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## Control of cerebrovascular patterning by neural activity during postnatal development

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### Abstract

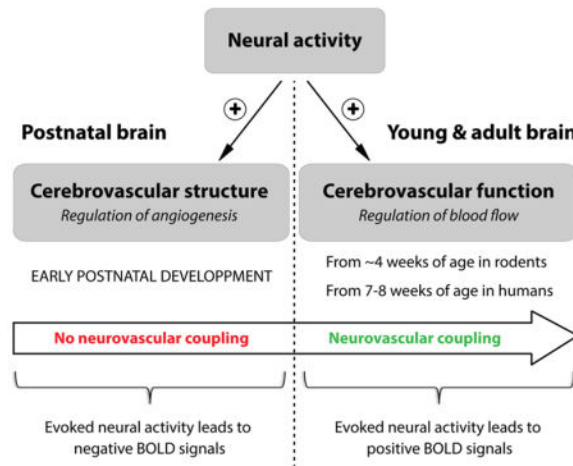
The brain represents only a small portion of the body mass and yet consumes almost a quarter of the available energy, and has a limited ability to store energy. The brain is therefore highly dependent on oxygen and nutrient supply from the blood circulation, which makes it vulnerable to vascular pathologies. Key vascular determinants will ensure proper brain maturation and function: the establishment of vascular networks, the formation of the blood-brain barrier, and the regulation of blood flow. Recent evidence suggests that the phenomenon of neurovascular coupling, during which increased neural activity normally leads to increased blood flow, is not functional until few weeks after birth, implying that the developing brain must rely on alternative mechanisms to adequately couple blood supply to increasing energy demands. This review will focus on these alternative mechanisms, which have been partly elucidated recently via the demonstration that neural activity influences the maturation of cerebrovascular networks. We also propose possible mechanisms underlying activity-induced vascular plasticity.

### Graphical abstract

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## Keywords

cerebrovascular patterning; neural activity; neurovascular unit; brain; endothelial cells

## I. Control of cerebrovascular patterning by neural activity

### I.a. Meeting energy demands: neurovascular interactions in the mature versus immature brain

In order to function properly, the brain relies heavily on the delivery of oxygen and nutrients from the blood stream (Attwell and Laughlin, 2001; Peters et al., 2004), requiring an adequate matching between metabolic demands of neural cells and blood supply. In the central nervous system (CNS), neural and vascular cells form a functionally integrated network, whereby neural activity and vascular dynamics are tightly coupled (Hamel, 2006; Lecrux and Hamel, 2011).

The anatomical substrate of neurovascular interactions in the brain is known as the ‘neurovascular unit’ (NVU), a complex multicellular system where neurons, astrocytes, microglia, pericytes and endothelial cells communicate to control the diameter of brain vessels and ensure an adequate delivery of oxygen and nutrients to neural tissues through the blood stream (Attwell et al., 2010; Cauli and Hamel, 2010; Chen et al., 2014; Fernandez-Klett et al., 2010; Hall et al., 2014; Hamel, 2006; Howarth, 2014; Lecrux and Hamel, 2011; Lo and Rosenberg, 2009; Petzold and Murthy, 2011). The NVU is also the anatomical substrate of the blood-brain barrier (BBB), a system which provides a tightly controlled environment, free of various toxins, pathogens, and with adequate chemical composition, for proper brain function (Andreone et al., 2015; Ben-Zvi et al., 2014; Saunders et al., 2014).

In the mature brain, the functional coupling between neural activity and cerebral blood flow (CBF) has been known for more than a century (Roy and Sherrington, 1890), as recently revisited (Sandrone et al., 2014). The increase in CBF following neural activity, also known as “neurovascular coupling”, has far-reaching implications in health and disease (Cauli and Hamel, 2010; Drake and Iadecola, 2007; Iadecola, 2004; Zlokovic, 2010), and represents the basis of functional brain imaging using blood oxygen level-dependent (BOLD) signals

(Devor et al., 2007; Devor et al., 2005; Hillman, 2014). In the immature brain, however, recent studies in rodents and humans have shown that the phenomenon of neurovascular coupling is not functional until few weeks after birth. While in adults sensory stimulation leads to a positive BOLD signal, reflecting a local increase in CBF, the identical stimulus in newborn infants or rat pups was shown to result in an inverted response with negative BOLD signals (Anderson et al., 2001; Born et al., 2002; Kozberg et al., 2013; Muramoto et al., 2002; Yamada et al., 2000). In these studies, negative BOLD signals were suggested to result from either decreased perfusion or increased oxygen consumption in response to sensory stimulation. The absence of a neurovascular coupling response to neuronal activation implies that, during early postnatal development, the immature brain must rely on alternative mechanisms to adequately match oxygen and nutrients supply with increasing energy demands. One potential mechanism during postnatal development could be the control of cerebrovascular patterning by neural activity.

### **I.b. Activity-induced vascular plasticity during postnatal development**

Both the nervous and vascular systems comprise highly branched and complex networks, and their patterning is initiated during development in a highly stereotyped fashion that is controlled by genetic programs, as reviewed elsewhere (Adams and Eichmann, 2010; Andreone et al., 2015; Carmeliet and Tessier-Lavigne, 2005; Tam and Watts, 2010). However, both networks exhibit a certain degree of plasticity and undergo dynamic remodeling after birth (Norman and O’Kusky, 1986). As early as embryonic day 10.5 (E10.5), the neural environment plays a critical role in the initial ingression and pruning/stabilization of blood vessels (Daneman et al., 2009; Haigh et al., 2003; Hogan et al., 2004). Along development, multiple cell types including neuroblasts, neuroepithelial radial glia, pericytes, microglia and astrocytes associate with blood vessels and influence their density/branching patterns (Arnold and Betsholtz, 2013; Lee and McCarty, 2014; Ma et al., 2012; Ma et al., 2013). For instance, reducing the proliferation of radial glial or astroglial progenitors during embryonic development led to a severe reduction in vascular density and branching frequency in the CNS at peri- and post-natal stages (Ma et al., 2012; Ma et al., 2013).

Whether electrical activity of neural cells influences the postnatal maturation of cerebrovascular networks remained elusive and controversial until recently. Almost thirty years ago, William T. Greenough and colleagues postulated that, during postnatal development, the brain adapts to increased metabolic demands by creating new vessels (Black et al., 1990; Black et al., 1987; Black et al., 1991). These milestone studies introduced the concept of vascular remodeling during maturation of the brain, however they did not establish a direct link between neural activity and vascular patterning after birth.

From studies in the rat cerebral cortex, the unique prevailing view was that requirements from expanding neural tissues influence the maturation of underlying capillary networks (Black et al., 1987; Sirevaag et al., 1988), and that high metabolic activity correlates with higher vascular density (Riddle et al., 1993). Moreover, several studies proposed the existence of anatomical relationships between neuronal and vascular modules within cortical columns in the rat somatosensory cortex (Cox et al., 1993; Patel, 1983). Such anatomical

parallelism suggests that neuronal and vascular modules may instruct each other to build a precise wired network for optimized local interactions, similar to the neurovascular congruency observed in the peripheral nervous system (Mukoyama et al., 2002). However, it was later demonstrated in the same species that cortical microvascular domains do not display any direct topological relationship with underlying columns (Woolsey et al., 1996). In line with this observation, recent studies using novel imaging and computational techniques, with three-dimensional (3-D) reconstructions of cerebrovascular networks, further demonstrated that the microvascular topology does not match the neuroarchitecture in the mouse cerebral cortex (Blinder et al., 2013; Lacoste et al., 2014; Tsai et al., 2009). Thus, in light of the fact that cortical columns are shaped after birth by neural activity (Erzurumlu and Kind, 2001; Li et al., 2013a; Narboux-Neme et al., 2012), it is possible that vascular network structure can also be influenced by neural activity.

The concept of neural activity-induced cerebrovascular plasticity during postnatal development was first introduced by earlier studies which postulated that sensory stimulation had a positive effect on brain angiogenesis (Argandona and Lafuente, 1996; Argandona and Lafuente, 2000; Black et al., 1987; Sirevaag et al., 1988). Therefore, after birth, sensory-related neural activity may refine cerebrovascular networks into their mature form, as it does for neuronal circuits (Katz and Shatz, 1996; Zhang and Poo, 2001). With the ability to simultaneously visualize and analyze neuronal and vascular modules, the direct effect of sensory neural activity on postnatal cerebrovascular development in the healthy brain was recently demonstrated in a study from our laboratory (Lacoste et al., 2014). We found that vascular density and branching, as well as endothelial cell proliferation, were decreased in layer IV of the primary somatosensory cortex when sensory input was reduced either by a complete deafferentation, by a genetic impairment of neurotransmitter release at thalamocortical synapses or by a selective reduction of sensory-related neural activity. In contrast, enhancement of sensory inputs led to an increase in vascular density and branching. Therefore, sensory-related neural activity appears necessary for vascular patterning, and changes in neural activity are sufficient to trigger changes in vascular structure. This implies that the postnatal maturation of brain vascular networks not only relies on angiogenic programs, but is also influenced by environmental stimuli.

Under pathological conditions in which neural activity is affected, the brain vascular structure may be regulated differently, particularly when these conditions occur during critical developmental periods. Excessive neural activity following hyperactivation of sensorimotor systems was recently shown to impair cerebrovascular network formation during a critical postnatal time (Whiteus et al., 2014). Whiteus et al. found a severe reduction of angiogenesis in the cerebral cortex following either intense locomotor exercise, persistent auditory stimulation, or following chemically-induced seizures in mice. This led the authors to propose that excessive neural activation during early childhood may trigger long-term deficits in microvascular networks with important consequences for brain function. In the adult rat brain however, previous studies with such hyperactivation paradigms evidenced increased angiogenesis in the cerebellum following vigorous locomotor exercise (Isaacs et al., 1992) or in the hippocampus after electroconvulsive seizures (Newton et al., 2006), thus emphasizing the difference between the “immature” and the “mature” brain in terms of vascular plasticity. Importantly, this angiogenic capability of

the adult brain might be of interest in ischemic conditions such as stroke. Indeed, it has been demonstrated that angiogenesis is increased in the penumbra of the ischemic adult mouse barrel cortex following enhancement of sensory-related neural activity by whisker stimulation (Whitaker et al., 2007), an effect which involves *vascular endothelial growth factor* (VEGF)/VEGFR2 signaling (Li et al., 2011) and which can be amplified by inhibition of de novo cholesterol synthesis by statins (Zhang et al., 2012).

## II. Possible mechanisms underlying activity-induced cerebrovascular plasticity

### II.a. What cell types could be involved?

The question remains whether neural activity affects angiogenesis directly via neurotransmitter and/or growth factor release by incoming axons, or indirectly via local pathways activated following neural activation that involve various cellular components of the NVU (Table 1).

**Neurons**—In the coupling between neural activity and vascular function in the cerebral cortex, the main neuronal players are neurons projecting from subcortical regions (e.g. basal forebrain, locus coeruleus) to the cortex (Cauli et al., 2004; Hamel, 2006), as well as local neurons (Cauli and Hamel, 2010; Lecrux et al., 2011). The cerebral cortex is densely innervated by projection neurons that release neurotransmitters such as acetylcholine (ACh, basal forebrain), noradrenaline (NA, locus coeruleus), serotonin (5-HT, raphe nuclei), or glutamate (Glu, thalamus). We recently demonstrated that proliferation of endothelial cells was decreased in layer IV of the barrel cortex following reduction of somatosensory inputs, pointing to a possible role of thalamocortical neurotransmission in the control of cortical angiogenesis (Lacoste et al., 2014). Local cortical pyramidal (excitatory) neurons and inhibitory interneurons are also recruited by somatosensory inputs (Lecrux et al., 2011), and in turn release vasoactive mediators which control vascular tone (Cauli and Hamel, 2010; Drake and Iadecola, 2007). However, the question remains whether these neural modules also release angiogenesis regulators upon neural activity changes. Interestingly, it has been shown that neuronal expression of VEGF increases in the hippocampus of rats subjected to environmental enrichment (Cao et al., 2004). More recently, two reports using the postnatal mouse retina as a model system for studying neuro-vascular crosstalk identified novel neuronal signaling pathways that govern CNS angiogenesis (Joyal et al., 2014; Okabe et al., 2014). Joyal et al. demonstrated that a subset of retinal neurons (the ganglion cells) express a G protein-coupled receptor known as F2rl1 (or Par2) which, upon agonist stimulation, relocates to the nucleus via a microtubule-dependent shuttle to induce *Vegfa* expression, promoting neovascularization. Okabe et al. demonstrated that VEGFR2 is predominantly expressed by retinal neurons. Upon binding to neuronal VEGFR2, VEGF protein is engulfed in the cell while its receptor is being endocytosed, a mechanism that allows titration of extracellular VEGF to regulate vascularization around retinal neurons. Additionally, neurons communicate with other cell types of the NVU (i.e., glial cells and pericytes) and might therefore indirectly influence vascular plasticity, as detailed below.

**Glial cells**—Astrocytes are well positioned to mediate the effects of neural activity, being in close contact with both cerebral synapses and vessels. They express different classes of glutamate receptors and project specialized extensions ('endfeet') around vessels. It is well accepted that astrocytes respond to glutamate (Glu), via metabotropic glutamatergic receptors, by a rise in intracellular calcium ( $[Ca^{2+}]_i$ ) from internal stores (Lind et al., 2013; Winship et al., 2007; Zonta et al., 2003), triggering signaling cascades which lead to the production of glial messengers involved in the control of CBF (Howarth, 2014), including epoxyeicosatrienoic acids (EETs) (Alkayed et al., 1996b). In addition to their role as vasodilators (Alkayed et al., 1996a; Alkayed et al., 1997), EETs are pro-angiogenic lipids. Indeed, *in vitro* evidence demonstrated that EETs are as potent as VEGF in inducing endothelial cells proliferation and tube formation (Munzenmaier and Harder, 2000; Potente et al., 2003; Pozzi et al., 2005; Zhang and Harder, 2002). More recently, a recent genetic study demonstrated that astrocytes are essential for the normal postnatal development of cerebral cortex vasculature (Ma et al., 2012). In addition, astrocytes are well known to release pro-angiogenic VEGF *in vivo* (Stone et al., 1995; West et al., 2005), and they can also promote angiogenesis via release of sonic hedgehog (shh) in ischemic conditions *in vitro* (Li et al., 2013b). Future studies should investigate the precise mechanisms through which neural activity controls the release of astroglial angiogenesis modulators *in vivo*, and their effects on cerebrovascular patterning. The other glial cell type of the brain, microglia, could also be involved in activity-induced vascular plasticity. Microglia are considered to be the brain's resident macrophages (Neumann et al., 2009). However, microglia also play critical roles in postnatal brain maturation during vascular development (Arnold and Betsholtz, 2013) and in experience-dependant neuronal plasticity during critical periods (Tremblay and Majewska, 2011; Tremblay et al., 2011). Microglia appear associated with blood vessels as early as E10.5 when vessels start to ingress into the neuroepithelium (Ginhoux et al., 2010) and they promote vascular branching in the embryo (Fantin et al., 2010) as well as postnatally (Kubota et al., 2009). Recent studies suggested that microglia regulate vascular branching and proliferation via release of soluble mediators which either promote or inhibit vascular network formation (Arnold and Betsholtz, 2013; Stefater et al., 2011). More recently, microglia have been shown to facilitate angiogenesis *in vitro* via release of *tumor necrosis factor- $\alpha$*  (TNF- $\alpha$ ), which upregulates endothelial ephrin-A3 and ephrin-A4 (Li et al., 2014). Moreover, microglia are endowed with neurotransmitter receptors including iono- and metabo-tropic Glu receptors (Pocock and Kettenmann, 2007) and are reactive to neural activity (Hung et al., 2010; Wake et al., 2009). However, it remains to be determined whether microglial angiogenesis regulators are released upon neuronal activation.

**Pericytes**—Long considered as support cells for the endothelium, pericytes are also involved in the proper vascularization of the brain (ElAli et al., 2014), in the formation and maintenance of the blood-brain barrier (Armulik et al., 2010; Daneman et al., 2010; Mae et al., 2011), and in the regulation of capillary diameter in response to neural activity (Fernandez-Klett et al., 2010; Hall et al., 2014; Hamilton et al., 2010; Itoh and Suzuki, 2012). Whether pericytes also play a role in activity-induced cerebrovascular structural plasticity needs to be clarified, but several lines of evidence advocate in favor of such possibility. Pericytes and endothelial cells interact via signalling pathways which are



instrumental for vascular network formation, as extensively reviewed elsewhere (Armulik et al., 2005; ElAli et al., 2014). To only give one example, angiopoietin-1 secreted by pericytes binds to the endothelial-specific Tie2 receptor to promote maturation and stabilization of the microvascular endothelium (Suri et al., 1996). Interestingly, pericytes are sensitive to neurotransmitters such as glutamate, noradrenalin and acetylcholine (Hall et al., 2014; Peppiatt et al., 2006; Puro, 2007; Wu et al., 2003), and they are responsive to electrical stimulation (Peppiatt et al., 2006). Moreover, like in astrocytes, neurotransmitters cause a rise in  $[Ca^{2+}]_i$  in pericytes (Kawamura et al., 2004; Kawamura et al., 2003). In microvessels of the postnatal brain, the  $[Ca^{2+}]_i$  elevation induced in pericytes by synaptic release of Glu was shown to result in nuclear accumulation of NFATc3, a transcription factor involved in vascular development and maturation (Filosa et al., 2007). Future studies could investigate the ability of pericytes to secrete angiogenesis regulators upon neural activity changes.

## II.b. What endothelial gene(s) could be involved?

As best illustrated by the NVU, activity-dependent regulation of vascular structure is an integrative process recruiting broad cellular networks. Knowing that the endothelium represents the ultimate effector undergoing structural changes, future studies could focus on investigating the genes that are regulated within endothelial cells upon modulation of neural activity. This approach is timely, since technological advances in gene analysis allow for a better understanding of cerebrovascular cell transcriptome (Ozkan et al., 2012; Zhang et al., 2014). Such approaches could be used to investigate candidate genes and identify new molecular players of vascular plasticity.

One endothelial-specific gene whose upregulation has already been linked to neural activity is named “vascular early response gene”, or *Verge* (Mirza et al., 2013; Regard et al., 2004). In developing tissues, *Verge* is constitutively expressed (mRNA and protein) in the endothelium and is associated with angiogenesis, whereas in the adult brain it is regulated as an immediate early gene induced by electrical or chemical seizures and by focal ischemia. In cultured endothelial cells, *Verge* is induced by growth factors and hypoxia. Since *Verge* induction is associated with remodeling of the actin cytoskeleton, the authors proposed that *Verge* might play a role in activity-dependent changes of brain vasculature (Regard et al., 2004). It would be interesting to test whether changes in *Verge* expression occur in the cerebral cortex following neuronal activation in a physiological context, and assess the effects of its modulation on cerebrovascular plasticity.

Other candidate genes that might be involved in activity-induced vascular plasticity include genes postnatally expressed in the endothelium and known to affect vascular remodeling. For instance, homeobox transcription factors are promising candidates involved in the control of vascular remodeling (Gorski and Walsh, 2000). Expression of members of HOX A, HOX B, and HOX D clusters has been detected in endothelial cells and involved in the balance between resting and angiogenic state of the endothelium. *HOX D3* is highly expressed in proliferating endothelial cells that are induced to form tubes in vitro (Boudreau et al., 1997), and constitutive expression of *HOX B3* in the chick chorioallantoic membrane leads to increased angiogenesis (Myers et al., 2000). Receptor neuropilin-1 (*Nrp1*) and its co-receptor VEGFR2 (*Kdr*) have recently been associated to postnatal angiogenesis and

vascular remodeling after ischemic challenge (Gelfand et al., 2014). Another interesting candidate is *Bail* (brain-specific angiogenesis inhibitor-1), a G-protein coupled receptor present in vessels of the mouse brain throughout development with higher expression at the tips of angiogenic sprouts (Ozkan et al., 2012). Interestingly, while endothelial-specific expression of *Bail* has to be confirmed, *Bail* appears predominantly expressed in dense microvascular networks of the cerebral cortex at P14 (Ozkan et al., 2012), which is an age when cerebrovascular growth and remodeling are very active (Lacoste et al., 2014). Future studies could investigate whether candidates or new endothelial genes are regulated by neural activity *in vivo* during postnatal development.

### II.c. Could hypoxia be involved?

As mentioned earlier in this review, the absence of neurovascular coupling responses is characteristic of the early postnatal brain. Evoked neural activity in the immature brain results in decreased perfusion and/or increased oxygen extraction, underlying negative BOLD signals (Anderson et al., 2001; Born et al., 2002; Kozberg et al., 2013; Muramoto et al., 2002; Yamada et al., 2000). Therefore, in the absence of blood flow regulation, it is possible that the developing brain recruits alternative mechanisms to meet its increasing metabolic needs. Indeed, after birth, the maturation of neuronal networks involves energy-consuming processes such as neurogenesis, synaptogenesis, maturation of astrocytes, and changes in brain cytoarchitecture. Thus, in the young brain, neuronal network activation might generate a local and transient hypoxic state. Interestingly, hypoxia-induced growth factors represent an important driving force of vascular development during embryogenesis (Haigh et al., 2003; James et al., 2009; Provis et al., 1997; Raab et al., 2004; Stone et al., 1995) but also during postnatal cerebrovascular remodeling (Rey and Semenza, 2010), particularly in pathological conditions such as ischemia and cancer (Silpanisong and Pearce, 2013). Local reduction in oxygen tension leads to activation of transcription factors ‘hypoxia-inducible factors’ (HIFs) that signal in the nucleus following heterodimerization. Activated HIFs regulate the expression of virtually all the key angiogenic factors, including VEGF, angiopoietin-2 and placental growth factor upon binding to their hypoxia response elements (Jiang et al., 1997; Rey and Semenza, 2010; Semenza et al., 1997). Future studies could investigate whether sensory stimulation, which leads to increased vascular density and branching in the cerebral cortex (Lacoste et al., 2014), is due to a local hypoxic state that triggers hypoxia-induced angiogenic factors.

### III. Future questions

While the postnatal maturation of brain vascular networks begins to draw most attention, it remains unknown whether the emergence of neuronal function influences brain angiogenesis before birth. In the embryo, before maturation of sensory organs, neural activity exists and has been identified as a spontaneous phenomenon contributing to the refinement of CNS connectivity (Katz and Shatz, 1996; Meister et al., 1991; Weliky and Katz, 1999). Yet, whether this type of electrical activity influences cerebrovascular patterning during embryogenesis remains to be determined. A recent study investigating stimulus-induced vascular remodeling described a melanopsin-dependent fetal light response regulating the regression of hyaloid vessels in the developing mouse eye (Rao et al., 2013). However, the



contribution, if any, of stimulus-independent neural activity to cerebrovascular patterning before birth remains an uncharted territory, raising the need for future investigation. Moreover, it is also not known whether postnatal vasculogenesis might be regulated by neural activity in the brain. In this context, postnatal recruitment of bone marrow-derived endothelial progenitor cells and their incorporation to growing vessels (Ribatti et al., 2001) may also be investigated following modulation of neural activity. Future studies could also investigate the influence of neural activity on cerebrovascular patterning during adulthood. Knowing that environmental changes can affect the neuronal connectivity in the mature brain (Bavelier et al., 2010; May, 2011), they might as well affect vascular networks. In line with this hypothesis, studies in adult rodents have shown that whisker stimulation can enhance angiogenesis in the penumbra surrounding the ischemic core following middle cerebral artery occlusion in adult mice (Li et al., 2011; Whitaker et al., 2007), suggesting that sensory stimulation might be beneficial in stroke. Other questions remain unanswered, for instance concerning the indirect effect of CBF on brain angiogenesis. Knowing that mechanical forces affect angiogenesis (Hofer et al., 2013), it is also possible that the regulation of CBF by neural activity remotely influences cerebrovascular patterning. As already assessed *in vitro* and in skeletal muscle (Hansen-Smith et al., 2001; Wilkins et al., 2014), future investigations will be needed to shed light on stretch-induced angiogenesis in the brain.

#### IV. Conclusion

Evidence gathered in this review highlight the role of neural activity in promoting the maturation of cerebrovascular networks during postnatal development. We propose a new model in which, in the absence of coupling between neural activity and cerebral blood flow, the immature brain might recruit alternative mechanisms around microvessels in order to meet the increasing metabolic demands triggered by developing neural tissues (Figure 1). This model, which involves different cell types in the NVU, as well as their ability to regulate vascular growth and plasticity, opens a new area in neurovascular research.

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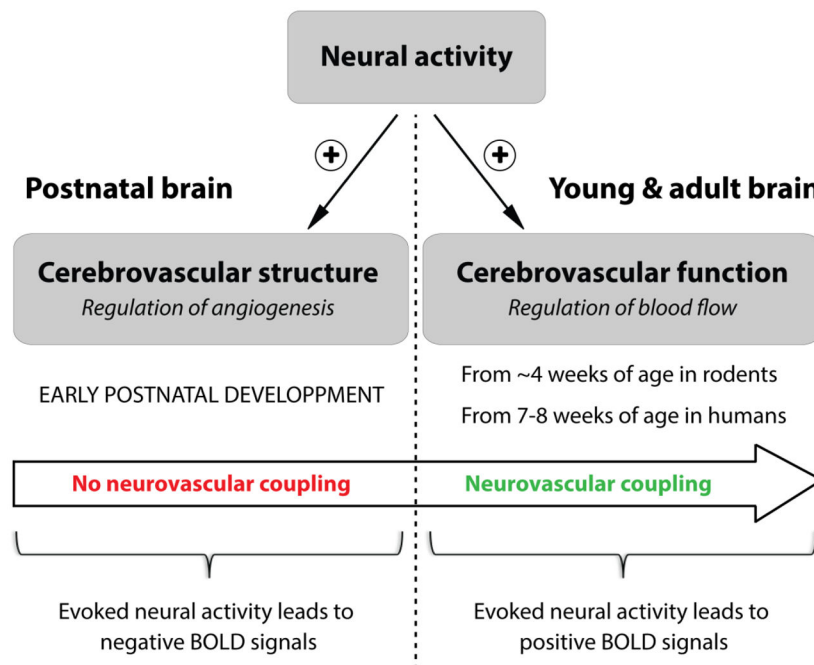


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**Highlights**

- Neural activity controls cerebral blood flow in the mature brain
- Neural activity is not coupled to cerebral blood flow in the early postnatal brain
- Neural activity controls cerebrovascular patterning in the early postnatal brain
- A model is proposed to explain how metabolic needs are met early after birth



**Figure 1. A new model for the role of neural activity in postnatal cerebrovascular development**  
 In the mature brain, a functional coupling between increased neural activity and increased cerebral blood flow (“neurovascular coupling”) ensures proper brain function. In the immature brain, however, the phenomenon of neurovascular coupling is not functional until few weeks after birth. Here, we propose a model in which, in the absence of coupling between neural activity and cerebral blood flow, the immature brain might recruit alternative mechanisms around microvessels in order to meet the increasing metabolic demands triggered by developing neural tissues

**Table 1**

Contribution of different cell type in the neurovascular unit to cerebrovascular development.

Cell type	Released factors	Effect on vascular patterning	References
Pericytes	Ang1	Vessel stabilization	Suri et al., 1996
Astrocytes	VEGF-A	Pro-angiogenic	Stone et al., 1995 Munzenmaier & Harder, 2000 Zhang & Harder, 2002 Potente et al., 2003 Pozzi et al., 2005 West et al., 2005 Li et al., 2013
	EETs		
	Shh		
	Ang1	Vessel stabilization	
Neurons	Ang1	Vessel stabilization	Cao et al., 2004 Joyal et al., 2014
	VEGF-A	Pro-angiogenic	
Microglia	TNF $\alpha$	Pro-angiogenic	Stefater et al., 2011 Arnold & Betsholtz, 2013 Li et al., 2014
	VEGF-C,D	Pro-/anti-angiogenic	
	Wnt5a, Wnt11	Anti-angiogenic	

Ang1, *angiopoietin-1*; EETs, *epoxyeicosatrienoic acids*; Shh, *sonic hedgehog*; VEGF, *vascular endothelial growth factor*; Wnt, *wingless integration site*.