. Similarly, the proteoglycan NG2, which is often used as a marker for pericytes [58] (Figure 9), is also expressed in arteriolar smooth muscle. This data raises questions about the utility of a mouse line that links the expression of the proteoglycan NG2 to the fluorescent protein DsRed [97,98] for studies on pericytes. The intermediate filament desmin, however, is expressed in arteriolar smooth muscle but not pericytes of the brain [5,61] (Figure 9B–D). In principal one can use an intersectional, dual-recombinase strategy [24] to form a

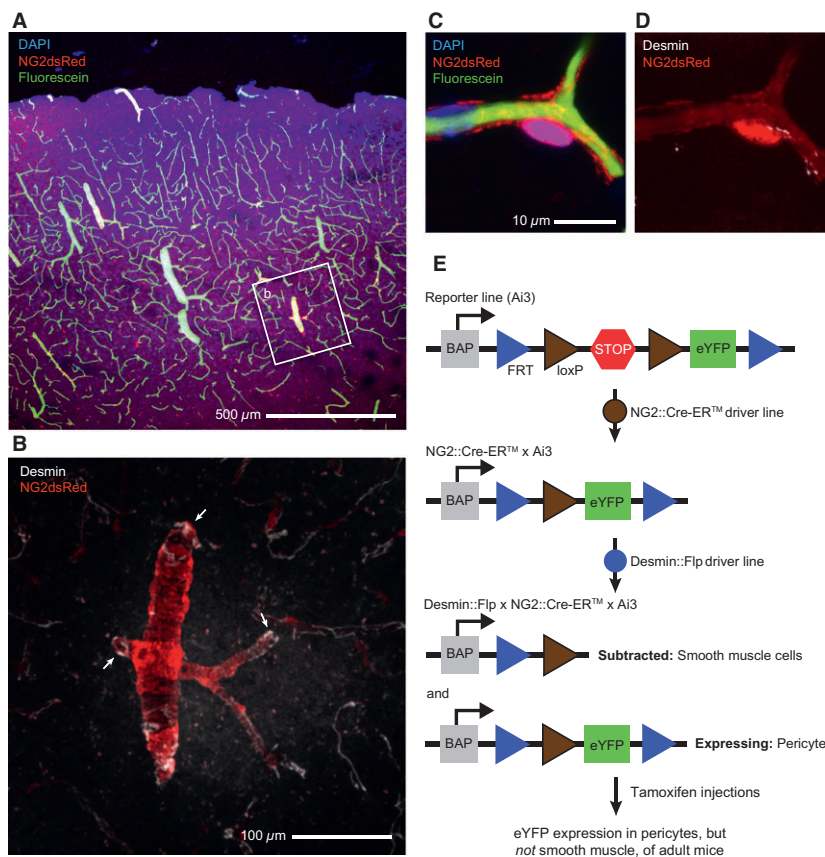


Figure 9. Pericytes are a potential means for the brain to control its own blood supply. The data are maximum projections through stacks of confocal images from a transgenic mouse brain that expressed NG2::DsRed (red), that is, the fluorescent protein DsRed is linked to expression of NG2 [97]. The animal was perfused with a fluorescein-labeled albumin gelatin [86] (green) and sections were labeled with α -desmin antibody (white) and stained for DAPI (blue) to identify cell nuclei. **(A)** Overview with areas noted for detailed analysis. Projection across 37 sections in 1.05 μm steps. The fluorescein channel is saturated and desmin channel is excluded. **(B)** Illustration of a penetration arteriole, with staining for both NG2 and the desmin (arrows point to expression at cuts through lumen); other channels are excluded. Projection across 43 sections in 1.0 μm steps. **(C, D)** High magnification view of microvessels and, presumably, pericytes with lack of labeling for desmin. Projection is across 32 sections in 1.0 μm steps. **(E)** Proposed dual-recombinase strategy to form a transgenic animal that labels pericytes but not smooth muscle. We cross the Ai3 reporter mouse [51] (Jax: 007903) with the tamoxifen-inducible NG2::CreERTM driver mouse [98] (Jax: 008538), and then a hypothetical desmin::Flp driver mouse. The expressing cells should be pericyte, as indicated, as well as oligodendrocyte precursors and sparse labeled neurons.

transgenic animal that labels only pericytes. We provide an example two-step scheme (Figure 9E) that is based on an initial activation of fluorescent protein expression in the Ai3 reporter line [51] through breeding with the existing NG2-CreERTM driver line [98]. The resulting bigenic progeny are then bred with a hypothetical desmin-Flp driver line to subtract fluorescent protein expression from arterial smooth muscle, while leaving pericyte expression intact.

Relation of Microlesions to Human Disease

The lesions generated by the occlusion of single-penetrating arterioles bear remarkable similarity in location and shape to microinfarcts seen in the aging human brain [78]. While most data supporting the existence of microinfarcts in humans comes from neuropathological studies [93] (Figure 10A), there is mounting evidence to suggest that these lesions can be detected and tracked during life using high-field MRI [89] (Figure 10B) and most recently clinical MRI [88]. Several groups have shown that vascular cognitive impairment is strongly correlated with an increased incidence of microinfarcts [29,37,39,82]. Thus, improved methods to detect microinfarcts by MRI are essential in the treatment of dementia, which must be identified before the onset of clinical symptoms. We suggest that rodent models of vascular cognitive impairment will be integral to the design of protocols for early detection through MRI imaging and mitigation of continued spread of dysfunction through pharmacological intervention [49].

A second and no less perilous form of microscopic lesion is the cerebral microbleed [65,71,90,95]. These are caused by rupture of microvessels, generating lesions that exist on a similar size scale as microinfarcts as seen with high-field MRI (Figure 10C). Microbleeds increase the likelihood of dementia, and like microinfarcts, their role in the pathogenesis of vascular cognitive impairment remains poorly understood. Microbleeds can be modeled in rodents through the use of nonlinear absorption of laser pulses to breach the vascular wall [67]. *In vivo* imaging has demonstrated the ability of microbleeds to compress the surrounding tissue but, remarkably, this leads to minimal impact on neuronal structure and function [14,73]. In light of clinical evidence for microbleeds in even middle-aged adults [71], it is imperative to pursue optically induced microinfarcts and microbleeds as experimental platforms to model their human counterparts in a controlled manner. It is especially relevant to pursue models of lesions to white matter in rodents, given the prevalence of white matter lesions in the human brain [53].

EPILOG

Continued progress toward our understanding of the neurovascular system will depend in part on the adoption of new

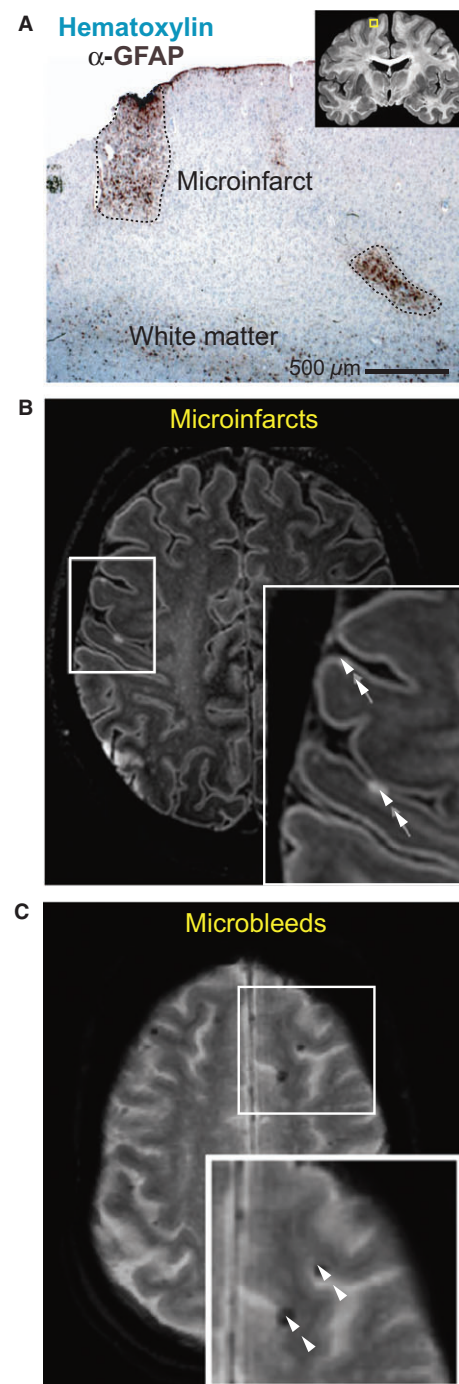


Figure 10. Neuropathological evidence of “invisible” lesions in the aging human brain. **(A)** Example of gray matter microinfarcts in a light-level microscopy neuropathological study by Sofroniew and Vinters [84]. **(B)** Detection of cerebral microinfarcts with FLAIR MRI at seven Tesla. Adapted from Brundel *et al.* [11]. **(C)** Detection of cerebral microbleeds with T2* gradient echo MRI at 3T. Adapted from De Reuck *et al.* [16].

tools. Studies on the logic of vasoactive transmitters will gain from the use of cell-based indicators of amine and peptide transmitters [57] and expanded use of structural and

functional markers of vascular function [33]. The detection of relatively small changes in the diameter of microvessels will gain from the use of sub-diffraction imaging techniques [20]. Lastly, studies on biomedical issues that concern microlesions in white matter and the mechanisms of vascular dementia will gain from nonlinear optical techniques for deep imaging that exploit windows in the absorption spectrum of water at long wavelengths [34,47].

PERSPECTIVE

We have reviewed the architectural principles of the cerebral cortical vascular system. Experimental and theoretical evidence suggests that penetrating arterioles are weaknesses in the vascular supply chain. Obstruction of these arterioles in the aging human brain may be key contributors to vascular cognitive impairment, and thus warrants further study both clinically and in animal models.

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