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COMMENTARY

Neurovascular coupling develops alongside neural circuits in the postnatal brain

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ABSTRACT

In the adult brain, increases in local neural activity are accompanied by increases in regional blood flow. This relationship between neural activity and hemodynamics is termed neurovascular coupling and provides the blood flow-dependent contrast detected in functional magnetic resonance imaging (fMRI). Neurovascular coupling is commonly assumed to be consistent and reliable from birth; however, numerous studies have demonstrated markedly different hemodynamics in the early postnatal brain. Our recent study in J. Neuroscience examined whether different hemodynamics in the immature brain are driven by differences in the underlying spatiotemporal properties of neural activity during this period of robust neural circuit expansion. Using a novel wide-field optical imaging technique to visualize both neural activity and hemodynamics in the mouse brain, we observed longer duration and increasingly complex patterns of neural responses to stimulus as cortical connectivity developed over time. However, imaging of brain blood flow, oxygenation, and metabolism in the same mice demonstrated an absence of coupled blood flow responses in the newborn brain. This lack of blood flow coupling was shown to lead to oxygen depletions following neural activations – depletions that may affect the duration of sustained neural responses and could be important to the vascular patterning of the rapidly developing brain. These results are a step toward understanding the unique neurovascular and neurometabolic environment of the newborn brain, and provide new insights for interpretation of fMRI BOLD studies of early brain development.

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Local neural activity in the adult brain leads to large increases in local blood flow. These increases in blood flow seemingly overcompensate for local oxygen consumption, leading to increases in the local concentration of both oxygenated (HbO) and total hemoglobin (HbT) and decreases in deoxygenated hemoglobin (HbR) due to a washout effect.[21,22,33] This decrease in the concentration of HbR is the basis of the fMRI BOLD response and it has been commonly assumed that these responses are in place and consistent from birth. Although some neonatal fMRI and near infrared spectroscopy (NIRS) studies have reported adult-like responses,[3,31,49] many studies in both humans and rodent models have reported differences in hemodynamic responses in the early postnatal brain compared to adults.[1,9,26,38,57] Such findings have raised doubts over the use of hemodynamic-dependent imaging modalities to study developing neural connectivity. Our recent study in J. Neuroscience is the first to image neural and hemodynamic activity simultaneously across the early postnatal mouse cortex, providing a unique look at the formation of neural circuitry alongside the establishment of neurovascular coupling.

Our work on this subject began with a study in rats at postnatal days 12–23 (P12–23), which demonstrated that somatosensory (hindpaw) stimulation does not evoke increases in local blood flow at P12.[26] At around P15, small, immature responses that were localized to the capillary bed were detected, later recruiting dilation of larger arteries feeding the responding region until reaching adult-like levels of evoked hyperemia by P23. This result indicated that the machinery to fully actuate hemodynamic responses might still be developing postnatally. By varying stimulus amplitude, another effect was identified: strong stimuli in younger animals (P12-P13)

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evoked large increases in systemic blood pressure, which led to uniform increases in cortical blood flow. This result indicated a state of immature autoregulation, and might explain the breadth of different neonatal hemodynamic responses reported in human fMRI and NIRS studies. When stimuli of lower amplitudes were used in our youngest age group, arteries across the cortex were seen to constrict in response to hindpaw stimulation, leading to a non-localized hemodynamic response inverted with respect to adult responses. Similar hemodynamic time-courses in response to whisker stimulation were demonstrated in neonatal mice by Zehendner et al.[57]

These results led us to hypothesize that neurovascular coupling itself was developing in the early postnatal brain. However, the question whether the postnatal changes in the hemodynamic response that we had observed could be explained solely by changes in underlying neural activity remained.

While the development of neural circuits has been studied extensively at a structural level,[30,52] functional studies using traditional electrophysiological techniques have been spatially limited. In our recent study in J. Neuroscience, we overcame this limitation by utilizing P7-P13 and adult mice expressing GCaMP, a fluorescent calcium indicator, in pyramidal neurons in layers 2/3 and 5 of the cortex,[13] providing an optical read-out of neural firing. Using high-speed optical imaging through a bilateral thinned-skull craniotomy, we simultaneously mapped both neural and hemodynamic activity in parallel across the cortical surface in mice of varying postnatal age.[27]

Cortical responses to hindpaw stimulation evolved from unilateral neural responses, localized to the somatosensory cortex at P7, to bilateral neural responses that initiate within the same region of the cortex before spreading to recruit other areas in adults. Neural responses at P7 were also much slower than those in older mice, consistent with postnatal increases in myelination,[57] and also sustained for less than the duration of the stimulus (4 seconds). Spontaneous neural activity in the newborn brain echoed these spatial distributions with rare spontaneous events remaining localized and unilateral in younger mice, gradually spreading to recruit other regions bilaterally with increasing age (P10 and older). In the adult brain, complex bilateral patterns of brain-wide spontaneous neural activity were observed, consistent with the results of previous studies using voltage-sensitive dyes in the adult brain, and interpreted to represent brain-wide network activity.[15,34,37,39]

In the same mice, hemodynamics across development mirrored those seen in rats: No localized increases in blood flow were observed at P7, despite the presence of strong, localized neural activity. By P10, only small correlated increases in blood flow were observed. This relative lack of vascular responses to neural activity was consistent in awake and anesthetized mice, and for both stimulus-evoked and spontaneous neural activity.

These findings clearly demonstrate that neurovascular coupling is still maturing in the postnatal mouse brain. In fact, this conclusion is highly consistent with the extensive neural and vascular development that is known to occur in the postnatal brain[14,20,23,25,41] as elaborated below:

Arteries and veins only begin to take on their unique characteristics postnatally, which may limit the reactivity of cortical arteries.[43] Additionally, nitric oxide is an important mediator of vascular dilation, and endothelial nitric oxide synthase (eNOS) expression increases significantly postnatally.[10] Of note, recent work from our laboratory has suggested that the vascular endothelium plays an important role in the propagation of hemodynamic responses.[12] The resting tone of cerebral vasculature is also essential to the ability of vessels to dynamically regulate their diameter, and the aminergic systems which regulate resting vascular tone mature postnatally.[17]

Astrocytes, cells that surround both synapses and cerebral vasculature, have been proposed as important mediators of neurovascular coupling.[4,50] These cells are still maturing postnatally, establishing gap junction coupling across the cortex at postnatal day 11, fully maturing by postnatal day 21 in terms of size, branching, and connectivity, and reaching adult density levels by postnatal day 50 in rats.[6,44,48]

Pericytes have recently been demonstrated to play a role in neurovascular coupling.[18,19] These cells are also important in cerebrovascular patterning, stabilizing vessels during angiogenesis, which may limit their capacity to act as neurovascular coupling mediators. Additionally, pericytes share a basement membrane with endothelial cells in the adult brain; however, they may only establish these gap junctions postnatally.[16]

Finally, neural networks rapidly expand and refine postnatally with an initial period of synaptogenesis and axonal growth[11,30,51,52] followed by a period of

synaptic pruning.[23] During this period, the brain physically expands with the cortical surface area of the rodent brain more than doubling within the first 20 d of life.[53] These changes all suggest that the baseline metabolic demand of the newborn and early developing brain is different from that of the adult brain. Our results show that, in mice, establishment of coupling to hemodynamics occurs during the same developmental phase as neural network expansion.

An important question raised by our results is whether neural firing in the absence of hemodynamic responses in the neonatal brain has metabolic consequences. Prior studies have demonstrated higher levels of non-oxidative metabolism in the newborn brain compared to adults.[5,7] Although adult hemodynamics have been shown to occur independently of oxygen and glucose availability,[32,55] we sought to understand whether our results could be explained by a lower demand for oxygen to support neural activity in the early postnatal brain.

To examine the dynamics of oxygen use in the postnatal brain, flavin adenide dinuclueotide (FAD) was imaged. FAD is a cofactor in oxidative metabolism, which is fluorescent in its oxidized form and not in its reduced form[45] Stimulus-evoked increases in FAD fluorescence were observed at P7, indicating an increase in the rate of oxygen consumption. This FAD increase grew in both amplitude and duration with increasing age, and closely matched the truncated duration of the neural response to a 4 second stimulation in age-matched animals. These FAD increases both confirm an evoked demand for oxygen in the postnatal brain, and suggest that immature blood flow responses are not a consequence of oxygen-dependent signaling. Furthermore, in the immature brain, increases in FAD fluorescence were followed by decreases in fluorescence below baseline levels. This observation is consistent with the depletion of available oxygen and slowing of oxidative phosphorylation, suggesting that oxygen availability might place a limitation on the ability of the newborn brain to sustain prolonged neural activity. Prior studies have noted a prolonged refractory period after neural stimulation in early postnatal animals,[57] an observation which may relate to the inability of the newborn brain to dynamically regulate its oxygen supply.

Neurally-evoked cortical oxygen depletions were confirmed through analysis of hemoglobin oxygenation levels. After subtraction of a global hemodynamic component, local blood oxygenation levels in the immature brain were found to dip below baseline in response to neural activity – consistent with local oxygen consumption, and localized 'negative BOLD' responses reported in some of the earliest fMRI studies of the human infant brain.[9] Analysis of spontaneous 'resting-state' neural activity in the postnatal brain yielded similar results: After subtraction of global hemodynamic trends, spike-triggered averaging demonstrated localized deoxygenations occurring following spontaneous neural firing in P7-P8 mice. Hyperemia responses to spontaneous neural activity established in parallel with the development of stimulus-evoked neurovascular responses.

We conclude that without adult-like neurovascular coupling, the newborn brain experiences a unique metabolic state in which neural activity can lead to relative hypoxias in activated regions. In the mouse brain, this state coincides with extensive neural network expansion, seemingly a time of ever increasing oxygen demands. Although the machinery to actuate full hemodynamic responses may not be in place at this time of brain maturation, it is also possible that there may be some developmental benefit to this unique metabolic state. For example, hypoxia is known to be an important signaling mechanism in angiogenesis, with markers of hypoxia reliably triggering blood vessel growth into regions of high oxygen demand.[42,46] Recent studies have demonstrated that regional differences in the level of postnatal neural activity affect local vascular density.[28,54] The relative hypoxias identified in our work may provide a mechanism by which vascular growth is driven into frequently utilized regions thereby optimizing blood delivery in the mature brain, a mechanism also recently proposed by Lacoste & Gu.[29] Additional measurements of oxygen dynamics in the postnatal brain, including assessment of baseline oxygen availability, the role of capillary density, fetal hemoglobin and the trajectories of these factors over time, will provide a clearer picture of the developing brain's unique metabolic state.

The results of this study suggest that fMRI data should be interpreted with caution in younger subjects. Although our studies were performed in mice, and it is thus challenging to determine equivalent ages in humans, fMRI studies of term infants and even toddlers have reported smaller amplitude BOLD responses and/or inverted BOLD responses.[1,2,8,36,40,47,56] Our

results suggest that, depending on developmental stage and the contribution of global or systemic hemodynamic effects, fMRI results could exhibit a range of dynamics, with varying representation of underlying neural activity during early brain development. Oxygen consumption in the absence of hyperemia would be expected to manifest as small, negative BOLD responses. The developing ability of the brain to generate functional hyperemia would gradually counteract these negative BOLD responses, ultimately leading to positive responses. Although these considerations may complicate fMRI analysis, analyzed objectively, such trends could potentially chart a trajectory of normal postnatal neurovascular development. Accordingly, fMRI may prove to be a valuable tool to assess neurovascular development in the human brain and provide new biomarkers of disease states in which altered neurovascular development might play a role.[24]

In summary, our study brings new insights into the functional development of the postnatal brain, both in terms of the pattern of neural circuit elaboration and the development of neurovascular coupling. We demonstrated the use of wide-field GCaMP imaging to study the establishment of long-range neural connectivity in the postnatal brain, and showed that these patterns of increasing connectivity are consistent across both stimulus-evoked and resting state conditions.[35] Throughout this period of neural circuit expansion, the early postnatal brain has a seemingly more delicate oxygenation balance than the adult brain, which could have important implications for the usage of oxygen therapies in premature and term infants. Lastly, this study demonstrates that the brain is still developing the ability to actuate hemodynamic responses postnatally, despite the presence of oxygen-consuming neural activity.

A critical next step in this research will be to map these observed stages of neurovascular development to human brain maturation. We propose that this aspect of postnatal brain maturation may play an unexplored role in abnormal brain development, and that reinterpretation of fMRI data could yield novel biomarkers for both normal and abnormal trajectories of human brain development.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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