Experience Dependent Plasticity of Neurovascularization

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Abstract

Experience powerfully shapes structural and functional organization of neurons during development and in adulthood. Recent experiments in the mouse primary somatosensory cortex now suggest that experience is also a critical factor in shaping neurovasculature and promoting angiogenesis. These results support the universality of brain plasticity and show that all structural cellular components in the brain, from neuron and glia to epithelia, are shaped by experience.

Being one of the most energy demanding organs in the mammalian body the brain requires a constant supply of blood. As evidenced by the negative impact that ischemia has on the neuronal activity (Dirnagl et al., 1999), sufficient blood supply is paramount for the maintenance and support of brain function. It may come as no surprise then that increases in neuronal activity are reflected in the organization of the surrounding vasculature and that angiogenesis (i.e. the formation of new blood vessels) is required for learning and memory (Kerr et al., 2010); An increase in angiogenesis and the number of synapses has been shown to occur in visual cortex of young and adult rats following enriched environment experience (Black et al., 1987). During a critical postnatal developmental period excessive stimulation
induces a reduction of blood vessel sprouting and endothelial proliferation, which in turn leads to blood vessel growth arrest in the somatosensory, auditory and motor cortices (Whiteus et al., 2014). These anti-angiogenic effects are also shown to lead to hypoxia in the corresponding overstimulated brain regions, which in turn is associated with dendritic spine loss. These findings suggest that brain plasticity is not restricted to (synapses between) neurons, but is a feature of the cerebrovasculature, and that these processes are functionally coupled.

In a recent study Lacoste and colleagues have addressed the effects of sensory deprivation and stimulation on the cerebrovasculature in the barrel cortex subregion of the somatosensory cortex (Lacoste et al., 2014). In rodents, the barrel cortex receives and processes sensory information coming from the whiskers, which the animals use in order to explore their environment (Voigts et al., 2014) and is heavily innervated by cerebral vasculature (Stewart et al., 2013). This part of the rodent brain is used extensively as a model system to study neuronal plasticity (e.g. Glazewski and Fox, 1996; Allen et al., 2003; Celikel et al., 2004; Erzurumlu and Gaspar, 2012) in part due to the ease with which cortical plasticity can be induced during development as well as in adulthood. By simply plucking or clipping (a subset of) the animal’s facial whiskers, sensory input to the barrel cortex is altered, leading to long-lasting changes in neuronal representations of touch in the barrel cortex (Celikel et al., 2004). Although the effects of whisker deprivation on the neuronal population have been widely studied in the past decades, the cerebrovasculature has been mostly neglected.

In order to investigate the effects of sensory deprivation or stimulation on cerebrovascular plasticity in barrel cortex, Lacoste et al. employed quantitative vascular mapping in transgenic mouse lines that allowed them to analytically describe the effects of sensory
experience on vascular organization \textit{ex vivo}. The main hypothesis the authors sought to
experimentally answer was that if vascular plasticity and neuronal plasticity are correlated,
then alterations of the neuronal activity through whisker stimulation or deprivation should be
reflected in the barrel cortex vascular network.

Since studies on neurovasculature are scarce, Lacoste \textit{et al.} first described development of the
vasculature in the barrel cortex. Using transgenic mice expressing green fluorescent protein
(GFP) in endothelial cells to visualize the vasculature, and the red fluorophore tdTomato
under the serotonin transporter promoter to identify barrel formation, the authors
reconstructed the cerebrovasculature in tangential sections of the barrel cortex. They found
that vascular density and branching increase until P14. Both vascular metrics are reduced
with further development although they are maintained at a steady level into young
adulthood. The peak of barrel cortex vascularization around P14 is particularly of interest as
the mouse barrel cortex undergoes rapid plasticity during this period as the LIV-LII/III
synapses are formed (Erzurumlu and Gaspar, 2012). Whether increased synaptogenesis
might potentially result in angiogenesis or increased angiogenesis ultimately leads to
synaptogenesis is unknown and will benefit from mapping developmental changes in
vascularization at higher temporal resolutions.

In order to experimentally test the hypothesis that brain activity controls, or otherwise alters,
the organization of the cerebrovasculature, Lacoste and colleagues have used four
complimentary experimental designs. First whisker follicles were lesioned shortly after birth,
abolishing both the associated neuronal cytoarchitecture and neuronal activity. In these
animals, clear disruptions of the barrel field could be distinguished, along with a reduction of
the density and branching of the vascular bed. A second group consisted of transgenic mice
(RIM DKO<sup>Scnt</sup> mice) that have reduced glutamatergic neurotransmitter release at their thalamocortical synapses, leading to diminished neuronal activity and a lack of LIV barrel structures (Narboux-Nême et al., 2012). In these animals, the neurovasculature had reduced vessel density and branch points, indirectly suggesting the conclusion that altered activity along the somatosensory axis results in changes in cortical angiogenesis.

The first two experimental manipulations were designed to abolish or otherwise alter the sensory processing during key developmental stages. However, these manipulations also altered both anatomical and functional neuroarchitecture, making the link between the neuronal activity and neurovasculature more ambiguous. To causally link the vascular plasticity to neuronal activity, in a third experiment, the authors employed whisker plucking, a method commonly used to decrease synaptic input into the barrel cortex (Allen et al., 2003). In these animals, Lacoste and colleagues found that complete unilateral sensory deprivation led to a reduction in vascular density, vascular branching and angiogenesis in the contralateral hemisphere. In future experiments sparing one or more whiskers while depriving others will be required to answer whether experience dependent angiogenesis involves competitive processes and if so whether whisker sparing and deprivation result in complimentary changes of neurovascular organization within the same barrel cortex.

As the authors aimed to unravel the relationship between cerebrovascular plasticity and neuronal activity, they designed one final experiment where whisker stimulation in the absence of whisker deprivation was employed to increase neural activity throughout the barrel cortex. Ex vivo imaging showed that daily unilateral whisker stimulation in vivo results in increased branching and vessel density in the contralateral barrel cortex. Taken together,
Lacoste and colleagues have shown that the cerebrovasculature is “plastic”, and that the neurovascular plasticity is correlated with (and possibly regulated by) neuronal activity.

The origin and specificity of cerebrovascular plasticity

Just like any good research article, the research reviewed herein leaves the reader with follow-up questions: What is the origin of cerebrovascular plasticity? Are the changes in vascularization specific to the cortical column whose activity is selectively altered? What is the role of competition in shaping the neurovasculature?

During development anatomical and functional organization of neural circuits rapidly change. Moreover, early cortical development occurs in the absence of correlated input originating from the sensory periphery as the thalamocortical projections do not mature until the end of the first postnatal week (Erzurumlu and Gaspar, 2012). Therefore causally linking the neural activity to the plasticity of cortical vasculature will require experiments that allow normal neural development while biasing the overall neural activity by direct interrogation of the cortical circuits. This could be achieved by targeted expression of light-gated cation and anion channels to selectively facilitate and suppress the neural activity in a cortical column-specific manner, ideally within the same animal and across neighbouring cortical columns. If the findings of Lacoste et al. are any indication, the expectation is that vessel branching, density and angiogenesis would be increased following direct cortical neuronal activation and decreased upon suppression of the neuronal spiking in otherwise intact animals. These results will confirm the necessity of a change in cortical neuronal activity to drive neurovascular plasticity and will shed light onto whether the neurovascular plasticity is specific to the cortical column being stimulated. Since alterations of neuronal activity upon
whisker deprivation are known to follow a distinct time course across cortical layers

(Glazewski and Fox, 1996), neurovascular plasticity might actually be cortical layer specific.

Experiments employing whisker sparing and longitudinal 3D imaging of vasculature across
cortical layers in living animals will be necessary to empirically answer the hypothesis that
neurovascular plasticity follows a distinct experience-dependent time-course across different
layers.

Role of competition in shaping cerebrovasculature

Lacoste and colleagues have employed unilateral whisker deprivation and whisker deflection
experiments that altered the sensory evoked responses throughout the contralateral barrel
cortex. Although these protocols enable unambiguous characterization of the sensory
experience (or the lack thereof) of a given barrel cortical column, they also eliminate the
competition between spared (or stimulated) and deprived (or non-stimulated) columns.

Blood vessel length and branching are likely to play distinct, yet complementary, roles in
shaping the vascular bed according to the needs of the surrounding neuronal population, and
likely to reflect the spatial distribution of neuronal activity. Increases in vessel length and
potentially diameter are required to reach more distant areas of the brain whereas branching
likely serves to deliver more blood locally. Determining the factors that collectively shape
the vasculature will require experiments that entail the introduction of competition between
neuronal populations. For instance, upon depriving all but two neighbouring whiskers
vascular branching and length are likely to increase specifically towards the spared whisker’s
principal cortical column, while branching originating from the spared column and targeting a deprived column is expected to be reduced.

Potential mechanisms of neurovascular plasticity

In their study, Lacoste and colleagues briefly described the effects of sensory deprivation on angiogenesis. Apoptosis (i.e. programmed cell death) could be modulated by experience to counterbalance the role of angiogenesis in shaping neurovasculature; Apoptosis could keep runaway angiogenesis in check and potentially serve to selectively remove redundant blood vessels while blood vessels serving metabolically active groups of neurons might increase in length and branching. In future studies inclusion of apoptosis markers might be necessary in order to gain a mechanistic insight into the role of apoptosis in relation to angiogenesis during neurovascular plasticity.

Independent from whether apoptosis and/or angiogenesis primarily shape the neurovascular network, it is likely that neural activity results in the release of diffusible factors, e.g. glutamate and growth factors, which bind to surface receptors on vascular endothelial cells to promote neurovascular changes. In addition, vasodilation and increased blood flow lead to mechanical stretch, leading to activation of stretch-activated channels. Independent from the trigger signal, intracellular signalling pathways, e.g. MAPK and HIF, are likely to translate trigger signals into long-lasting vascular reorganization. Unravelling the molecular mechanisms underlying neurovascular plasticity will not only help to mechanistically explain the observations of Lacoste and colleagues but also could help to shed light to vasculature changes associated with aging and stroke.

Broader implications
Plasticity of neurovasculature has overarching implications for the organization and function of neuronal circuits in health and disease. With angiogenesis contributing to the animal performance in learning and memory tasks even in adulthood (Kerr et al., 2010), the role of experience dependent plasticity of neurovasculature outlasts its contribution to the rapid and extensive neuronal plasticity observed in the barrel cortex during development. Considering that angiogenesis diminishes after postnatal day 14 (Lacoste et al., 2014), even though experience dependent neuronal plasticity is preserved well into adulthood, albeit at a slower rate (Glazewski and Fox, 1996; Erzurumlu and Gaspar, 2012), the neurovascular plasticity is likely to play a complementary, rather than an independent, role in shaping adult cortical circuits in most brain states. One potential exception to this proposition are clinical conditions like stroke and neurodegenerative disorders where large-scale changes in neuronal activity trigger changes in coordination of blood-flow. Being able to control the plasticity of neurovasculature in brain disorders could hence lead to the development of promising therapies enhancing, otherwise enabling, neural recovery.

An increased understanding of (the mechanisms behind) neurovascular plasticity may also have implications for the interpretation of data obtained using functional MRI experiments. Changes in the BOLD signal are often attributed to changes in neural activity, and it is now becoming increasingly clear that the plastic nature of the neurovasculature provides a mechanistic link between changes in neural activity and the BOLD signal as observed in fMRI studies.

In conclusion, the study by Lacoste et al. shows that neurons are not the only plastic players in the brain and that the cerebral vasculature changes during murine cortical development in an experience dependent manner. Follow-up studies should focus on cortical
and laminar specificity of neurovascular plasticity, and aim to introduce competition across
cortical columns to provide further insight into the origin and specificity of plastic changes in
neurovasculature.
References


