WIREs Wiley Interdisciplinary Reviews

Article Title:

Postnatal Development of Cerebrovascular Structure and the Neurogliovascular Unit

Article Type:

○ OPINION	○ PRIMER	○ OVERVIEW
◉ ADVANCED REVIEW	○ FOCUS ARTICLE	○ SOFTWARE FOCUS

Authors:

Vanessa Coelho-Santos[1,2]
0000-0002-9450-6103
[1]Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute, Seattle, Washington, USA.
[2]Department of Pediatrics, University of Washington, Seattle, Washington, USA.
vcsantos@uw.edu
The author declares no conflict of interest.

Andy Y. Shih[1,2]*
0000-0002-7839-392X
[1]Center for Developmental Biology and Regenerative Medicine, Seattle Children's Research Institute, Seattle, Washington, USA.
[2]Department of Pediatrics, University of Washington, Seattle, Washington, USA.
andy.shih@seattlechildrens.org
The author declares no conflict of interest.

Abstract

The unceasing metabolic demands of brain function are supported by an intricate 3-dimensional network of arterioles, capillaries and venules, designed to effectively distribute blood to all neurons and provide shelter from harmful molecules in the blood. The development and maturation of this microvasculature involves a complex interplay between endothelial cells with nearly all other brain cell types (pericytes, astrocytes, microglia, and neurons), orchestrated throughout embryogenesis and the first few weeks after birth in mice. Both the expansion and regression of vascular networks occur during the postnatal period of cerebrovascular remodeling. Pial vascular networks on the brain surface are dense at birth and are then selectively pruned during the postnatal period, with the most dramatic changes occurring in the pial venular network. This is contrasted to an expansion of subsurface capillary networks through the induction of angiogenesis. Concurrent with changes in vascular structure, the integration and crosstalk of neurovascular cells lead to establishment of blood-brain barrier integrity and neurovascular coupling to ensure precise control of macromolecular passage and metabolic supply. While we still possess a limited understanding of the rules that control cerebrovascular development, we can begin to assemble a view of how this complex process evolves, as well as identify gaps in knowledge for the next steps of research.

Introduction

The brain represents only ~2% of the body's total mass, yet consumes ~20% of its resting energy production. This corresponds to a 10-fold higher rate of energy consumption, compared to an equivalent volume of tissue in the rest of the body.[1] The high metabolic demand of the brain is fuelled by a constant flow of blood, supplied through a pervasive network of small arterioles, capillaries, and venules. The brain's microvasculature is remarkable in several ways. First, it is densely packed into brain tissue. Every cubic millimetre of brain tissue contains a meter of total vascular length.[2] Extrapolated over the entire brain, this means that ~400 miles of vasculature is held within the adult human brain, most of which is comprised of miniscule capillaries. Second, the microvasculature in the cerebral cortex is organized such that every cell is no more than 15 micrometers away from a capillary.[3] This ensures that every brain cell is adequately supplied with oxygen and nutrients, including those distant from an arteriole perfusion source. Third, the brain vasculature is unique in its ability to form a blood-brain barrier (BBB), which tightly regulates the passage of molecules in and out of the brain. These barrier properties are first established during embryogenesis, but continue to be refined after birth. Fourth, the brain is a highly dynamic organ with fluctuating energy demands. The microvasculature must also be able to communicate with neurons to dilate or constrict during the regulation of cerebral blood supply.

There is still much to be learned about how the cerebrovasculature develops to become an efficient system for blood supply. However, existing data has already revealed an evolving growth and refinement in microvascular structure, which is contrasted to the marked stability of vascular networks of the adult brain.[4-6] This process involves interplay between endothelial cells and mural cells (smooth muscle cells and pericytes) as chief engineers of the vessel wall, and then the gradual incorporation of other brain cell types (glia and neurons) and structural components (basement membrane) to further specialize vascular structure and function. In this review, we will explore the process of cerebrovascular development with a primary focus on changes in vascular network structure in the initial month after birth, primarily in rodent models. To appreciate the end product, we first present the microvasculature of the adult mouse cerebral cortex, which is a well-studied 3-dimensional network with distinct microvascular zones. We then detail the establishment of this network during postnatal development and how it gains its key physiological roles, with a primary focus on BBB integrity. We further describe endothelial interactions with other cell types to support the sculpting and refinement of the capillary network. Finally, we discuss how live imaging could help grow our knowledge on postnatal vascular development. This review draws primarily on data from rodent cerebral cortex, and thus timing of events in other CNS regions, and other model organisms, may differ.

The design of a mature brain microvascular network

To illustrate the structural features of brain microvasculature, we turn to the adult cerebral cortex. At the brain surface, pial arterioles lie within the subarachnoid space of the brain meninges **(Fig. 1A)**. These arterioles range from tens to hundreds of micrometers in diameter and are comprised of an endothelial layer and internal elastic lamina, surrounded by 2-3 cell layers of concentric, ring-link contractile smooth muscle cells. These arterioles also have an outer adventitial layer comprised mostly of collagen fibers and perivascular nerves. This wall composition enables arterioles to dilate or constrict under the control of peripheral nerve ganglia or intrinsic brain neurons, which enables to modulation of cerebrovascular tone and brain perfusion.[7]

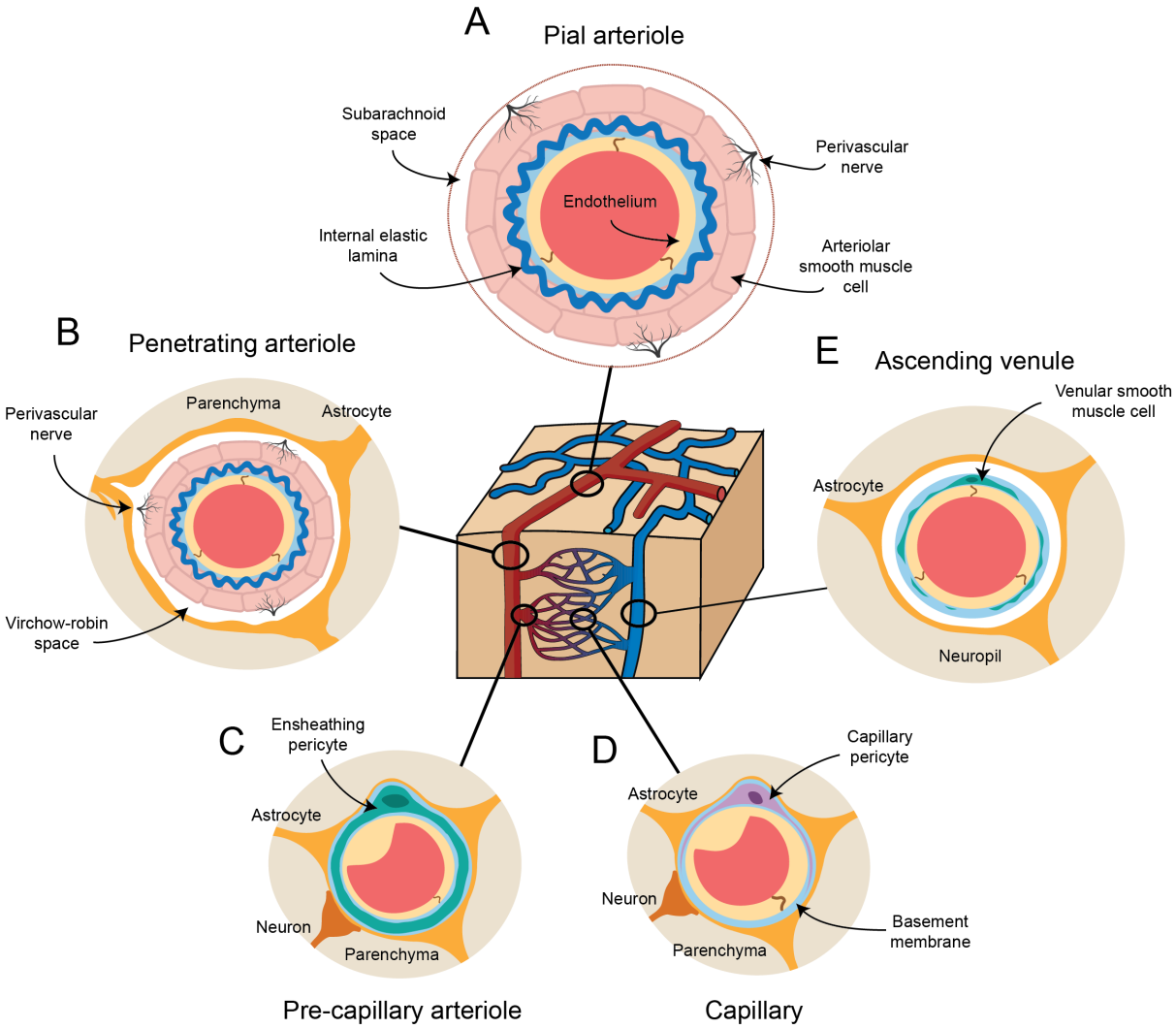

Figure 1. Neurovascular unit of the adult rodent brain. The cellular components that comprise the vascular wall across different microvascular zones in cerebral cortex. This schematic shows cross-sectional views of the vascular wall composition at the level of pial arterioles on the brain surface **(A)**, and downstream penetrating arterioles **(B)**, pre-capillary arterioles **(C)**, capillaries **(D)**, and ascending venules **(E)** within the brain parenchyma.

On a broader structural level, pial arterioles exist as an interconnected two-dimensional network composed of the branches of the major cerebral arteries (middle, anterior or posterior cerebral arteries), and shorter anastomotic connections linking these major arterial branches (**Fig. 2A, red**).[8] Collateral arterioles further connect the distal ends of the cerebral arteriole territories to link the perfusion across distinct arterial domains. The pial arteriole network offers a number of intriguing attributes. First, it facilitates the distribution of blood supply across the cortex when serving the demands of local neuronal activity, *i.e.* neurovascular coupling. That is, blood flow can be more easily shifted from less active tissues to tissues with metabolic need through a multitude of flow routes [9]. Second, in the case of brain ischemia due to focal arterial occlusion, the anastomotic connections allow blood to be re-routed from normally flowing arterioles into the under-perfused tissues.[10, 11]

Penetrating arterioles branch from the leptomeningeal network and plunge into the cortical depth to bridge blood flow from the brain surface with capillary networks of the brain parenchyma (**Fig. 1B and 2B**).[12] Penetrating arterioles range from 10-30 micrometers in diameter, and like pial arterioles, are composed of an endothelial layer surrounded by a single cell thick layer of smooth muscle. They

are surrounded by a cerebrospinal fluid-filled Virchow-Robin space in the upper layers of cortex, which is a continuation of the subarachnoid space.[13, 14] As the penetrating arteriole descends into cortex, the Virchow-Robin space disappears and the glial limitans is formed as the endfeet of parenchymal astrocytes come in close apposition to the vessel wall. Penetrating arterioles are also highly dynamic in their response to neuronal activity, but the conveyance of signals during neurovascular coupling differ from pial arterioles, and remain an active area of investigation.[15]

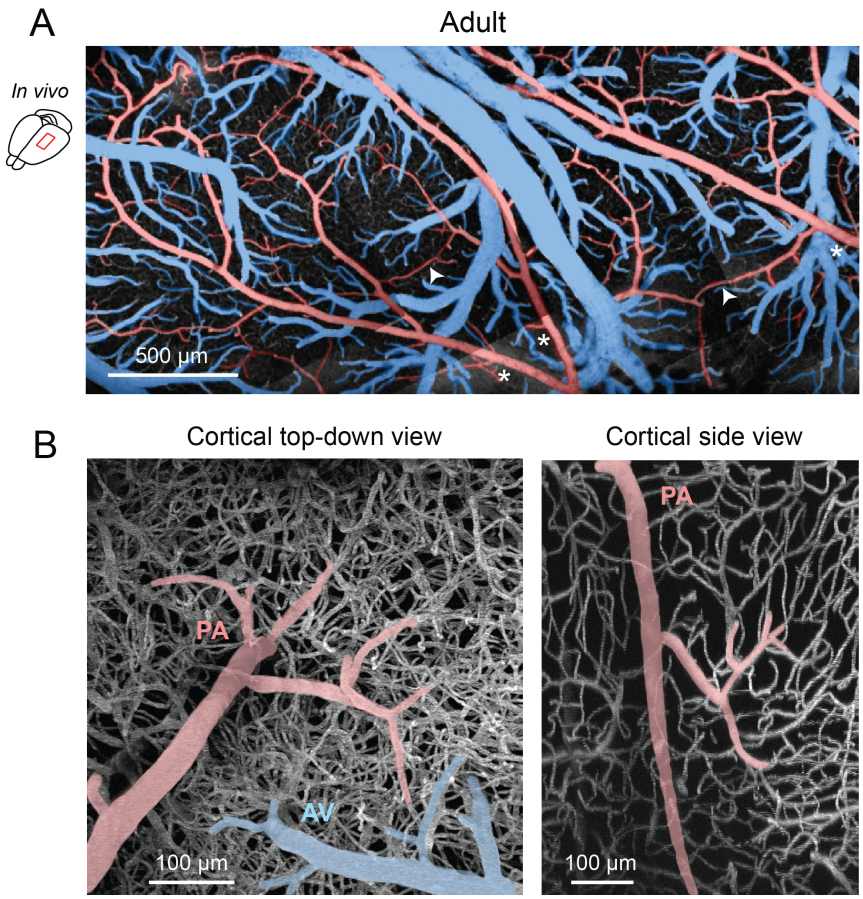

Figure 2. Microvasculature of the adult rodent cortex captured by *in vivo* imaging. (A) An image of the cerebral cortex surface captured by *in vivo* two-photon microscopy through a cranial window in an adult rat. Pial arteriole networks are pseudocolored in red, and pial venular networks are in blue. Major branches of the middle cerebral artery traverse the entirety of the window (white asterisks), and smaller anastomotic connections link these major branches to create an interconnected web (white arrowheads; two examples shown). **(B, left)** Highly magnified view of a pial arteriole descending into the parenchyma as a penetrating arteriole (PA; red), and an ascending venule (AV; blue) emerging from cortex and draining into a pial venule. The dense subsurface capillary network (white) is also visible in this image because the image is a projection across 700 µm of total cortical depth. **(B, right)** The same penetrating arteriole in the left panel is shown from a side view, revealing its descent into the cortex. A pre-capillary arteriole offshoot branches from the penetrating arteriole and ramifies into the capillary network.

Pre-capillary arterioles branch from the penetrating arterioles at different depths below the pial surface (**Figure 1C and 2B**). Pre-capillary arterioles are smaller in diameter (5-8 micrometers) than the parent penetrating arteriole, yet still covered by contractile mural cells with a hybrid morphology of smooth muscle cells and pericytes (termed ensheathing pericytes in our past work).[16] This zone is relatively small and occupies the first 2-3 branches before the microvasculature further ramifies into a dense 3-dimensional network of capillaries. Capillaries are the predominant vessel type in the brain, representing more than 90% of the total cerebrovascular length, and interweave with neurons and glia

of the brain parenchyma to facilitate much of the nutrient and gas exchange (**Figure 1D and 2B**). They are composed of thin endothelial tubes (average 4 μm in diameter), covered by true capillary pericytes, with protruding ovoid cell bodies that extend long thin processes.[17]

After transiting the capillary bed, blood is then routed back to the brain surface via post-capillary venules that empty into ascending venules, which have thin, stellate mural cells that incompletely cover the vascular wall (**Figure 1E and 2B, left**).[17] At the pial surface, the blood circulates through a network of venules that are interconnected much like the leptomeningeal arteriolar system, but still maintain a tree-like structure (**Fig. 2A, blue**). In the rodent brain, there are 2-3 ascending venules for every penetrating arteriole, suggesting multiple outputs for blood from the brain parenchyma.[18, 19] Blood then empties into pial venules and larger draining veins and sinuses on the brain surface.

The cortical vasculature has been studied across multiple species, from rodent [3, 8] and cats [20], to non-human primates [21] and human.[22, 23] Remarkably, apart from some difference such as arteriole:venous ratio [24], the same hierarchical organization of vessels seems to be preserved across phylogeny. Thus, the basic building principles and mechanisms for blood allocation is similar between model organisms and the human brain.

Development of microvascular architecture

How the vasculature is shaped during brain development remains less clear, though a good amount of knowledge has amassed.[25, 26] New vessels can be generated by two different processes, vasculogenesis and angiogenesis. Vasculogenesis is the process by which new vessels are formed *de novo* from vascular precursor cells (angioblasts). In contrast, angiogenesis corresponds to the growth and branching of existing blood vessels to expand the complexity of vascular networks. During embryogenesis, the process of vasculogenesis establishes the first vascular networks, which are then expanded *via* angiogenesis.[27]

In the rodent brain, vascularization starts with the formation of the perineural vascular plexus via vasculogenesis at the ventral neural tube at approximately embryonic day (E) 7.5–8.5.[25] Then, at E9.5 the first angiogenic vascular sprouts from perineural vascular plexus invade the neuroepithelium from the pial surface. The invasion is followed by branching, arborization and migration of vascular sprouts from the pial network toward the ventricles where angiogenic factors, such as vascular endothelial growth factor (VEGF), are highly expressed.[25, 28] Hypoxia is the principal regulator of VEGF expression, as it is a direct transcriptional target of both hypoxia-inducible factors (HIF)-1α and HIF-2α.[29, 30] Deficiency in HIF-1α and its dimerization partner HIF-1β results in embryonic lethality at around days E9.5 and E10.5, with severe defects in vessel formation, particularly in the yolk sac.[31, 32] During normal brain development, oxygenation is lower in the non-vascularized cortex at E10.5 than in the already vascularized ventral forebrain, and oxygen levels increase as the cortex becomes vascularized.[33] However, there is a scarcity of data on how tissue oxygen content contributes to vascular growth postnatally.

At the time of birth, the pattern of interconnected pial arterioles has resemblance to what it will become in the adult brain (**Fig. 1C and Fig. 2**).[34-36] Major branches of the cerebral arterioles are present, and the arterial tree has many short anastomotic connections, as seen in the adult brain. Further, collaterals arterioles that link the distal ends of separate cerebral arteriole domains are already present, as their formation occurs between E15.5 to E18.5.[37] Postnatal remodeling of the pial

vasculature primarily involves pruning about one half the anastomotic connections within the branches of the arterial trees, as well as pruning of collaterals linking the major cerebral arteries.[37] At P7, the pial arterioles alter their diameter, possibly due to adjustments in vascular tone, and lengthen to accommodate the growing cortex.

Interestingly, large differences in collateral artery density are seen between certain strains of mice, underscoring the importance of genetic factors that govern vascular patterning and density. In particular, Faber and colleagues have shown that BALB/c mice exhibit 60% fewer collaterals between middle and anterior cerebral artery territories than C57Bl/6 mice.[37] This difference was a result of reduced collateral formation during embryogenesis rather than postnatal vascular pruning. Subsequent studies have identified genes, such as VEGF-A[38] and chloride intracellular channel-4[39], that positively regulate collaterogenesis and increase collateral density during adulthood. Collaterals are critical for maintenance of blood supply in occlusive disease, and accordingly, mouse strains endowed with greater collateral density are more resilient to stroke injury caused by middle cerebral artery obstruction.[40] Understanding the genetic basis of collaterogenesis may yield clinical biomarkers to identify patients with higher susceptibility to stroke injury due to collateral deficiency, and provide a means to harness collaterogenesis for stroke prevention and treatment.

In contrast to arterioles, the pial venular network begins as a dense vascular plexus covering most of the cortical surface at birth (**Fig. 3 and Fig. 4A**) [34, 35, 41]. This plexus is composed of small diameter, short distance loops that have been referred to as a superficial "capillary-like" network, though they appear distinct from true parenchymal capillaries [35]. By P7-P14, this dense plexus is then gradually pruned away in regions intervening the major draining venules over the course of 1-2 weeks. As a result, the branches of the venous network become more evident and blood flow within the remaining vessels increases to more effectively drain blood.

The remodeling of pial venules during brain development bears marked resemblance to vascular changes in the developing mammalian lung and the avian chorioallantoic membrane (CAM).[42, 43] The key process to note is intussusceptive angiogenesis, where two indentations on opposing sides of the lumen grow and gradually connect to create a cylindrical pillar-like structure within the lumen. This results in the creation of two daughter branches separated by the pillar. Intussusceptive pillars may then further widen to change the structure of the two daughter vessels. Imaging data from brain suggests the presence of similar events occur during pial venule development[34], evident as the formation of small black "holes" in a vascular tube (**Fig. 4A,B**), similar to that noted by imaging studies of Djonov and colleagues in the chicken CAM [44], and more recently in the zebrafish caudal vein plexus.[45] However, rather than increase vascular network complexity, this intussusceptive process results in pruning of the initially amorphous venous plexus into a hierarchical tree-like structure for effective blood drainage.

The transduction of mechanical forces by blood flow and pressure can strongly influence the remodeling process through alteration of endothelial structure and organization.[46, 47] By collecting detailed vasodynamic information using repeated two-photon imaging, Letourneur *et al.* found a positive relationship between the shear rate of blood flow, a metric proportional to shear stress force, and the subsequent diameter of venules one day later. That is, venules with low shear rate were likely to be pruned away, while those with high shear rate widened and stabilized within the network.[34] Similarly, repeated imaging of mid-brain microvessels in zebrafish revealed that flow in vessels destined to be pruned was lower and more variable that in microvessels that persisted within the network.[48] Flow-dependent pruning must be essential for removal of redundant connections and

promotion of efficient routing of cerebral blood flow. Since, improper venous remodeling can impact on cerebral circulation by impairing cerebrovascular output, further studies on how mechanical forces govern cerebrovascular development are warranted.

Beneath the brain surface, the penetrating arterioles and ascending venules are present at birth, though their characteristics continue to be refined. The penetrating arterioles are initially thin and capillary-like within the first few days after birth, but then their lumen expand and walls muscularize to become more arteriole-like by P10.[49] Penetrating arterioles give off branches to perfuse downstream capillaries. However, there is little data on the development of the pre-capillary arteriole zone, which recent studies have shown to be critical for the initiation of neurovascular coupling.[50, 51]

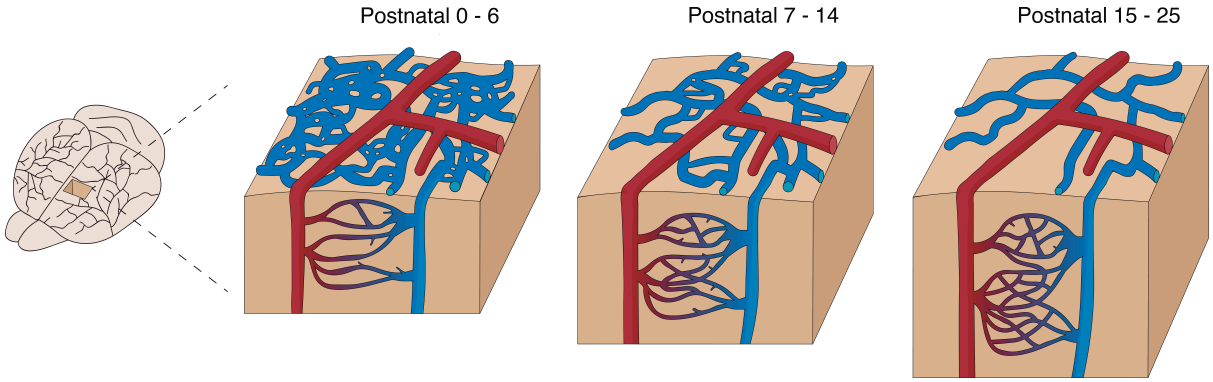

Figure 3. Postnatal remodeling of cortical vasculature in rodents: Refinement above, expansion below. Arteries are represented in red and veins in blue. At birth, the pial surface is covered by a dense plexus of veins that undergoes pruning over the initial few weeks postnatally. Pial arteriolar structure is generally unchanged, but there is regression of some anastomotic connections. Concurrently, the capillary bed undergoes massive proliferation through generation of new angiogenic sprouts. By postnatal day 15 to 25, the capillary bed is almost formed and rates of endothelial proliferation decrease.

The capillary network at birth is sparse and incomplete compared to the dense networks of the adult brain (**Fig. 3**). The numerous ongoing angiogenic and regression events result in non-flowing endothelial tubes that may contain only a trickle of blood plasma until they become patent. Over the course of weeks, the cortical capillary network undergoes dramatic expansion *via* angiogenesis.[4, 27, 52] The length of capillary branches and individual endothelial cells also elongates to keep pace with the rapidly expanding cortical volume.[53] Detailed histological analyses of endothelial sprouting have revealed bursts of angiogenesis that occur at different stages and cortical layers.[49] From P5 to P25, new capillary loops are often formed by short distance sprouting without the need for proliferating endothelial cells. That is, single endothelial tip cells can reach to connect with other capillary branches through the extension of their cellular processes.[4] *In vivo* imaging of postnatal vascular development has revealed that many of the nascent angiogenic sprouts are eliminated, with only a small subset forming lasting connections within the capillary network.[4] However, it was not clear from data of Harb *et al.* whether eliminated vessels were true capillaries, or "capillary-like" venules near the brain surface, which are known to regress as discussed above. Nevertheless, capillary refinement by regression is still greatly outweighed by angiogenesis. By P15 to P25, the extent of capillary angiogenesis begins to subside and capillary density stabilizes.[4, 54, 55] During this stabilization, the proliferation rates of both pericytes and endothelial cells decline.[4]

In light of data from other vertebrate models (zebrafish)[56] and mammalian organs (heart and retina) [57, 58] showing that angiogenic sprouts tend to emerge from venules, the microvascular zone in which nascent angiogenic sprouts emerge in the postnatal brain needs further characterization. Venous sprouts can in fact give rise to arterioles through migration and reprogramming of endothelial cells, though in the mammalian postnatal brain this seems to be less likely with arterioles pial and penetrating arteriole structures already defined. The underlying reason for why veins are a source of angiogenic sprouts remains obscure. Decreased tendency to rupture due to lower intravascular pressure[59], or decreased oxygen content surrounding venules are logical hypotheses for this phenomenon.

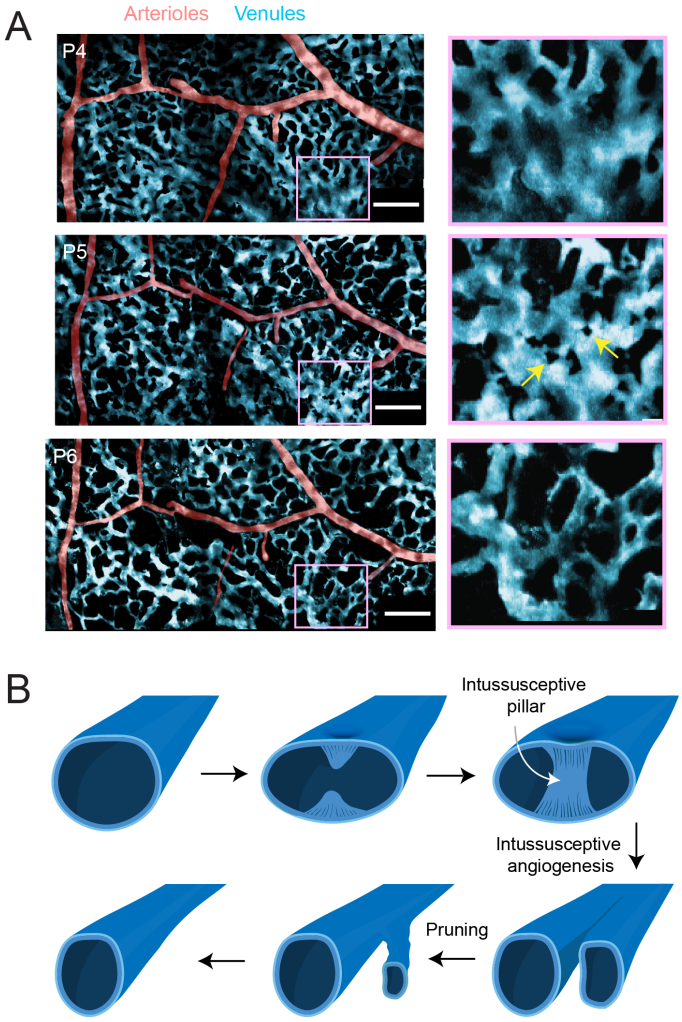

Figure 4. Evidence for intussusceptive remodeling in the developing pial vein plexus. (A) Image montages from *in vivo* two-photon microscopy provide a wide-field view of pial arteriole and venule remodeling between P4 to P6. Arteries are pseudocolored in red and veins in blue. The inset shows a region of the vein plexus that forms small holes (yellow arrows) that grow in size and depth over a day. These regions are devoid of blood plasma because they do not label with an intravenously injected fluorescent dye that enables visualization during microscopy, consistent with an intussusceptive pillar. Images adapted from Letourneur *et al.*[34]. **(B)** A schematic depicting a hypothesized mechanism for venous remodeling in the developing mammalian brain, involving intussusceptive angiogenesis and then pruning.

Gray and white matter tissues require different levels of metabolic supply. It is estimated that white matter, which is densely packed with energy efficient myelinated axons, requires 1/4 to 1/3 the energy used by gray matter. It is therefore not surprising that capillary networks of white matter are less

dense compared to gray matter. Interestingly, developmental studies have revealed that capillary density in gray matter doubles during the period of birth to P20, while capillary density in white matter structures increases to a far lesser extent, or even decrease with age [55].

Sidebar title: Relating animal models to human development

Early-life development is characterized by dramatic changes that can impact brain function over an entire lifespan. Like with rodents, a considerable portion of human brain development occurs after birth, with individual regions of the brain maturing at different rates.[52] Nevertheless, cross-comparisons of developmental benchmarks between rodents and humans, including the timing of neuroanatomical changes, neurogenesis, synaptogenesis, gliogenesis, and myelination, have revealed that the rodent brain at P1 to P5 roughly corresponds roughly to 23 to 32-weeks of gestation in the humans.[60] P10 in rodents roughly corresponds to 40-weeks of gestation in human development. Thus, studying rodent models just within the first 2-weeks following birth provides access to a wide range of events relevant to prenatal and human neonatal brain development.

Postnatal refinement of the blood-brain barrier

The capillary endothelium creates an efficient physical and biochemical barrier between the blood and the brain.[61] Through tight regulation of intracellular trafficking and dynamics of intercellular junctions, endothelial cells control several aspects of BBB function, including transport of micro- and macro-nutrients, receptor-mediated signalling, leukocyte trafficking, and osmoregulation. The multi-step process of BBB development does not occur with endothelial cells alone (**Fig. 5**). The recruitment and crosstalk of other cell types (pericytes, microglia, astrocytes, neurons) in the neurogliovascular unit are required for refinement and maturation of the BBB. In particular, astrocytes are only fully integrated after birth suggesting that BBB development extends into the postnatal period in rodents. Excellent reviews have been written about the complex molecular and cellular events involved in barriergenesis[26, 62, 63], much of which has been characterized during embryogenesis. Here, we aim to highlight some aspects of this process that suggests that BBB maturation continues to occur postnatally.

When endothelial cells first invade the CNS, there are features of endothelial permeability, including fenestrae, upregulated rates of transcytosis [64, 65] and also high levels of leukocyte adhesion molecules.[66] While endothelial tight junction structure are already present at E12, detailed ultrastructural analyses in late embryonic (E15-18) to postnatal stages (P1) have revealed an increase in their density and complexity until adulthood.[66, 67] Measurements of transendothelial electrical resistance (TEER) and vascular permeability *in situ* from fetal to postnatal rat brain vasculature revealed a gradual sealing of the BBB from E17 to P33.[68] Ultrastructural analyses of capillaries walls in rat corpus callosum, which were already covered by pericytes, showed a numerous pinocytotic vesicles at P1 through P7 with high permeability to intravenously injected ferritin. At approximately P14 there is a decrease in vesicle abundance and ferritin leakage, concomitant with increasing coverage of the capillary wall by astrocytic endfeet. During this maturation, the peri-capillary spaces are reduced in size and replaced by an astrocytic-basement membrane interface [65].

Endothelial expression of the efflux transporter Pg-P (P8) [66] and ABC-transporters [69] increases during postnatal development, indicating greater capacity for molecular efflux from the brain. The glucose transporter, GLUT1, is already expressed in early embryonic stages in capillary tight barrier structures. However major changes in the regional pattern of GLUT1 distribution occurs at P3 until 2

months of age.[55, 70] Moreover, combined FACS purification with GeneChip analysis was used to generate a transcriptional profile of highly purified CNS endothelial cells across different ages. Comparing the ages ranges of P2-P8 with P60-70, endothelial cells exhibited differences in genes that control angiogenesis and formation of the BBB, suggesting a continued maturation of the BBB postnatally.[66]

Building the wall: from a vascular network to a neurogliovascular unit

The assembly of the neurogliovascular unit involves interaction with pericytes, astrocytes, neurons and non-cellular structural components such as the basement membrane (**Fig. 5**). This process initiates at embryonic stages, but is completed in early postnatal stages of development. Below, we discuss the multicellular dynamics that occur postnatally to form a functional vascular network.

Pericyte-endothelial dynamics: angiogenesis and vessel stabilization

Pericytes are mural cells embedded in the basement membrane of brain capillaries, directly abluminal to the endothelium.[71] Communication between the endothelial cells and pericytes is essential for the establishment of a number of cerebrovascular functions, including vascular structure and BBB integrity. This communication is mediated by signaling at direct cell-cell contacts occurring within peg and socket interactions, as well as through the release of growth factors and the modulation of the extracellular matrix. Pericytes may also be linked to the endothelium through gap junctions, tight junctions, and focal adhesions junctions.[72] One ultrastructural imaging study revealed pericyte-endothelial 'gap junctions' in embryonic rat brain capillaries[73], which may be required for endothelium-induced differentiation of mural cells.[74] Numerous signaling pathways underlie pericyte-endothelial crosstalk for the development of vascular structure and barriergenesis, and these have been reviewed in detail previously.[72]

Pericytes are present on the brain endothelium as early as E10, and contribute to a large expansion of capillaries that occurs between E14.5 and E18.5 [75]. These early pericytes or pericyte progenitors express established marker genes *Pdgfrb* and *Cspg4*, but still lack other markers such as *Anpep* (CD13) seen in the adult brain, suggesting an ongoing maturation of their phenotype. At the time of birth, the morphological characteristics of brain pericytes are distinct from that in the adult brain (**Fig. 5**). Stereotyped protruding cell body of pericytes are less conspicuous, and cellular processes fully enwrap the endothelium, as opposed to the partial coverage seen in adulthood.[65] It is widely appreciated that pericytes promote quiescence and inhibition of endothelial growth [76, 77]. However, in early development pericytes must also be supportive of rapid capillary network expansion. Indeed, a recent study showed that pericyte ablation in postnatal retina led to impaired endothelial sprouting and patterning via disruption of local VEGF/VEGFR signaling between pericytes and endothelial cells.[78]

There are two seemingly opposing views to how pericytes and endothelial cells coordinate the formation of new capillaries during sprouting angiogenesis. The predominant concept is that endothelial cells lead the process, and pericytes lag behind. Endothelial tip cells first migrate from existing capillary networks through a VEGF-dependent mechanism, and penetrate into the tissue.[79] Endothelial stalk cells then follow the tip cell and proliferate to further extend the angiogenic sprout. Tip cells release the growth factor PDGF-B, which is deposited in dimeric form (PDGF-BB) on the surrounding vascular extracellular matrix and forms the signal for pericyte recruitment to the nascent

endothelial tube, leading to subsequent vessel stabilization and maturation.[80-82] Crosstalk between pericytes and endothelial cells then promotes vascular stability and increase in BBB integrity by increasing expression of endothelial tight junction proteins and reduced transcellular transport through caveolae.[66, 83] This concept suggests that there is a window of endothelial plasticity and BBB immaturity until pericytes are invested into new capillaries. In retina, this window can be significant, lasting up to several days, before pericytes invade the endothelial plexus.[84]

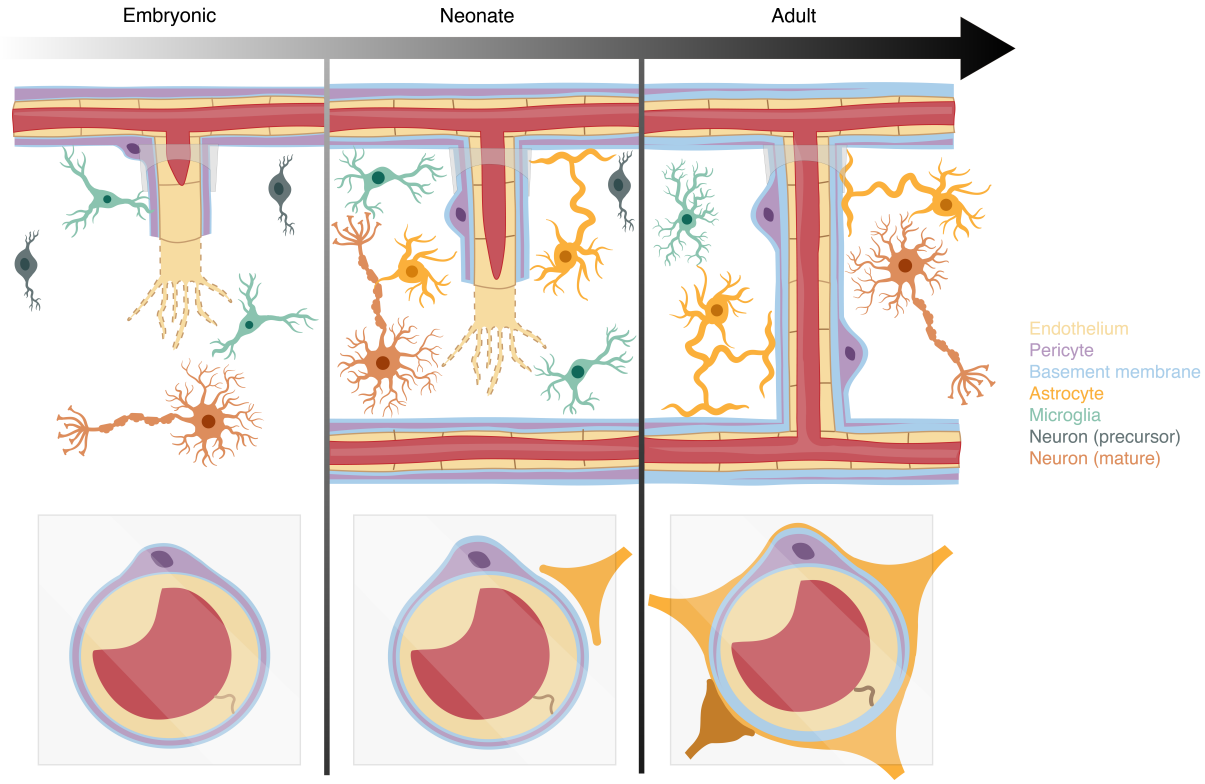

Figure 5. Maturation of the neurogliovascular unit. The upper panels are a schematic depicting the gradual integration of different cell types and the basement membrane in brain capillaries. The lower panels show a cross-sectional view of the capillary wall at each developmental stage. Developmental periods (embryonic and neonate) show an angiogenic sprout with extensive filopodia. This sprout connects with an existing part of the capillary network and becomes patent. Increased tight junction density, basement membrane thickness, and astrocytic endfoot investment contributes to BBB maturation.

A second view is that pericytes lead the angiogenic process by invading the parenchyma, followed in turn by endothelial cells.[85] In this case, pericytes may be a source of VEGF that provides the guiding signal for endothelial cells.[86] This concept implies that pericytes are already present on angiogenic sprouts and capable of delivering signals to rapidly promote BBB integrity and vessel stabilization, thereby limiting the window for vessel immaturity. It further raises the possibility that pericytes have some role in deciding the location of new angiogenic sprouts in existing capillary networks, and in guiding or bridging the sprout through the brain parenchyma. This mechanism may be more relevant to angiogenesis occurring in vascular networks already abundant in pericytes. However, these opposing views come from diverse model systems and organs, and likely differ in various angiogenic contexts. Recent data from Payne *et al.* reveal a potential middle ground where pericyte and endothelial tip cells co-migrate, taking turns to lead the new capillary sprout.[87] The relative position of pericytes and endothelial cells was hypothesized to be dependent upon fluctuations in the availability of ligands such as PDGF-BB at the angiogenic front.

While most of the vascular growth within the capillary network is attributed to sprouting angiogenesis, a second means to increase capillary network complexity is through intussusception or "splitting angiogenesis".[42] While, intussusceptive angiogenesis has been characterized in other developing organs, such as the lung, it remains unclear whether it contributes significantly to development of brain capillary networks.

Pericytes contact the majority of the endothelium via long processes that run longitudinally along the vessel axis. These processes allow a single pericyte to contact and communicate with hundreds of micrometers of capillary length.[17] The processes incompletely enwrap the underlying endothelium, and studies often report the extent of this overlap as "coverage", which ranges ~ 50-70% coverage in the normal brain. The extent to which pericytes cover the endothelium is developmentally regulated by PDGF-B/PDGFRbeta signaling.[88] While mice that are null for the *Pdgfb* or *Pdgfrb* null genes have near complete absence of brain pericytes, and are perinatal lethal [82, 89], mice experiencing partial disruption in PDGF-B/PDGFRbeta signaling are viable and useful for postnatal developmental studies. For example, $PDGFB^{ret/ret}$ mice harbor a mutation to PDGF-B that inhibits ligand binding to the vascular basement membrane, thereby removing the signal for pericyte recruitment. These mice have pericyte coverage of only 25% of the endothelium and exhibit aberrant capillary dilations and increased transcellular BBB leakage.[83] They also exhibit fewer branch points in the capillary network, consistent with a role for pericytes in capillary patterning. Using hypomorphs that collectively exhibit a range of severities in pericyte loss, pericyte coverage was found to correlate with BBB integrity, where greater pericyte loss was associated with worsened leakage through transcellular routes.[66] Transcriptomic studies further revealed that pericytes suppressed pathways for endothelial leakage, including genes involved in promotion of immune cell trafficking.

Pericytes also regulate endothelial expression of major facilitator super family domain containing 2a (MFSD2A), and lipid transporting protein at the plasma membrane. Genetic ablation of *Mfsd2a* leads to BBB leakage due to increased transcytosis from embryonic to adult stages.[90] Recent work also showed that the spatiotemporal expression of a cell adhesion receptor, CD146, is important for pericyte recruitment/attachment and BBB maturation.[91] Pericyte-specific deletion of CD146 causes BBB breakdown and impairment in pericyte coverage.

The vascular basement membrane: More than just cement.

The microvascular basement membrane is a thin layer of extracellular matrix, composed of structural proteins, glycoproteins and proteoglycans, located between the endothelium and perivascular endfeet of the parenchyma. Pericytes that are located within this intermediate space are completely embedded within the basement membrane. The basement membrane anchors cells within the neurogliovascular unit and forms a physical barrier to regulate cellular migration, such as leukocyte trafficking. The basement membrane also serves critical roles in facilitating cell-cell communication at the vascular wall. For example, it provides a scaffold to retain signalling molecules such as PDGF-BB and VEGF crucial for angiogenesis and pericyte recruitment. Bioactive components such as laminin are also required for cell differentiation and migration. The basement membrane is evident as early as E20 in rat embryos but still ill-defined at birth. Its density and thickness increases with postnatal development suggesting continued formation by perivascular cells (**Fig. 5**).[65, 92] In mice, laminin and agrin are the earliest proteins to appear at the vascular wall around P0, reaching the peak levels as early as P7.[93] This early basement membrane and its junctions with astrocytic endfeet become critical

for establishing the polarization of proteins, such as the water channel aquaporin-4 (AQP4), at the vascular interface.

Highlighting the importance of basement membrane for vascular integrity, alterations and disruption of the basement membrane can lead to changes in the endothelial cell cytoskeleton, which in turn affects tight junction protein structure and BBB integrity. Accordingly, laminin α2 subunit-null mice at P21 exhibit BBB hyperpermeability correlated with significant decreases in VE-cadherin, claudin-5, and occludin, as well as lower pericyte and astrocytic endfoot coverage.[94] More specifically, Yao and colleagues showed that the laminin produced by astrocytes is needed for polarization of astrocytic endfeet, pericyte differentiation and maintenance of BBB properties.[95] Additionally, it has been shown that endothelial laminin, laminin α4, regulates vascular integrity at embryonic/neonatal stage, and its deletion leads to impaired microvessel maturation causing haemorrhages during the embryonic and neonatal period.[96] Mutations in collagen type IV, which causes alterations in the basement membrane, similarly leads intracerebral haemorrhage in both mice and humans during development.[97]

Astrocyte-endothelial crosstalk: Barriergenesis and vascular remodeling

In the adult brain, astrocytes form a layer of fine lamellae, called endfeet, that is closely opposed to the abluminal surface of the endothelium and basement membrane.[98] This layer forms a physical barrier at the vascular wall, and also facilitates the neurovascular cross-talk needed for BBB maturation. Astrocytes first become prominent during the perinatal period, coincident with the onset of expansion of cortical capillary networks (**Fig. 5**).[99, 100] Some findings show that after populating the cortex, astrocytes continue to proliferate locally during the first 3-weeks of postnatal development.[101] In the rat brain, increase in astrocyte density proceeds until P50, when adult density levels are reached.[102, 103] Also, endfoot expression of AQP4, a channel for water transport across the BBB with implications in glymphatic drainage, was weakly expressed at P7 and showed a sharp increase in expression towards adulthood.[93, 104, 105] It has been suggested that pericytes may be needed to attract or maintain astrocyte endfoot coverage of the vessels, as pericyte-deficient mice exhibit defects in association and polarization of astrocytic endfeet.[83]

Astrocytes seem to also serve a critical role in regulating vascular architecture, as the astrocytes interact closely with blood vessels by P5-P7, and inhibition of astroglial proliferation results in a drastic reduction in the density and branching of cortical blood vessels.[100] Loss of astrocyte investment also leads to abnormal endothelial proliferation and enlargement of vessel diameter. Further, the expression level of several of the proteins normally present at the perivascular astrocyte endfeet is very low in immature astrocytes, including the canonical astrocyte marker glial fibrillary acidic protein.[93]

Astrocytes also orchestrate the postnatal formation and reorganization of vascular scaffolds for cellular migration. Detailed gain and loss of function studies have shown that astrocyte release of VEGF influenced the formation of blood vessels in the rostral migratory stream, which is used as a scaffold for neuroblasts migrating to the olfactory bulb.[106] *In vivo* downregulation of VEGF specifically in the astrocytes affected vascular development and led to complications in neuronal migration. Curiously, postnatal expression of VEGF by astrocytes is unique to the rostral migratory stream, as expression in cerebral cortex is higher in neurons at early stages of development.[106] However, as vascular beds stabilize around P24, the proportion of astrocytes expressing VEGF increases in cortex relative to neurons.[107]

Microglia-endothelial contact

Microglia are the resident innate immune cells of the brain, and represent ~10-15% of all glia in the brain.[108] Studies from developing brain and retina suggest that microglia cells colonize prior to tissue vascularization, but then come in close contact with the microvasculature during the vasculogenic and angiogenic process.[109] Microglia interact with endothelial tip cells to coordinate anastomotic connections within the capillary bed (**Fig. 4**). They are often seen at sites where two tip cells with filopodia come in close contact and anastomotic connections are likely to occur.[110] Rymo and colleagues further probed the role of microglia in combined *in vivo* and *ex vivo* studies of mouse retina and an aortic ring model.[111] First, by examining mice with microglial deficiency (M-CSF/CSF-1 deficient mice), they showed that microglial absence led to a sparser vascular network. Then using aortic ring cultures, they showed that microglia could stimulate vessel sprouting and branching via release of a soluble, microglial-derived molecule. Together, this indicates a bi-directional communication between microglia and endothelial tip cells during the formation of new vascular connections. With respect to underlying mechanisms of microglia-endothelial communication, work from Tammela and colleagues suggested that a subpopulation of microglial cells express VEGF-C, which is able to activate VEGFR-3 in tip cells to reinforce Notch signaling.[109] This contributes to a phenotype change of the endothelial cell at sites of vascular fusion.

In addition to roles in regulation of vascular growth, microglial cells associated with brain capillaries in the early postnatal stage exhibit a phagocytic, amoeboid morphology. Over a period of 3-weeks, this shape then shifts to the classic morphological profile of ramified, resting surveillant microglial seen in the normal adult brain.[112, 113] Amoeboid microglia are able to uptake and retain intravenously injected dyes, suggesting that they serve as front-line protection from exogenous substances that may enter the pericapillary space by transendothelial transport during brain development.[65]

Neuronal activity alters the development of capillary structure

The microvascular architecture of the brain is shaped by neural activity during the postnatal period.[114, 115] Lacoste and colleagues showed that enhancement of neural activity between P0 to P5 by gentle whisker stimulation (15 minutes per day over 8 days) lead to increased vascular density and branching in the barrel field of sensory cortex.[114] Conversely, vascular density and branching were decreased when vibrissal sensory input was reduced by genetic impairment of neurotransmitter release at thalamocortical synapses, or by whisker plucking. Whiteus and colleagues also examined the relation between neural activity and vascular structure and reported that excessive stimulation and repetitive neural activation during P15 to P25 caused decreases in vascular density by reducing endothelial proliferation and sprouting.[115] This effect was observed using a variety of stimulation paradigms, ranging from treadmill exercise to auditory stimulation. While these two seminal studies seem contradictory at first, the contrasting outcomes may be explained by the extent of stimulation given, where Lacoste *et al.* used more physiologically relevant stimulation and Whiteus *et al.* aimed to test the effects of over-stimulation. It seems that physiological stimulation strengthens the neurovascular link, while over-stimulation disrupts this link and leads to lasting deficits in microvascular density. In contrast to the plasticity of the capillary bed, a recent study demonstrated that the structure of pial arteriole networks was unaffected by sensory deprivation.[116] The absence of vibrissal input from P2 to P30, due to continual whisker plucking, had no effect on the branching or pial arterioles, the density of penetrating arterioles, or anastomotic connections, suggesting that this system is already set prior to birth.

The construction of vascular and neuronal network is guided by similar mechanisms during development.[117]s Many signaling molecules important for axonal guidance, such as netrins, semaphorins, slits, nogo, and ephrins, are also able to influence vascular growth through attractive or repulsive cues. For example, recent studies discovered that a membrane protein RTN4 (previously NOGO-A), which inhibits axonal growth in adults, also acts as a negative regulator of angiogenesis during postnatal CNS development.[118] RTN4 is expressed in neurons in the postnatal brain (P10), with close proximity to vasculature endothelial tip cells and their filopodial protrusions. Genetic ablation or antibody-mediated neutralization of RTN4 in P4 or P8 mice led to a significant increase in the number of endothelial tip cells at P10, and the addition of new patent capillary branches in the microvascular network.[119] Another common pathway that influences both neural and vascular development is WNT signalling. WNTs are secreted glycoproteins with well-established roles in development of neuronal circuitry.[120] In vascular development, WNT/β-catenin signaling has dual effects on angiogenesis and BBB development through embryogenesis to postnatal periods. Loss of WNT 7a/b or WNT receptor, Frizzled 8, reduces vessel density and capillary bed structure, and also results in vascular leakage due to decreased tight junction protein expression and structural integrity.[121-123]

A well-studied positive regulator of angiogenesis is VEGF signaling. As discussed above, in postnatal rats (P8 – P13), VEGF is primarily expressed by neurons. Since VEGF release can be modulated by neural activity, it is can serve as a neurovascular link for regulation of angiogenesis.[124] Between P13 and P24, VEGF expression decreases in neurons and increase in astrocytes. Since astrocytes are ideally positioned to sense neuronal activity and interact with the vasculature, this shift in expression may continue to shape vascular architecture concurrent with the maturation of astrocyte-endothelial signals required for vascular tone and neurovascular coupling.[125]

Functional imaging studies of the developing human brain have observed patterns of hemodynamic responses that differ from adult responses. In adults, the increase of local neural activity is nearly always accompanied by increases in local blood flow to the active brain area, *i.e.* functional hyperemia. In contrast, the developing brain exhibits absent or even inverted vascular responses to neural activity.[126, 127] To better understand this phenomenon, Kozberg and colleagues examined the spatiotemporal dynamics of oxidative phosphorylation (inferred by fluorescence changes in flavoprotein) concurrent with neuronal activity in P7 mice [126]. They concluded that neural activity led to local oxygen consumption, but concurrent vascular responses were insufficient to supply additional oxygen. This was postulated to create conditions of local tissue oxygen depletion, which could reduce thresholds for hypoxic injury, indicating a period of vulnerability during early development. On the other hand, local oxygen reduction may be necessary as an angiogenic trigger to link the most active brain regions with the greatest capillary density. Nevertheless, absolute levels of tissue oxygen reduction during brain activity in neonates remain to be measured in detail, and this could be facilitated with the advent of new phosphorescent tissue oxygen probes.[128]

New techniques in imaging of live mouse pups have created fertile ground for understanding how neurovascular coupling is established, concurrent with maturation of the vascular architecture and neurovascular unit. Indeed, capillary networks in the adult brain provide retrograde hyperpolarization for upstream arteriolar dilation, and the architecture needed to convey these endothelial signals may be lacking in early development [129]. Further, the essential cellular elements for coupling, including astrocytes and pericytes, are also not fully integrated [130].

Open questions.

Collective research on cerebrovascular development has emphasized key topics for future emphasis. These include, but are not limited to:

(1) The mechanisms that establish the microarchitecture of brain capillaries. Research suggests that developing capillary networks are modifiable by neural activation/inhibition in the perinatal period and that these signals set the branching pattern and density of capillaries that ultimately serve blood flow in the adult brain. It will be critical to understand the mechanism and endothelial cues underlying this approach for modulating capillary structure and function, and whether it can be harnessed to promote microcirculatory function in human disease.

(2) The basis of neurovascular coupling in the developing brain remains understudied. Given that functional hyperemia is distinctly regulated in the infant brain, the interpretation of fMRI signals that rely on blood flow and oxygenation state as a surrogate for neural activity requires careful evaluation. Further, it is important to understand if the apparent lack of vascular reactivity despite robust neural activation creates conditions of oxygen deficiency in the infant brain. Does lowered oxygen content lead to increased vulnerability to hypoxia, or is this a normal angiogenic signal to link neuronal demand to vascular supply?

(3) *The perinatal refinement of blood-brain barrier integrity is poorly characterized.* It remains controversial as to whether humans are born with a fully functional BBB.[131] This is important to clarify because differences in permeability to drugs and other pharmacological agents may have inadvertent effects in treatments of nursing mothers and neonates. Further, while much is known about structural elements and genetics of the BBB, there remains limited information on the crosstalk that occurs between cells of the neurovascular unit that leads to BBB maturation. In the developing brain, there are both signals that promote cell proliferation, as well as those that support vascular maturation. It remains unclear how these processes coexist to produce a functional vascular system.

A window to visualize the developing brain

While mouse brain development has been extensively studied using histology and *in vitro* approaches, quantitative characterization of morphological changes over time remains a challenge. *Ex vivo* and *in vitro* studies are unable to replicate the complexity of neurovascular connections and the dynamics of perfused blood vessels in the intact brain. *In vivo* imaging through cranial imaging windows using two-photon microscopy (TPM) has emerged in recent years as one approach to overcome this limitation [4, 34]. In addition to providing cellular to subcellular resolution of microvessels below the brain surface, TPM allows longitudinal assessment of physiological dynamics and remodeling over days (**Fig. 6**). The use of thinned-skull windows reduces the likelihood of inducing inflammation or exposing the brain to air, which can change the trajectory of cerebrovascular development.[34] In combination with powerful genetic approaches currently available to fluorescently label and manipulate neurovascular cells [132], TPM holds promise for understanding the orchestration of cerebrovascular development *in vivo*. It further allows the opportunity to track the maturation of tight junction complexes, concurrent with assessments of BBB permeability[133], and vascular reactivity to neuronal metabolic demand.

In vivo imaging provides an additional advantage of noninvasive optical manipulation of cell types at specific times in the developmental process to probe the effects of their absence in cerebrovascular development. Recent studies have shown the possibility of precision optical ablation of a variety of cell types, including pericytes [134, 135], microglial cells [136], astrocytic endfeet [137], and neurons [135] in the adult brain. This approach has yet to be applied to study the role of specific cell types during cerebrovascular development. It is a powerful alternative to genetic ablation studies, i.e. using diphtheria receptor and toxin induced apoptosis, as it does not disrupt cell types in a global fashion, and does not rely on the availability of Cre drivers, which are often not entirely specific for the cell type of interest.

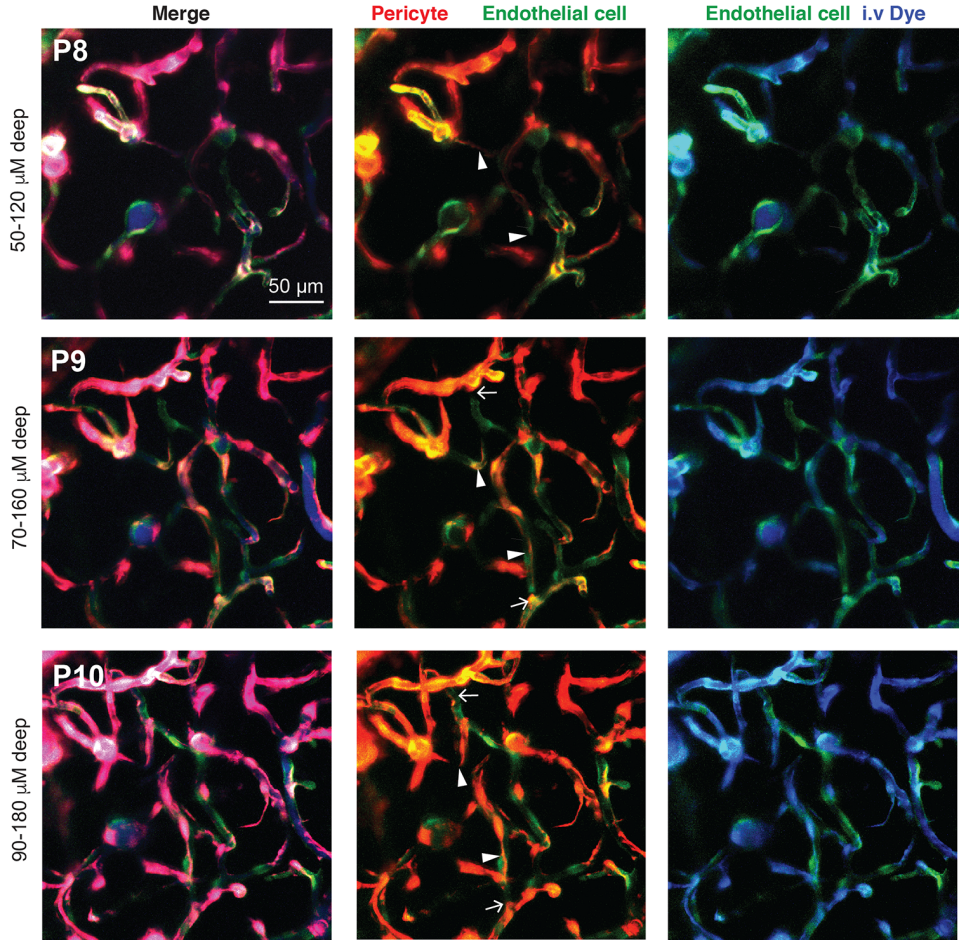

Figure 6. *In vivo* two-photon imaging of capillary network expansion in the postnatal brain. **(Upper row)** Capillaries visualized through a chronic, thinned-skull window in a P8 mouse pup. The mouse is double transgenic for Tie2-GFP and PDGFRbeta-tdTomato, allowing concurrently visualization of endothelial cells (green) and pericytes (red). An intravenous dye (Alexa 680-dextran; 2MDa) was injected intravenously to label the blood plasma (i.e. dye). To aid in visualization, the middle panel shows only endothelial cells with pericytes, and the right panel shows only endothelial cells and the i.v. dye. Note that many capillaries are covered by pericytes, but some regions still lack pericyte coverage/contact (middle panel). Also, many capillaries have not yet lumenized, *i.e.* not filled with the i.v. dye (right panel). Further, angiogenic sprouts are clearly visible in this expanding network (white arrowheads). **(Middle row)**. The same region of cortex was re-examined 1 day later. The angiogenic sprouts have grown further and are now integrated into an existing part of the capillary network (white arrow). **(Lower row)**. The nascent capillary branches formed over the previous two days are still present, and further increase in pericyte coverage (middle panel).

Conclusion

Research in cerebrovascular development has revealed that vascular architecture remains remarkably plastic and modifiable in the first few weeks of like. This process involves refinement of vascular networks at the pial surface, concurrent with increasing growth and density of sub-surface capillary networks. It has also demonstrated the complex interplay of cell types required to assemble a functional neurovascular unit. In collating the data, we have highlighted open questions worth examining with respect to development of neurovascular structure, reactivity, and integrity. Innovative *in vivo* imaging approaches have enabled researchers to begin tracking complex developmental processes in the living brain. A better understanding of cerebrovascular development will improve our knowledge of how the vasculature serves the immense task of feeding the brain in adulthood, and may also unveil approaches to leverage developmental programs for the improvement of vascular function in diseases of the adult and aging brain.

Acknowledgments

This work was supported by grants from the NIH/NINDS (R01NS097775, R21NS106138) and NIH/NIA (R21AG63031). We also thank Tiago Figueiredo for creating artwork used in Figures 1, 3, 4B, 5 and the graphic abstract (www.behance.net/TiagoFigueiredoGD).

References

1. Mink JW, Blumenschine RJ, Adams DB. Ratio of central nervous system to body metabolism in vertebrates: its constancy and functional basis. *American Journal of Physiology* 1981, 241:R203-R212.
2. Tsai PS, Kaufhold J, Blinder P, Friedman B, Drew P, Karten HJ, Lyden PD, Kleinfeld D. Correlations of neuronal and microvascular densities in murine cortex revealed by direct counting and colocalization of cell nuclei and microvessels. *Journal of Neuroscience* 2009, 18:14553-14570.
3. Blinder P, Tsai PS, Kaufhold JP, Knutsen PM, Suhl H, Kleinfeld D. The cortical angiome: An interconnected vascular network with noncolumnar patterns of blood flow. *Nature Neuroscience* 2013, 16:889-897.
4. Harb R, Whiteus C, Freitas C, Grutzendler J. In vivo imaging of cerebral microvascular plasticity from birth to death. *Journal of Cerebral Blood Flow & Metabolism* 2012, 33:146-156.
5. Drew PJ, Shih AY, Driscoll JD, Knutsen PM, Davalos D, Blinder P, Akassoglou K, Tsai PS, Kleinfeld D. Chronic optical access through a polished and reinforced thinned skull. *Nature Methods* 2010, 7:981-984.
6. Cudmore RH, Dougherty SE, Linden DJ. Cerebral vascular structure in the motor cortex of adult mice is stable and is not altered by voluntary exercise. *Journal of Cerebral Blood Flow & Metabolism* 2017, 37:3725-3743.
7. Hamel E. Perivascular nerves and the regulation of cerebrovascular tone. *Journal of Applied Physiology* 2006, 100:1059-1064.
8. Blinder P, Shih AY, Rafie CA, Kleinfeld D. Topological basis for the robust distribution of blood to rodent neocortex. *Proceedings of the National Academy of Sciences USA* 2010, 107:12670-12675.

9. Devor A, Tian P, Nishimura N, Teng IC, Hillman EM, Narayanan SN, Ulbert I, Boas DA, Kleinfeld D, Dale AM. Suppressed neuronal activity and concurrent arteriolar vasoconstriction may explain negative blood oxygenation level-dependent signaling. *Journal of Neuroscience* 2007, 27:4452-4459.
10. Shih AY, Friedman B, Drew PJ, Tsai PS, Lyden PD, Kleinfeld D. Active dilation of penetrating arterioles restores red blood cell flux to penumbral neocortex after focal stroke. *Journal of Cerebral Blood Flow & Metabolism* 2009, 29:738-751.
11. Schaffer CB, Friedman B, Nishimura N, Schroeder LF, Tsai PS, Ebner FF, Lyden PD, Kleinfeld D. Two-photon imaging of cortical surface microvessels reveals a robust redistribution in blood flow after vascular occlusion. *Public Library of Science Biology* 2006, 4:258-270.
12. Nishimura N, Schaffer CB, Friedman B, Lyden PD, Kleinfeld D. Penetrating arterioles are a bottleneck in the perfusion of neocortex. *Proceedings of the National Academy of Sciences USA* 2007, 104:365-370.
13. Jones EG. On the mode of entry of blood vessels into the cerebral cortex. *J Anat* 1970, 106:507-520.
14. Roggendorf W, Cervos-Navarro J. Ultrastructure of arterioles in the cat brain. *Cell Tissue Res* 1977, 178:495-515.
15. Iadecola C. The Neurovascular Unit Coming of Age: A Journey through Neurovascular Coupling in Health and Disease. *Neuron* 2017, 96:17-42.
16. Grant RI, Hartmann DA, Underly RG, Berthiaume A-A, Bhat NR, Shih AY. Organizational hierarchy and structural diversity of microvascular pericytes in adult mouse cortex. *Journal of Cerebral Blood Flow & Metabolism* 2017, 39:411-425.
17. Hartmann DA, Underly RG, Grant RI, Watson AN, Lindner V, Shih AY. Pericyte structure and distribution in the cerebral cortex revealed by high-resolution imaging of transgenic mice. *Neurophotonics* 2015:041402.
18. Nguyen J, Nishimura N, Iadecola C, Schaffer CB. Single venule occlusions induced by photodisruption using femtosecond laser pulses cause decreased blood flow in rat cortex. In: *Society for Neurocience*. San Diego: Society for Neuroscience; 2007.
19. Shih AY, Blinder P, Tsai PS, Friedman B, Stanley G, Lyden PD, Kleinfeld D. The smallest stroke: Occlusion of one penetrating vessel leads to infarction and a cognitive deficit. *Nature Neuroscience* 2013, 16:55-63.
20. O'Herron P, Chhatbar PR, Levy M, Shen Z, Schramm AE, Lu Z, Kara P. Neural correlates of single-vessel haemodynamic responses in vivo. *Nature* 2016, 534:378-382.
21. Weber B, Keller AL, Reichold J, Logothetis NK. The microvascular system of the striate and extrastriate visual cortex of the macaque. *Cerebral Cortex* 2008, 18:2318-2330.
22. Duvernoy HM, Delon S, L. VJ. Cortical blood vessels of the human brain. *Brain Research Bulletin* 1981, 7:519-579.
23. Lauwers F, Cassot F, Lauwers-Cances V, Puwanarajah P, Duvernoy H. Morphometry of the human cerebral cortex microcirculation: General characteristics and space-related profiles. *NeuroImage* 2008, 39:936-948.
24. Hartmann DA, Hyacinth HI, Liao FF, Shih AY. Does pathology of small venules contribute to cerebral microinfarcts and dementia? *Journal of Neurochemistry* 2017, in press.
25. Tata M, Ruhrberg C, Fantin A. Vascularisation of the central nervous system. *Mech Dev* 2015, 138 Pt 1:26-36.
26. Lee HS, Han J, Bai HJ, Kim KW. Brain angiogenesis in developmental and pathological processes: regulation, molecular and cellular communication at the neurovascular interface. *FEBS J* 2009, 276:4622-4635.
27. Risser L, Plouraboue F, Cloetens P, Fonta C. A 3D-investigation shows that angiogenesis in primate cerebral cortex mainly occurs at capillary level. *International Journal of Developmental Neuroscience* 2009, 27:185-196.
28. Bautch VL, James JM. Neurovascular development: The beginning of a beautiful friendship. *Cell Adh Migr* 2009, 3:199-204.

29. Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, Semenza GL. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol* 1996, 16:4604-4613.
30. Levy AP, Levy NS, Wegner S, Goldberg MA. Transcriptional regulation of the rat vascular endothelial growth factor gene by hypoxia. *J Biol Chem* 1995, 270:13333-13340.
31. Maltepe E, Schmidt JV, Baunoch D, Bradfield CA, Simon MC. Abnormal angiogenesis and responses to glucose and oxygen deprivation in mice lacking the protein ARNT. *Nature* 1997, 386:403-407.
32. Ryan HE, Lo J, Johnson RS. HIF-1 alpha is required for solid tumor formation and embryonic vascularization. *EMBO J* 1998, 17:3005-3015.
33. Lange C, Turrero Garcia M, Decimo I, Bifari F, Eelen G, Quaegebeur A, Boon R, Zhao H, Boeckx B, Chang J, et al. Relief of hypoxia by angiogenesis promotes neural stem cell differentiation by targeting glycolysis. *EMBO J* 2016, 35:924-941.
34. Letourneur A, Chen V, Waterman G, Drew PJ. A method for longitudinal, transcranial imaging of blood flow and remodeling of the cerebral vasculature in postnatal mice. *Physiological Reports* 2014, 2:e12238.
35. Wang DB, Blocher NC, Spence ME, Rovainen CM, Woolsey TA. Development and remodeling of cerebral blood vessels and their flow in postnatal mice observed with in vivo videomicroscopy. *Journal of Cerebral Blood Flow & Metabolism* 1992, 12:935-946.
36. Bär T, Miodoński A, Budi Santoso AW. Postnatal development of the vascular pattern in the rat telencephalic pia-arachnoid. A SEM study. *Anatomical embryology* 1986, 174:215-223.
37. Chalothorn D, Faber JE. Formation and maturation of the native cerebral collateral circulation. *J Mol Cell Cardiol* 2010, 49:251-259.
38. Clayton JA, Chalothorn D, Faber JE. Vascular endothelial growth factor-A specifies formation of native collaterals and regulates collateral growth in ischemia. *Circulation Research* 2008, 103:1027-1036.
39. Chalothorn D, Zhang H, Smith JE, Edwards JC, Faber JE. Chloride intracellular channel-4 is a determinant of native collateral formation in skeletal muscle and brain. *Circulation Research* 2009, 2015:89-98.
40. Zhang H, Prabhakar P, Sealock R, Faber JE. Wide genetic variation in the native pial collateral circulation is a major determinant of variation in severity of stroke. *Journal of Cerebral Blood Flow & Metabolism* 2010, 30:923-934.
41. Fehér G, Schulte ML, Weigle CG, Kampine JP, Hudetz AG. Postnatal remodeling of the leptomeningeal vascular network as assessed by intravital fluorescence video-microscopy in the rat. *Brain Research: Developmental Brain Research* 1996, 91.
42. Burri PH, Hlushchuk R, Djonov V. Intussusceptive angiogenesis: its emergence, its characteristics, and its significance. *Developmental Dynamics* 2004, 231:474-488.
43. Ackermann M, Houdek JP, Gibney BC, Ysasi A, Wagner W, Belle J, Schittny JC, Enzmann F, Tsuda A, Mentzer SJ, et al. Sprouting and intussusceptive angiogenesis in postpneumonectomy lung growth: mechanisms of alveolar neovascularization. *Angiogenesis* 2014, 17:541-551.
44. Djonov VG, Galli AB, Burri PH. Intussusceptive arborization contributes to vascular tree formation in the chick chorio-allantoic membrane. *Anat Embryol (Berl)* 2000, 202:347-357.
45. Karthik S, Djukic T, Kim JD, Zuber B, Makanya A, Odriozola A, Hlushchuk R, Filipovic N, Jin SW, Djonov V. Synergistic interaction of sprouting and intussusceptive angiogenesis during zebrafish caudal vein plexus development. *Scientific Reports* 2018.
46. Skalak TC, Price RJ. The role of mechanical stresses in microvascular remodeling. *Microcirculation* 1996, 3:143-165.
47. Ranade SS, Qiu Z, Woo SH, Hur SS, Murthy SE, Cahalan SM, Xu J, Mathur J, Bandell M, Coste B, et al. Piezo1, a mechanically activated ion channel, is required for vascular development in mice. *Proc Natl Acad Sci U S A* 2014, 111:10347-10352.
48. Chen Q, Jiang L, Li C, Hu D, Bu JW, Cai D, Du JL. Haemodynamics-Driven Developmental Pruning of Brain Vasculature in Zebrafish. *Plos Biology* 2012, 10:e1001374-e1001374.
49. Rowan RA, Maxwell DS. Patterns of vascular sprouting in the postnatal development of the cerebral cortex of the rat. *Am J Anat* 1981, 160:247-255.

50. Hall CN, Reynell C, Gesslein B, Hamilton NB, Mishra A, Sutherland BA, O'Farrell FM, Buchan AM, Lauritzen M, Attwell D. Capillary pericytes regulate cerebral blood flow in health and disease. *Nature* 2014, 508:55-60.
51. Rungta RL, Chaigneau E, Osmanski BF, Charpak S. Vascular Compartmentalization of Functional Hyperemia from the Synapse to the Pia. *Neuron* 2018, 99:362-375.
52. Norman MG, O'Kusky JR. The growth and development of microvasculature in human cerebral cortex. *J Neuropathol Exp Neurol* 1986, 45:222-232.
53. Keep RF, Jones HC. Cortical microvessels during brain development: a morphometric study in the rat. *Microvasc Res* 1990, 40:412-426.
54. Walchli T, Mateos JM, Weinman O, Babic D, Regli L, Hoerstrup SP, Gerhardt H, Schwab ME, Vogel J. Quantitative assessment of angiogenesis, perfused blood vessels and endothelial tip cells in the postnatal mouse brain. *Nat Protoc* 2015, 10:53-74.
55. Zeller K, Vogel J, Kuschinsky W. Postnatal distribution of Glut1 glucose transporter and relative capillary density in blood-brain barrier structures and circumventricular organs during development. *Brain Res Dev Brain Res* 1996, 91:200-208.
56. Bussmann J, Wolfe SA, Siekmann AF. Arterial-venous network formation during brain vascularization involves hemodynamic regulation of chemokine signaling. *Development* 2011, 138:1717-1726.
57. Red-Horse K, Ueno H, Weissman IL, Krasnow MA. Coronary arteries form by developmental reprogramming of venous cells. *Nature* 2010, 464:549-553.
58. Xu C, Hasan SS, Schmidt I, Rocha SF, Pitulescu ME, Bussmann J, Meyen D, Raz E, Adams RH, Siekmann AF. Arteries are formed by vein-derived endothelial tip cells. *Nature Communications* 2014, 5:5758.
59. Red-Horse K, Siekmann AF. Veins and Arteries Build Hierarchical Branching Patterns Differently: Bottom-Up versus Top-Down. *Bioessays* 2019, 41:e1800198.
60. Semple BD, Blomgren K, Gimlin K, Ferriero DM, Noble-Haeusslein LJ. Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog Neurobiol* 2013, 106-107:1-16.
61. Cardoso FL, Brites D, Brito MA. Looking at the blood-brain barrier: molecular anatomy and possible investigation approaches. *Brain Res Rev* 2010, 64:328-363.
62. Saunders NR, Liddelow SA, Dziegielewska KM. Barrier mechanisms in the developing brain. *Front Pharmacol* 2012, 3:46.
63. Obermeier B, Daneman R, Ransohoff RM. Development, maintenance and disruption of the blood-brain barrier. *Nat Med* 2013, 19:1584-1596.
64. Yoshida Y, Yamada M, Wakabayashi K, Ikuta F. Endothelial fenestrae in the rat fetal cerebrum. *Brain Res Dev Brain Res* 1988, 44:211-219.
65. Xu J, Ling EA. Studies of the ultrastructure and permeability of the blood-brain barrier in the developing corpus callosum in postnatal rat brain using electron dense tracers. *J Anat* 1994, 184 (Pt 2):227-237.
66. Daneman R, Zhou L, Kebede AA, Barres BA. Pericytes are required for blood-brain barrier integrity during embryogenesis. *Nature* 2010, 468:562-566.
67. Kniesel U, Risau W, Wolburg H. Development of blood-brain barrier tight junctions in the rat cortex. *Brain Res Dev Brain Res* 1996, 96:229-240.
68. Butt AM, Jones HC, Abbott NJ. Electrical resistance across the blood-brain barrier in anaesthetized rats: a developmental study. *J Physiol* 1990, 429:47-62.
69. Ek CJ, Wong A, Liddelow SA, Johansson PA, Dziegielewska KM, Saunders NR. Efflux mechanisms at the developing brain barriers: ABC-transporters in the fetal and postnatal rat. *Toxicology Letters* 2010, 197:51-59.
70. Harik SI, Hall AK, Richey P, Andersson L, Lundahl P, Perry G. Ontogeny of the erythroid/HepG2-type glucose transporter (GLUT-1) in the rat nervous system. *Brain Res Dev Brain Res* 1993, 72:41-49.
71. Sweeney MD, Ayyadurai S, Zlokovic BV. Pericytes of the neurovascular unit: key functions and signaling pathways. *Nature Neuroscience* 2016, 19:771-783.
72. Armulik A, Abramsson A, Betsholtz C. Endothelial/pericyte interactions. *Circulation Research* 2005, 97:512-523.

73. Fujimoto K. Pericyte-endothelial gap junctions in developing rat cerebral capillaries: a fine structural study. *Anatomical record* 1995, 242:562-565.
74. Hirschi KK, Burt JM, Hirschi KD, Dai C. Gap junction communication mediates transforming growth factor-beta activation and endothelial-induced mural cell differentiation. *Circulation research* 2003, 93:429-437.
75. Jung B, Arnold TD, Raschperger E, Gaengel K, Betsholtz C. Visualization of vascular mural cells in developing brain using genetically labeled transgenic reporter mice. *Journal of Cerebral Blood Flow & Metabolism* 2017, Epub ahead of print.
76. Orlidge A, D'Amore PA. Inhibition of capillary endothelial cell growth by pericytes and smooth muscle cells. *Journal of Cell Biology* 1987, 105:1455-1462.
77. Durham JT, Surks HK, Dulmovits BM, Herman IM. Pericyte contractility controls endothelial cell cycle progression and sprouting: insights into angiogenic switch mechanics. *American journal of physiology: Cell physiology* 2014, 307:C878-C892.
78. Eilken HM, Diéguez-Hurtado R, Schmidt I, Nakayama M, Jeong HW, Arf H, Adams S, Ferrara N, Adams RH. Pericytes regulate VEGF-induced endothelial sprouting through VEGFR1. *Nature Communications* 2017, 8:1574.
79. Gerhardt H, Golding M, Fruttiger M, Ruhrberg C, Lundkvist A, Abramsson A, Jeltsch M, Mitchell C, Alitalo K, Shima D, et al. VEGF guides angiogenic sprouting utilizing endothelial tip cell filopodia. *Journal of Cell Biology* 2003, 161:1163-1177.
80. Hellström M, Gerhardt H, Kalén M, Li X, Eriksson U, Wolburg H, Betsholtz C. Lack of pericytes leads to endothelial hyperplasia and abnormal vascular morphogenesis. *The journal of cell biology* 2001, 153:543-553.
81. Lindblom P, Gerhardt H, Liebner S, Abramsson A, Enge M, Hellstrom M, Backstrom G, Fredriksson S, Landegren U, Nystrom HC, et al. Endothelial PDGF-B retention is required for proper investment of pericytes in the microvessel wall. *Genes and Development* 2003, 17:1835-1840.
82. Lindahl P, Johansson BR, Leve´en P, Betsholtz C. Pericyte loss and microaneurysm formation in PDGF-B-deficient mice. *Science* 1997, 277:242-245.
83. Armulik A, Genové G, Mäe M, Nisancioglu MH, Wallgard E, Niaudet C, He L, Norlin J, Lindblom P, Strittmatter K, et al. Pericytes regulate the blood-brain barrier. *Nature* 2010, 468:557-561.
84. Benjamin LE, Hemo I, Keshet E. A plasticity window for blood vessel remodelling is defined by pericyte coverage of the preformed endothelial network and is regulated by PDGF-B and VEGF. *Development* 1998, 125:1591-1598.
85. Amselgruber WM, Schafer M, Sinowatz F. Angiogenesis in the bovine corpus luteum: an immunocytochemical and ultrastructural study. *Anatomia, histologia, embryologia* 1999, 28:157-166.
86. Reynolds LP, Grazul-Bilska AT, Redmer DA. Angiogenesis in the corpus luteum. *Endocrine* 2000, 12:1-9.
87. Payne LB, Zhao H, James CC, Darden J, McGuire D, Taylor S, Smyth JW, Chappell JC. The Pericyte Microenvironment during Vascular Development. *Microcirculation* 2019, Epub ahead of print.
88. Hellström M, Kalén M, Lindahl P, Abramsson A, Betsholtz C. Role of PDGF-B and PDGFR-beta in recruitment of vascular smooth muscle cells and pericytes during embryonic blood vessel formation in the mouse. *Development* 1999, 126:3047-3055.
89. Soriano P. Abnormal kidney development and hematological disorders in PDGF beta-receptor mutant mice. *Genes and Development* 1994, 8:1888-1896.
90. Ben-Zvi A, Lacoste B, Kur E, Andreone BJ, Mayshar Y, Yan H, Gu C. Mfsd2a is critical for the formation and function of the blood-brain barrier. *Nature* 2014, 509:507-511.
91. Chen J, Luo Y, Hui H, Cai T, Huang H, Yang F, Feng J, Zhang J, Yan X. CD146 coordinates brain endothelial cell-pericyte communication for blood-brain barrier development. *Proc Natl Acad Sci U S A* 2017, 114:E7622-E7631.
92. Donahue S, Pappas GD. The fine structure of capillaries in the cerebral cortex of the rat at various stages of development. *Am J Anat* 1961, 108:331-347.

93. Lunde LK, Camassa LM, Hoddevik EH, Khan FH, Ottersen OP, Boldt HB, Amiry-Moghaddam M. Postnatal development of the molecular complex underlying astrocyte polarization. *Brain Struct Funct* 2015, 220:2087-2101.
94. Menezes MJ, McClenahan FK, Leiton CV, Aranmolate A, Shan X, Colognato H. The extracellular matrix protein laminin alpha2 regulates the maturation and function of the blood-brain barrier. *J Neurosci* 2014, 34:15260-15280.
95. Yao Y, Chen ZL, Norris EH, Strickland S. Astrocytic laminin regulates pericyte differentiation and maintains blood brain barrier integrity. *Nat Commun* 2014, 5:3413.
96. Thyboll J, Kortesmaa J, Cao R, Soininen R, Wang L, Iivanainen A, Sorokin L, Risling M, Cao Y, Tryggvason K. Deletion of the laminin alpha4 chain leads to impaired microvessel maturation. *Mol Cell Biol* 2002, 22:1194-1202.
97. Gould DB, Phalan FC, Breedveld GJ, van Mil SE, Smith RS, Schimenti JC, Aguglia U, van der Knaap MS, Heutink P, John SW. Mutations in Col4a1 cause perinatal cerebral hemorrhage and porencephaly. *Science* 2005, 308:1167-1171.
98. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. *Nat Rev Neurosci* 2006, 7:41-53.
99. Marshall CA, Suzuki SO, Goldman JE. Gliogenic and neurogenic progenitors of the subventricular zone: who are they, where did they come from, and where are they going? *Glia* 2003, 43:52-61.
100. Ma S, Kwon HJ, Huang Z. A functional requirement for astroglia in promoting blood vessel development in the early postnatal brain. *PLoS One* 2012, 7:e48001.
101. Ge WP, Miyawaki A, Gage FH, Jan YN, Jan LY. Local generation of glia is a major astrocyte source in postnatal cortex. *Nature* 2012, 484:376-380.
102. Seregi A, Keller M, Hertting G. Are cerebral prostanoids of astroglial origin? Studies on the prostanoid forming system in developing rat brain and primary cultures of rat astrocytes. *Brain Res* 1987, 404:113-120.
103. Stichel CC, Muller CM, Zilles K. Distribution of glial fibrillary acidic protein and vimentin immunoreactivity during rat visual cortex development. *J Neurocytol* 1991, 20:97-108.
104. Agre P, King LS, Yasui M, Guggino WB, Ottersen OP, Fujiyoshi Y, Engel A, Nielsen S. Aquaporin water channels--from atomic structure to clinical medicine. *Journal of Physiology* 2002, 542:3-16.
105. Iliff JJ, Wang M, Liao Y, Plogg BA, Peng W, Gundersen GA, Benveniste H, Vates GE, Deane R, Goldman SA, et al. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Science Translational Medicine* 2012, 4:147ra111.
106. Bozoyan L, Khlghatyan J, Saghatelyan A. Astrocytes control the development of the migration-promoting vasculature scaffold in the postnatal brain via VEGF signaling. *J Neurosci* 2012, 32:1687-1704.
107. Ogunshola OO, Stewart WB, Mihalcik V, Solli T, Madri JA, Ment LR. Neuronal VEGF expression correlates with angiogenesis in postnatal developing rat brain. *Brain Res Dev Brain Res* 2000, 119:139-153.
108. Nayak D, Roth TL, McGavern DB. Microglia development and function. *Annual Review of Immunology* 2014, 32:367-402.
109. Tammela T, Zarkada G, Nurmi H, Jakobsson L, Heinolainen K, Tvorogov D, Zheng W, Franco CA, Murtomäki A, Aranda E, et al. VEGFR-3 controls tip to stalk conversion at vessel fusion sites by reinforcing Notch signalling. *Nature Cell Biology* 2011, 13:1202-1213.
110. Fantin A, Vieira JM, Gestri G, Denti L, Schwarz Q, Prykhozhij S, Peri F, Wilson SW, Ruhrberg C. Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. *Blood* 2010, 116:829-840.
111. Rymo SF, Gerhardt H, Wolfhagen Sand F, Lang R, Uv A, Betsholtz C. A two-way communication between microglial cells and angiogenic sprouts regulates angiogenesis in aortic ring cultures. *PLoS One* 2011, 6:e15846.
112. Arcuri C, Mecca C, Bianchi R, Giambanco I, Donato R. The Pathophysiological Role of Microglia in Dynamic Surveillance, Phagocytosis and Structural Remodeling of the Developing CNS. *Front Mol Neurosci* 2017, 10:191.

113. Zusso M, Methot L, Lo R, Greenhalgh AD, David S, Stifani S. Regulation of postnatal forebrain amoeboid microglial cell proliferation and development by the transcription factor Runx1. *J Neurosci* 2012, 32:11285-11298.
114. Lacoste B, Comin CH, Ben-Zvi A, Kaeser PS, Xu X, Costa Lda F, Gu C. Sensory-related neural activity regulates the structure of vascular networks in the cerebral cortex. *Neuron* 2014, 83:1117-1130.
115. Whiteus C, Freitas C, Grutzendler J. Perturbed neural activity disrupts cerebral angiogenesis during a postnatal critical period. *Nature* 2014, 505:407-411.
116. Adams MD, Winder AT, Blinder P, Drew PJ. The pial vasculature of the mouse develops according to a sensory-independent program. *Scientific Reports* 2018, 8:9860.
117. Carmeliet P, Tessier-Lavigne M. Common mechanisms of nerve and blood vessel wiring. *Nature* 2005, 436:193-200.
118. Wälchli T, Pernet V, Weinmann O, Shiu JY, Guzik-Kornacka A, Decrey G, Yüksel D, Schneider H, Vogel J, Ingber DE, et al. Nogo-A is a negative regulator of CNS angiogenesis. *Proceedings of the National Academy of Sciences* 2013, 110:E1943-1952.
119. Walchli T, Ulmann-Schuler A, Hintermuller C, Meyer E, Stampanoni M, Carmeliet P, Emmert MY, Bozinov O, Regli L, Schwab ME, et al. Nogo-A regulates vascular network architecture in the postnatal brain. *J Cereb Blood Flow Metab* 2017, 37:614-631.
120. Inestrosa NC, Varela-Nallar L. Wnt signalling in neuronal differentiation and development. *Cell and Tissue Research* 2015, 359:215-223.
121. Liebner S, Corada M, Bangsow T, Babbage J, Taddei A, Czupalla CJ, Reis M, Felici A, Wolburg H, Fruttiger M, et al. Wnt/beta-catenin signaling controls development of the blood-brain barrier. *Journal of Cell Biology* 2008, 183:409-417.
122. Zhou Y, Wang Y, Tischfield M, Williams J, Smallwood PM, Rattner A, Taketo MM, Nathans J. Canonical WNT signaling components in vascular development and barrier formation. *Journal of Clinical Investigation* 2014, 124:3825-3846.
123. Daneman R, Agalliu D, Zhou L, Kuhnert F, Kuo CJ, Barres BA. Wnt/beta-catenin signaling is required for CNS, but not non-CNS, angiogenesis. *Proc Natl Acad Sci U S A* 2009, 106.
124. Kim BW, Choi M, Kim YS, Park H, Lee HR, Yun CO, Kim EJ, Choi JS, Kim S, Rhim H, et al. Vascular endothelial growth factor (VEGF) signaling regulates hippocampal neurons by elevation of intracellular calcium and activation of calcium/calmodulin protein kinase II and mammalian target of rapamycin. *Cellular Signalling* 2008, 20:714-725.
125. Tran CHT, Peringod G, Gordon GR. Astrocytes Integrate Behavioral State and Vascular Signals during Functional Hyperemia. *Neuron* 2018, 100:1133-1148.
126. Kozberg MG, Ma Y, Shaik MA, Kim SH, Hillman EM. Rapid Postnatal Expansion of Neural Networks Occurs in an Environment of Altered Neurovascular and Neurometabolic Coupling. *Journal of Neuroscience* 2016, 36:6704-6717.
127. Zehendner CM, Tsohataridis S, Luhmann HJ, Yang JW. Developmental switch in neurovascular coupling in the immature rodent barrel cortex. *PLoS One* 2013, 8:e80749.
128. Esipova TV, Barrett MJP, Erlebach E, Masunov AE, Weber B, Vinogradov SA. Oxyphor 2P: A high-performance probe for deep-tissue longitudinal oxygen imaging. *Cell Metabolism* 2019, S1550-4131:30759-30759.
129. Longden TA, Dabertrand F, Koide M, Gonzales AL, Tykocki NR, Brayden JE, Hill-Eubanks D, Nelson MT. Capillary K+-sensing initiates retrograde hyperpolarization to increase local cerebral blood flow. *Nature Neuroscience* 2017, 20:717-726.
130. Harris JJ, Reynell C, Attwell D. The physiology of developmental changes in BOLD functional imaging signals. *Developmental cognitive neuroscience* 2011, 1:199-216.
131. Saunders NR, Dreifuss JJ, Dziegielewska KM, Johansson PA, Habgood MD, Mollgard K, Bauer HC. The rights and wrongs of blood-brain barrier permeability studies: a walk through 100 years of history. *Front Neurosci* 2014, 8:404.
132. Hartmann DA, Underly RG, Watson AN, Shih AY. A murine toolbox for imaging the neurovascular unit. *Microcirculation* 2015, 22:168-182.
133. Knowland D, Arac A, Sekiguchi KJ, Hsu M, Lutz SE, Perrino J, Steinberg GK, Barres BA, Nimmerjahn A, Agalliu D. Stepwise recruitment of transcellular and paracellular pathways underlies blood-brain barrier breakdown in stroke. *Neuron* 2014, 82:603-617.

134. Berthiaume AA, Grant RI, McDowell KP, Underly RG, Hartmann DA, Levy M, Bhat NR, Shih AY. Dynamic Remodeling of Pericytes In Vivo Maintains Capillary Coverage in the Adult Mouse Brain. *Cell Reports* 2018, 22:8-16.
135. Hill RA, Damisah EC, Chen F, Kwan AC, Grutzendler J. Targeted two-photon chemical apoptotic ablation of defined cell types in vivo. *Nature Communications* 2017, 8:15837.
136. Lou N, Takano T, Pei Y, Xavier AL, Goldman SA, Nedergaard M. Purinergic receptor P2RY12-dependent microglial closure of the injured blood-brain barrier. *Proceedings of the National Academy of Sciences* 2016, 113:1074-1079.
137. Kubotera H, Ikeshima-Kataoka H, Hatashita Y, Allegra Mascaro AL, Pavone FS, Inoue T. Astrocytic endfeet re-cover blood vessels after removal by laser ablation. *Scientific Reports* 2019, 9:1263.