Barrier Mechanisms in Neonatal Stroke

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1 Introduction

The concept that the fetal and newborn blood–brain barrier (BBB) is "immature," implying that the BBB is not formed, leaky or even absent has dominated the field for a long time. However, such a simplistic idea has been challenged in multiple species, demonstrating in naïve brain that many functional barrier elements are already in place in fetal brain and, together with placenta, are effectively preserving brain metabolism and limiting brain edema that is associated with entrance of peripheral components. It has also become apparent that BBB integrity does not linearly change during postnatal brain maturation and that infection, inflammation, brain trauma, or stroke during postnatal development exert age-specific "susceptibility signatures" of BBB disturbances, processes that we will discuss in this chapter.

2 Development of the BBB and Other Brain Barriers

There are multiple brain barriers, including the BBB, blood–CSF barrier across choroid plexus (BCSFB) and the pia-arachnoid barrier [1]. There is also a barrier only present in the embryo, the CSF-ventricular zone interface lining the ventricular system. Cumulatively, these barriers limit exchange of solutes and various molecules between compartments as well as establish efflux/influx mechanisms that control ionic, nutrient, glucose, and other gradients. In the adult, the BBB exists at all levels

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of the vasculature within the CNS, including the penetrating arteries and arterioles, the capillary bed, the postcapillary venules and the draining venules and veins [2, 3].

The development of the BBB is believed to be a multi-step process. Both the development and maintenance of the BBB are governed by intracellular endothelial mechanisms in conjunction with multiple extracellular mechanisms that link endothelial cells to the extracellular matrix (ECM), thereby supporting their proper positioning and functionality. Astrocytes, pericytes, and ECM components provide both structural and functional support to the BBB. BBB development starts mid-gestation and temporally occurs after establishment of a local microglial pool in the brain and before pericytes and astrocytes take their respective positions surrounding the vessels [4–7]. Thus, endothelial sprouts are guided by microglia/macrophages in fetal brain, largely via VEGF-dependent mechanisms [8]. Lack of or reduced number of PU.1 or SDF-1 microglia/macrophage leads to distorted vasculature and embryonic lethality [8]. Mice deficient in VEGF or VEGF receptor 2 (Flk-1−/−) fail vessel formation, which leads to embryonic lethality [9]. Lack of a single VEGF allele also leads to embryonic lethality despite partial blood vessel development [10]. Several VEGF-independent mechanisms that regulate embryonic angiogenesis and BBB formation were identified. Integrin/TGFβ signaling, for example, suppresses vascular branching/sprouting and prevents germinal matrix hemorrhage [11]. Excessive vascular sprouting, in turn, underlies cerebral hemorrhage in mice lacking αVβ8-TGFβ signaling in the brain [11].

Wnt-β-catenin signaling has been shown to be important in brain angiogenesis and BBB formation, but not in barrier formation in peripheral vessels [12]. The canonical Wnt pathway was demonstrated to play a key role in BBB formation via induction of the BBB genes, such as the nutrient transporter Glut-1 [13], while β-catenin was shown to regulate angiogenesis in the developing brain, in part by inducing expression of the death receptors Dr6 and Troy. Endothelial β-catenin was also shown to play a key role in embryonic and postnatal BBB maturation by regulating the formation of tight junctions (TJs) [14].

Pericytes contribute to BBB development and function in a number of ways, primarily by regulating capillary diameter and CBF and controlling BBB integrity [15]. They also contribute to vessel stability, possibly by affecting both TJs and transcytosis routes [5]. However, the existence and the role of multiple phenotypes are still poorly understood. Pericyte coverage gradually increases starting late gestation and continues through postnatal ages [4, 5]. Astrocytes are another cell population central to the maturing BBB in several ways [15]. Perivascular astrocytic end-feet at the abluminal side of brain vessels are polarized structures that are enriched with water channel aquaporin-4 (AQP-4) and several ion pumps. Astrocytes modulate TJs and are also an essential part of antioxidative mechanisms in the brain, recycling GSSG, which, as we discuss later, is of particular importance after stroke and other diseases. They also provide nutrition for neurons and neurotransmitter clearance and recycling.

Cells of the BBB/neurovascular unit do not exist in isolation. The ECM plays a key role both in the development and maintenance of the BBB under normal and disease conditions. Mutant ECM proteins, such as collagen-IVα1 isoform and, to a

lesser extent, α2 isoform adversely affect endothelial communications with the matrix, leading to mortality or hemorrhagic transformation [16]. During embryonic angiogenesis, TGFβ signaling suppresses vascular branching/sprouting and prevents hemorrhages [11]. Loss of TGFbR2/SMAD3 enhances vascular sprouting, branching, and hemorrhages during embryonic brain development but these effects are independent of BBB dysfunction [11]. Multiple lines of evidence show an important role for endothelial TGFβ/TGFbR2 signaling for vascular integrity during embryonic brain development [17]. Endothelial specific depletion of Alk5 leads to a similar cerebral hemorrhagic phenotype and embryonic lethality [17, 18], consistent with a requirement for both TGFbR1 and TGFbR2 proteins as a heteromeric receptor complex in endothelial cells. Mutations in other members of the TGFβ family, endoglin, (ENG) and activin receptor-like kinase 1(ALK1), were demonstrated as major contributors to intracerebral hemorrhages of brain arteriovenous malformations in children [19].

Although individual neurovascular unit components form at different paces during gestation, likely contributing to age-dependent susceptibility to infection, inflammatory stress and stroke, the fetal brain has surprisingly effective influx/efflux mechanisms. Efflux mechanisms are active across brain barriers in the developing brain. Multiple transcription factors involved in metabolic- or cell stress-induced detoxifying enzymes and transporters are expressed throughout embryonic brain development. For example, several proteins of the ATP-binding cassette (ABC) family, which reduce the entry of compounds from blood into the brain by active efflux, are already expressed in cerebral blood vessels and in choroid plexus in the fetal and neonatal rat brain [20]. Among ABC-transporters, Pgp/ABCB1 expression is lower in the fetus, whereas MRP1/ABCC1, MRP4/ABCC4, and BCRP/ABCG2 are expressed at comparable levels in fetal and adult brains. Data on amino acid transporters in endothelial cells in the developing brain are sparse but available data indicate that the function of several amino acid and inward transporters is higher than in the adult. For example, transporter mannose 6-phosphate receptor is expressed in developing rodent and rabbit brain but is progressively lost with age [21].

The BCSFB plays a prominent role in fetal and newborn brain. Ion pumps are active in the BCSFB. Essential nutrients and other molecules important for growth and differentiation of the brain enter the brain via uptake from the CSF. A recent transcriptome approach allowed insights into transport mechanisms at the developing mouse blood–CSF interface [22].

3 Blood–Brain Barrier and Adult Stroke

There is ample evidence of BBB disruption after acute stroke. Several recent reviews comprehensively discussed various mechanistic aspects of neurovascular state in adult stroke, including contribution of the ECM, its communication with endothelial cells, interaction between individual cell types, including endothelial cells,

astrocytes and pericytes, as well as various individual intracellular and intercellular mechanisms of adult stroke [23–25]; these aspects will not be discussed here. It is, however, important to mention that several long-term concepts in the stroke field are currently being revisited and reconsidered. Three most notable examples of revisited questions are the relative role of the paracellular compared to the transcellular route as paths for leukocyte trafficking [26], the ability of neutrophils to reach injured parenchyma and signal from within, rather than signal from the perivascular space [27], and beneficial, rather than purely toxic role of inflammation and microglial involvement in particular [25].

4 Maturation-Dependent Susceptibility of the BBB to Inflammation

Neuroinflammation is a characteristic feature of stroke progression in the adult and is a major contributor to brain injury [25]. Parenchymal, perivascular, and peripheral circulating cells independently and in concert contribute to stroke-induced production of inflammatory mediators and neuroinflammation [25] and activation of endothelial cells [28, 29]. Perivascular macrophages, microglial cells and mast cells, which are strategically positioned around brain vessels, further contribute to BBB disruption by induction and release of signaling molecules and proteases that promote vascular permeability.

A comparative study of the effects of recombinant interleukin-1 beta (IL-1β) injected into the brain parenchyma of adult, juvenile and newborn rats showed that while in the adult intense meningitis and disruption of the BCSFB, but not leukocyte recruitment, occur within 4 h, in the juvenile rats, a 500-fold lower IL-1β dose causes a large BBB leakage and neutrophil recruitment into the tissue around the injection site within the same time frame [30]. In the newborn rat (2-h-old rat), a similar low IL-1β dose gives rise to an increase in permeability in the meninges, but no increase in BBB permeability. The IL-1β-induced increases in vessel permeability in the meninges, parenchyma, and choroid plexus are neutrophil-dependent [30]. These findings demonstrate clear differences in the age-specific response of the BBB and barrier-type specific responsiveness to IL-1β. Another study utilizing prolonged systemic inflammation in early development in the rat showed induction of long-term changes in BBB permeability that ultimately led to white matter lesions [31]. LPS given at postnatal day 0 (P0)-P8 induced BBB permeability to small (sucrose and inulin) and large (protein) molecules during and immediately after the inflammatory response. Permeability to protein was increased only transiently whereas increased permeability to ^{14}C-sucrose and ^{14}C-inulin was more persistant, demonstrating size selective increases in BBB in the early postnatal period in response to prolonged systemic inflammation [31].

5 Models of Neonatal Focal Stroke and Hypoxia–Ischemia

While models of stroke in adult rodents have existed for several decades, models of perinatal focal arterial stroke have been established relatively recently. These models include permanent MCA ligation [32] and suture tMCAO in P7–P10 rats [33, 34] and P9 mice [35]. Varying the duration of tMCAO has allowed for induction of injuries of different severity [35] and MRI-based confirmation of recirculation following suture retraction in the tMCAO model [36] has allowed for studies of reperfusion, which frequently occurs in arterial stroke in term infants. The vast majority of experimental data related to ischemia-induced brain injury in neonates this far have been obtained using a hypoxia–ischemia (HI) model in P7–P9 rats and mice, a group of models that more closely mimic hypoxic-ischemic encephalopathy in term human babies than focal arterial stroke [37–40]. We and others recently reviewed the most important pathophysiological findings in the neonatal HI models [39, 41–43]. Thus, we will limit our discussion to available data on stroke and HI-induced changes in BBB permeability in the neonate [44, 45] and effects of neurovascular changes for injury and repair [46–48].

6 BBB Integrity After Neonatal Focal Stroke and Hypoxia–Ischemia

The data on BBB function after ischemia-related brain injury in the neonate are rather scant. Considering that endothelial TJs are already present during early embryonic development [49], specific BBB transporters are present in the brain endothelium during mid-gestation, with no fenestrations are observed at birth [50], that astrocytes and pericytes cover brain vessels in postnatal brain [5], at least to some extent, we examined whether neonatal stroke disrupts functional integrity of the BBB. To test if compared to adult stroke, the assumed more extensive magnitude of BBB disruption after stroke occurs in neonates, we determined BBB leakage in adult and P7 rats subjected to a 3-h tMCAO. We administered Evans Blue or fluorescent intravascular trace 3, 70 kDa dextran or TRITC-albumin at 24 after reperfusion and observed significant extravasation of Evans Blue, 70kD and TRITC-albumin in injured regions of adult rats. Extravasation of these molecules was significantly lower following acute tMCAO in P7 rats, showing a strikingly better-preserved BBB integrity in injured neonatal brains [44]. We also used Gd-DTPA-enhanced T1W to further test BBB permeability in neonates and showed that contrast enhancement in injured regions was negligible at 24 h, within 10 % [44]. Fig. 1a demonstrates increased leakage of 70 kDa dextran in injured adult brain and only minor leakage in injured neonatal rats. In contrast to tMCAO, permanent MCAO in P7 rats resulted in rapid BBB disruption and leukocyte extravasation [51], suggesting that

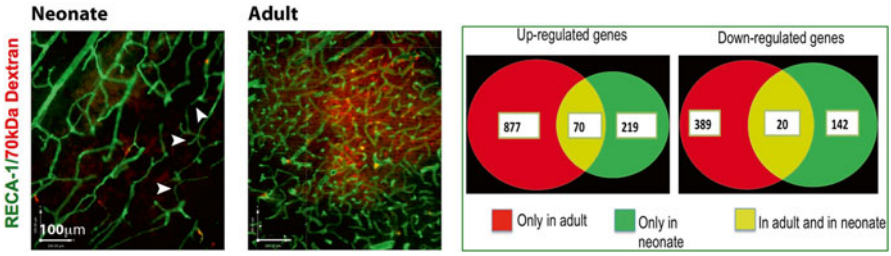

Fig. 1 Acute focal arterial stroke differentially affects BBB integrity in the adult and neonate. (**a**) Representative examples of the spatial distribution of intravenously administered TRITC-conjugated 70 kDa dextran in injured neonatal (*left*) and adult (*right*) brain regions 24 h after a 3-h tMCAO. (**b**) Differential effect of acute focal stroke on gene expression in endothelial cells of adult and neonatal rats. The endothelial transcriptome data were obtained in endothelial cells purified by sequential negative and positive selection from injured and contralateral tissue of adults and neonates 24 h after reperfusion. Shown are genes with >2-fold change in expression compared to that in contralateral hemisphere

persistent lack of cerebral microcirculation contributes to BBB collapse. Two studies that utilized tMCAO model in P10 rats observed increased BBB permeability during a sub-chronic injury phase, 72 h following tMCAO [52, 53]. The affected region was larger in spontaneously hypertensive pups [52] than in normotensive pups of the same age [53]. A recent study studied changes to the BBB in conjunction with CBF in a neonatal mouse HI model [45]. HI increased BBB permeability to small and large molecules, peaking at 6 h after the insult followed by normalization by 24 h. The opening of the BBB was associated with changes to BBB protein expression. Brain pathology was closely related to reductions in CBF during the hypoxia as well as the areas with compromised BBB [45]. Taken together, these data demonstrate a limited extent of BBB opening after ischemia-related injury in neonatal brain.

While the phenomenon of a better-preserved BBB integrity in neonatal stroke has been established, the underlying mechanisms are poorly understood. Comparative endothelial transcriptome data obtained in adult and neonatal rats subjected to tMCAO provided some mechanistic insight of age differences in BBB susceptibility to stroke. It appeared that, strikingly, the patterns of up- and down-regulated endothelial genes are largely non-overlapping between the two ages (Fig. 1b) [44]. Transcript levels of several adhesion molecules and ECM components were differentially affected by injury in immature and adult brain, including E-selectin and P-selectin. Gene expression of Mmp-9 was significantly upregulated in injured adults and, while high transcript levels of collagen type IV α1 (Col4a1) and Col4a2 remained unaltered in neonates, a significant increase of these two genes was evident in injured adult rats. Interestingly, transcripts of angiogenic regulators Vegfr-2 and Angpt2 were increased after stroke in adults but not in neonates [44]. Comparisons of protein expression of occludin, claudin-5, and ZO-1 between

adult and neonatal rats after tMCAO showed better-preserved expression in neonates than in adults [44]. Endothelial-ECM interaction via β1 integrins regulates the expression of claudin-5 and BBB tightness whereas other ECM proteins, like galectin-3, mediate integrin-induced stabilization of focal adhesions and activate cytokine receptors to enhance actions of growth factors [54]. Laminin degradation occurs after focal stroke in adults and causes detachment of astrocytic end-feet, disrupts BBB, and induces ICH [55], while in neonates, expression of this ECM protein is not reduced acutely [44]. The role of other ECM proteins in injured neonates is less studied but the opposite effects of galectin-3 in adult stroke and HI have been demonstrated [56, 57]. Together, these data suggest that intrinsic developmental differences in basement membrane and ECM formation may contribute to a better-preserved BBB integrity after acute neonatal arterial stroke.

Astrocytes and pericytes may contribute differently to neonatal and adult stroke. In adults, astrocyte swelling, mediated by water channels (aquaporin-4, AQP-4) present in astrocyte end-feet, is one of the mechanisms involved in the generation of cytotoxic edema during the early phase after stroke [58]. Retraction of astrocyte end-feet from the parenchymal basal lamina of vessels and cellular redistribution and/or degradation of AQP-4 at later stroke stages are involved in BBB leakage and formation of vasogenic edema [59]. While coverage of vessels with astrocytic end-feet begins before birth, it continues to increase during the first postnatal week [5]. However, these particular mechanisms have not yet been specifically characterized after neonatal stroke.

7 Leukocyte and BBB Integrity After Neonatal Stroke

Interactions between activated endothelial cells and peripheral leukocytes contribute to BBB disruption after stroke. Compared to the adult, neutrophil infiltration in neonates is negligible after tMCAO [44]. Following HI, neutrophil infiltration was shown limited [60] or brief [61]. Neuropenia was shown beneficial when induced before, not after, HI [60]. The exact mechanisms that restrict neutrophil infiltration in the injured neonatal brain are not well understood. The different or uncoordinated patterns of expression of adhesion molecules and MMPs between neonatal and adult rodents may be of critical importance. Particulars of chemokine gradients between the brain and the blood may affect neutrophil extravasation in the neonate [44]. For example, cytokine-induced neutrophil chemoattractant (CINC-1) is a major chemoattractant for neutrophils. Neutralization of peripheral CINC-1 following tMCAO in the adult halted neutrophil transmigration, reduced brain edema and protected [62] but CINC-1 neutralization in neonatal rats subjected to tMCAO promoted neutrophil infiltration. Alterations in the blood–brain gradient of CINC-1 not only led to increased presence of neutrophils in the brain parenchyma but also increased BBB permeability and injury volume [44], suggesting that neutrophils mediate BBB damage in association with transmigration.

Compared to adult stroke, infiltration of circulating monocytes across the BBB was also low during the acute phase after stroke in the neonate [63]. The exact mechanisms of low monocyte infiltration in the ischemic neonatal brain are not completely understood, and it remains unclear whether the higher resistance of the neonatal BBB to stroke is a cause or a consequence of reduced transmigration. Monocytes have been recently shown to have a dual role, elicit both inflammatory effects and maintain BBB integrity following cerebral ischemia [64]. T and B cell infiltration may be less profound [61] or transient [51] in injured neonates than in adults [65, 66] but there have been no studies that examined effects for BBB integrity.

8 BBB Integrity, Angiogenesis and Brain Repair After Neonatal Stroke

Physiological angiogenesis continues during the first two postnatal weeks in the rat brain [44]. Endothelial cell proliferation and endothelial tip cells are abundantly present [67, 68]. Interestingly, following stroke in P7 rats, angiogenesis is essentially arrested in injured brain regions up to 14 days after injury, and only subtle angiogenic response is detected in the ischemic boundaries in the cortex [69]. Figure 2 summarizes these findings. Considering that in adults endothelial cell proliferation and vascular outgrowth have been reported as soon as 24 h after stroke [70–72],

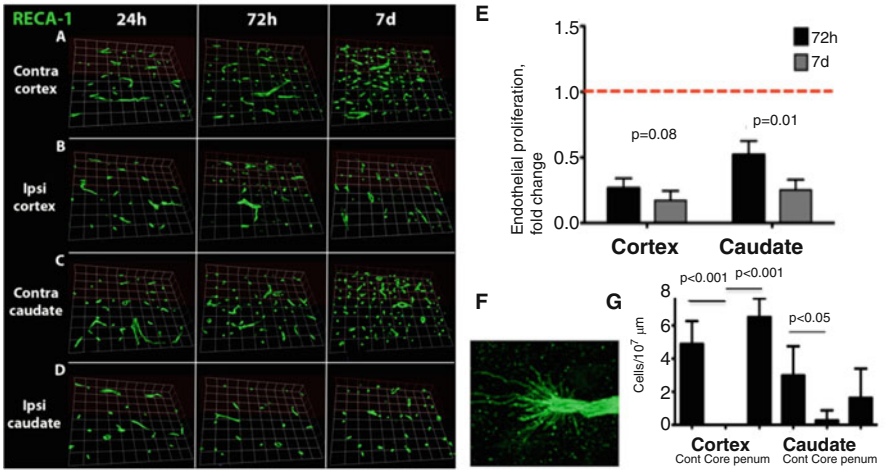

Fig. 2 Reduced vascular density and endothelial proliferation in sub-chronic injury phase after stroke in neonatal rats. (**a-d**) Examples of RECA-1+ vessel distribution in the ischemic core in the cortex (**a**, **b**) and caudate (**c**, **d**) at 24, 72 h, and 7 days after reperfusion. (**e**) Quantification of proliferating endothelial cells ($BrdU^+/RECA\text{-}1^+$) in the ischemic cortex and caudate. (**f**) Examples of PECAM-1^+ endothelial tip cells (defined by extended filopodia). (**g**) Quantification of the number of PECAM-1^+ endothelial tip cells in the contralateral and ischemic cortex and caudate

the response of neonatal brain to stroke differs to that in the adult in this aspect as well. Gain- and loss-of-function stroke studies in the adult have demonstrated that neuroblast migration occurs in association with remodeling of blood vessels [73], via a link between angiogenesis and neurogenesis within the "neurovascular niche," and that blockage of angiogenesis abolishes neurogenesis after adult stroke [73]. Brain vessels with active endothelial proliferation in the ischemic boundaries of the injured regions 14 days after neonatal stroke showed abnormal expression of the endothelial barrier antigen (EBA) [69], a protein necessary for proper BBB function in adolescent and adult rats [74–76] and which expression is maturation-dependent [77, 78]. It is tempting to speculate that the relatively preserved BBB after neonatal stroke may negatively impact angiogenesis and account for a delay in angiogenesis and ultimate endogenous neurogenesis, but the relationships between the processes are still poorly understood.

Consistent with the findings in injured P7 rats, the extent of angiogenesis was also limited 1 and 2 weeks after tMCAO in P10 [46, 48]. Angiogenesis was low despite rapid induction of first neuronal and, then, of astrocytic VEGF [34]. Angiogenesis and neurogenesis were enhanced by delayed administration of rhVEGF and disrupted by VEGFR2 inhibition, demonstrating the role of VEGF signaling for the maintenance of angiogenesis and repair in the injured ischemic core [46, 48].

9 Conclusions and Future Directions

Successful development of therapeutics for neonatal stroke depends on accurate understanding of the neurovascular interface. Recent studies have improved our understanding of the events at the BBB after neonatal ischemic injury by revealing that the developmental step of the BBB at the time of ischemic insult contributes to the differing patterns of brain damage between neonates and adults and that careful consideration should be given about whether the BBB is in fact disrupted, allowing therapies to reach an injured neonatal brain. However, it is important to recognize that there are too many unknowns. We know little about relationships between BBB integrity and white matter maturation after stroke. No studies systematically examined whether these processes are sex-dependent. There have been no studies looking in depth at the effects of hypothermia on neurovascular integrity in injured neonatal brain. Future studies should also shed light on relationships between neurovascular integrity and interaction with neuroprogenitors, endogenous or engrafted, and migration and differentiation of neural progenitors during stroke-induced neurogenesis. Although BCSFB is critically important for functioning of a neonatal brain, there have been no studies on addressing functionality of this barrier in injured neonatal brain.

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References

1. Engelhardt B, Sorokin L. The blood-brain and the blood-cerebrospinal fluid barriers: function and dysfunction. Semin Immunopathol. 2009;31(4):497–511.
2. Obermeier B, Daneman R, Ransohoff RM. Development, maintenance and disruption of the blood-brain barrier. Nat Med. 2013;19(12):1584–96.
3. Engelhardt B, Liebner S. Novel insights into the development and maintenance of the blood-brain barrier. Cell Tissue Res. 2014;355(3):687–99.
4. Armulik A, et al. Pericytes regulate the blood-brain barrier. Nature. 2010;468(7323):557–61.
5. Daneman R, et al. Pericytes are required for blood-brain barrier integrity during embryogenesis. Nature. 2010;468(7323):562–6.
6. Cahoy JD, et al. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. J Neurosci. 2008;28(1):264–78.
7. Zhang Y, Barres BA. Astrocyte heterogeneity: an underappreciated topic in neurobiology. Curr Opin Neurobiol. 2010;20(5):588–94.
8. Fantin A, et al. Tissue macrophages act as cellular chaperones for vascular anastomosis downstream of VEGF-mediated endothelial tip cell induction. Blood. 2010;116(5):829–40.
9. Shalaby F, et al. Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. Nature. 1995;376(6535):62–6.
10. Carmeliet P, et al. Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. Nature. 1996;380(6573):435–9.
11. Arnold TD, et al. Excessive vascular sprouting underlies cerebral hemorrhage in mice lacking alphaVbeta8-TGFbeta signaling in the brain. Development. 2014;141(23):4489–99.
12. Daneman R, et al. Wnt/beta-catenin signaling is required for CNS, but not non-CNS, angiogenesis. Proc Natl Acad Sci U S A. 2009;106(2):641–6.
13. Stenman JM, et al. Canonical Wnt signaling regulates organ-specific assembly and differentiation of CNS vasculature. Science. 2008;322(5905):1247–50.
14. Liebner S, et al. Wnt/beta-catenin signaling controls development of the blood-brain barrier. J Cell Biol. 2008;183(3):409–17.
15. Zlokovic BV. The blood-brain barrier in health and chronic neurodegenerative disorders. Neuron. 2008;57(2):178–201.
16. Gould DB, et al. Mutations in Col4a1 cause perinatal cerebral hemorrhage and porencephaly. Science. 2005;308(5725):1167–71.
17. Nguyen HL, et al. TGF-beta signaling in endothelial cells, but not neuroepithelial cells, is essential for cerebral vascular development. Lab Invest. 2011;91(11):1554–63.
18. Sridurongrit S, et al. Signaling via the Tgf-beta type I receptor Alk5 in heart development. Dev Biol. 2008;322(1):208–18.
19. Park SO, et al. Real-time imaging of de novo arteriovenous malformation in a mouse model of hereditary hemorrhagic telangiectasia. J Clin Invest. 2009;119(11):3487–96.
20. Ek CJ, et al. Efflux mechanisms at the developing brain barriers: ABC-transporters in the fetal and postnatal rat. Toxicol Lett. 2010;197(1):51–9.
21. Urayama A, et al. Mannose 6-phosphate receptor-mediated transport of sulfamidase across the blood-brain barrier in the newborn mouse. Mol Ther. 2008;16(7):1261–6.
22. Dziegielewska KM, et al. Fetuin in the developing neocortex of the rat: distribution and origin. J Comp Neurol. 2000;423(3):373–88.
23. Vates GE, et al. Pericyte constriction after stroke: the jury is still out. Nat Med. 2010;16(9):959; author reply 960.
24. Denes A, et al. Inflammation and brain injury: acute cerebral ischaemia, peripheral and central inflammation. Brain Behav Immun. 2010;24(5):708–23.
25. Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. Nat Med. 2011;17(7):796–808.
26. Knowland D, et al. Stepwise recruitment of transcellular and paracellular pathways underlies blood-brain barrier breakdown in stroke. Neuron. 2014;82(3):603–17.

27. Enzmann G, et al. The neurovascular unit as a selective barrier to polymorphonuclear granulocyte (PMN) infiltration into the brain after ischemic injury. Acta Neuropathol. 2013;125(3): 395–412.
28. Osborn L, et al. Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. Cell. 1989;59(6):1203–11.
29. Stanimirovic DB, et al. Increase in surface expression of ICAM-1, VCAM-1 and E-selectin in human cerebromicrovascular endothelial cells subjected to ischemia-like insults. Acta Neurochir Suppl. 1997;70:12–6.
30. Anthony DC, et al. Age-related effects of interleukin-1 beta on polymorphonuclear neutrophil-dependent increases in blood-brain barrier permeability in rats. Brain. 1997;120(Pt 3):435–44.
31. Stolp HB, et al. Long-term changes in blood-brain barrier permeability and white matter following prolonged systemic inflammation in early development in the rat. Eur J Neurosci. 2005;22(11):2805–16.
32. Renolleau S, et al. A model of transient unilateral focal ischemia with reperfusion in the P7 neonatal rat: morphological changes indicative of apoptosis. Stroke. 1998;29(7):1454–60; discussion 1461.
33. Derugin N, Ferriero DM, Vexler ZS. Neonatal reversible focal cerebral ischemia: a new model. Neurosci Res. 1998;32(4):349–53.
34. Mu D, et al. Regulation of hypoxia-inducible factor 1alpha and induction of vascular endothelial growth factor in a rat neonatal stroke model. Neurobiol Dis. 2003;14(3):524–34.
35. Woo MS, et al. Genetic deletion of CD36 enhances injury after acute neonatal stroke. Ann Neurol. 2012;72:961–70.
36. Derugin N, et al. Evolution of brain injury after transient middle cerebral artery occlusion in neonatal rat. Stroke. 2000;31:1752–61.
37. Rice 3rd JE, Vannucci RC, Brierley JB. The influence of immaturity on hypoxic-ischemic brain damage in the rat. Ann Neurol. 1981;9(2):131–41.
38. Sheldon RA, et al. Manipulation of antioxidant pathways in neonatal murine brain. Pediatr Res. 2004;56(4):656–62.
39. Northington FJ, Chavez-Valdez R, Martin LJ. Neuronal cell death in neonatal hypoxia-ischemia. Ann Neurol. 2011;69(5):743–58.
40. van Velthoven CT, et al. Stem cells for neonatal stroke- the future is here. Front Cell Neurosci. 2014;8:207.
41. Fernandez-Lopez D, et al. Mechanisms of perinatal arterial ischemic stroke. J Cereb Blood Flow Metab. 2014;34(6):921–32.
42. Mallard C, Vexler ZS. Modeling ischemia in the immature brain: how translational are animal models? Stroke. 2015;46(10):3006–11.
43. Hagberg H, et al. The role of inflammation in perinatal brain injury. Nat Rev Neurol. 2015;11(4):192–208.
44. Fernandez-Lopez D, et al. Blood-brain barrier permeability is increased after acute adult stroke but not neonatal stroke in the rat. J Neurosci. 2012;32(28):9588–600.
45. Ek CJ, et al. Brain barrier properties and cerebral blood flow in neonatal mice exposed to cerebral hypoxia-ischemia. J Cereb Blood Flow Metab. 2015;35(5):818–27.
46. Shimotake J, et al. Vascular endothelial growth factor receptor-2 inhibition promotes cell death and limits endothelial cell proliferation in a neonatal rodent model of stroke. Stroke. 2010;41(2):343–9.
47. Dzietko M, et al. VEGF treatment in a rodent model of neonatal stroke. In: PAS meeting. Boston; 2012.
48. Dzietko M, et al. Delayed VEGF treatment enhances angiogenesis and recovery after neonatal focal rodent stroke. Transl Stroke Res. 2013;4(2):189–200.
49. Kniesel U, Risau W, Wolburg H. Development of blood-brain barrier tight junctions in the rat cortex. Brain Res Dev Brain Res. 1996;96(1–2):229–40.
50. Engelhardt B. Development of the blood-brain barrier. Cell Tissue Res. 2003;314(1):119–29.
51. Benjelloun N, et al. Inflammatory responses in the cerebral cortex after ischemia in the P7 neonatal Rat. Stroke. 1999;30(9):1916–23; discussion 1923–4.

52. Wang R, et al. Albumin reduces blood-brain barrier permeability but does not alter infarct size in a rat model of neonatal stroke. Pediatr Res. 2007;62(3):261–6.
53. Dzietko M, et al. MRI as a translational tool for the study of neonatal stroke. J Child Neurol. 2011;26(9):1145–53.
54. Goetz JG, et al. Concerted regulation of focal adhesion dynamics by galectin-3 and tyrosine-phosphorylated caveolin-1. J Cell Biol. 2008;180(6):1261–75.
55. Fukuda S, et al. Focal cerebral ischemia induces active proteases that degrade microvascular matrix. Stroke. 2004;35(4):998–1004.
56. Doverhag C, et al. Galectin-3 contributes to neonatal hypoxic-ischemic brain injury. Neurobiol Dis. 2010;38(1):36–46.
57. Lalancette-Hebert M, et al. Galectin-3 is required for resident microglia activation and proliferation in response to ischemic injury. J Neurosci. 2012;32(30):10383–95.
58. Zador Z, et al. Role of aquaporin-4 in cerebral edema and stroke. Handb Exp Pharmacol. 2009;190:159–70.
59. Papadopoulos MC, et al. Aquaporin-4 facilitates reabsorption of excess fluid in vasogenic brain edema. FASEB J. 2004;18(11):1291–3.
60. Hudome S, et al. The role of neutrophils in the production of hypoxic-ischemic brain injury in the neonatal rat. Pediatr Res. 1997;41(5):607–16.
61. Bona E, et al. Chemokine and inflammatory cell response to hypoxia-ischemia in immature rats. Pediatr Res. 1999;45(4 Pt 1):500–9.
62. Yamasaki Y, et al. New therapeutic possibility of blocking cytokine-induced neutrophil chemoattractant on transient ischemic brain damage in rats. Brain Res. 1997;759(1):103–11.
63. Denker S, et al. Macrophages are comprised of resident brain microglia not infiltrating peripheral monocytes acutely after neonatal stroke. J Neurochem. 2007;100(4):893–904.
64. Gliem M, et al. Macrophages prevent hemorrhagic infarct transformation in murine stroke models. Ann Neurol. 2012;71(6):743–52.
65. Chu HX, et al. Immune cell infiltration in malignant middle cerebral artery infarction: comparison with transient cerebral ischemia. J Cereb Blood Flow Metab. 2014;34(3):450–9.
66. Catania A, Lipton JM. Peptide modulation of fever and inflammation within the brain. Ann N Y Acad Sci. 1998;856:62–8.
67. Ogunshola OO, et al. Neuronal VEGF expression correlates with angiogenesis in postnatal developing rat brain. Brain Res Dev Brain Res. 2000;119(1):139–53.
68. Iwai M, et al. Erythropoietin promotes neuronal replacement through revascularization and neurogenesis after neonatal hypoxia/ischemia in rats. Stroke. 2007;38(10):2795–803.
69. Fernandez-Lopez D, et al. Acute and chronic vascular responses to experimental focal arterial stroke in the neonate rat. Transl Stroke Res. 2013;4(2):179–88.
70. Beck H, Plate KH. Angiogenesis after cerebral ischemia. Acta Neuropathol. 2009;117(5):481–96.
71. Hayashi T, et al. Temporal profile of angiogenesis and expression of related genes in the brain after ischemia. J Cereb Blood Flow Metab. 2003;23(2):166–80.
72. Marti HJ, et al. Hypoxia-induced vascular endothelial growth factor expression precedes neovascularization after cerebral ischemia. Am J Pathol. 2000;156(3):965–76.
73. Ohab JJ, et al. A neurovascular niche for neurogenesis after stroke. J Neurosci. 2006;26(50):13007–16.
74. Ghabriel MN, et al. Immunological targeting of the endothelial barrier antigen (EBA) in vivo leads to opening of the blood-brain barrier. Brain Res. 2000;878(1–2):127–35.
75. Lu H, et al. Temporary disruption of the rat blood-brain barrier with a monoclonal antibody: a novel method for dynamic manganese-enhanced MRI. Neuroimage. 2010;50(1):7–14.
76. Saubamea B, et al. Heterogeneity in the rat brain vasculature revealed by quantitative confocal analysis of endothelial barrier antigen and P-glycoprotein expression. J Cereb Blood Flow Metab. 2012;32(1):81–92.
77. Rosenstein JM, et al. Immunocytochemical expression of the endothelial barrier antigen (EBA) during brain angiogenesis. Brain Res Dev Brain Res. 1992;66(1):47–54.
78. Sternberger NH, Sternberger LA. Blood-brain barrier protein recognized by monoclonal antibody. Proc Natl Acad Sci U S A. 1987;84(22):8169–73.