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Putting the Neuro in Neurovascular Coupling

Kyle P. Lillis, PhD

Differential Contribution of Excitatory and Inhibitory Neurons in Shaping Neurovascular Coupling in Different Epileptic Neural States

Lim H-K, You N, Bae S, et al. *J Cereb Blood Flow Metab*. 2021;41:1145-1161. doi:10.1177/0271678X20934071.

Understanding the neurovascular coupling (NVC) underlying hemodynamic changes in epilepsy is crucial to properly interpreting functional brain imaging signals associated with epileptic events. However, how excitatory and inhibitory neurons affect vascular responses in different epileptic states remains unknown. We conducted real-time in vivo measurements of cerebral blood flow (CBF), vessel diameter, and excitatory and inhibitory neuronal calcium signals during recurrent focal seizures. During preictal states, decreases in CBF and arteriole diameter were closely related to decreased γ-band local field potential (LFP) power, which was linked to relatively elevated excitatory and reduced inhibitory neuronal activity levels. Notably, this preictal condition was followed by a strengthened ictal event. In particular, the preictal inhibitory activity level was positively correlated with coherent oscillating activity specific to inhibitory neurons. In contrast, ictal states were characterized by elevated synchrony in excitatory neurons. Given these findings, we suggest that excitatory and inhibitory neurons differentially contribute to shaping the ictal and preictal neural states, respectively. Moreover, the preictal vascular activity, alongside with the γ-band, may reflect the relative levels of excitatory and inhibitory neuronal activity, and upcoming ictal activity. Our findings provide useful insights into how perfusion signals of different epileptic states are related in terms of NVC.

Mesoscopic Mapping of Ictal Neurovascular Coupling in Awake Behaving Mice Using Optical Spectroscopy and Genetically Encoded Calcium Indicators

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Unambiguously identifying an epileptic focus with high spatial resolution is a challenge, especially when no anatomic abnormality can be detected. Neurovascular coupling (NVC)-based brain mapping techniques are often applied in the clinic despite a poor understanding of ictal NVC mechanisms, derived primarily from recordings in anesthetized animals with limited spatial sampling of the ictal core. In this study, we used simultaneous wide-field mesoscopic imaging of GCamp6f and intrinsic optical signals (IOS) to record the neuronal and hemodynamic changes during acute ictal events in awake, behaving mice. Similar signals in isoflurane-anesthetized mice were compared to highlight the unique characteristics of the awake condition. In awake animals, seizures were more focal at the onset but more likely to propagate to the contralateral hemisphere. The HbT signal, derived from an increase in cerebral blood volume (CBV), was more intense in awake mice. As a result, the ""epileptic dip"" in hemoglobin oxygenation became inconsistent and unreliable as a mapping signal. Our data indicate that CBV-based imaging techniques should be more accurate than blood oxygen level dependent (BOLD)-based imaging techniques for seizure mapping in awake behaving animals.

Commentary

The next best hope for treatment of patients with drug-resistant epilepsy is often surgical resection. Because it is invasive and irreversible, substantial effort is being directed toward improving the pre-surgical planning to minimize the disruption caused by the resection and optimize the anti-epileptic efficacy of the surgery. In a general sense, the volume of brain targeted for resection is the region thought to contain the cell bodies of neurons that constitute the epileptic focus, from which seizures emerge. However, defining the focus is not always straightforward, in part because of deficiencies in the clinically available tools for

Massachusetts General Hospital, USA

identifying a seizure focus. For example, even the most invasive electrographic recording configurations provide incomplete spatial sampling of the brain. Imaging modalities such as single-photon emission computed tomography (SPECT) and functional magnetic resonance imaging (fMRI) afford a comprehensive image of the brain. However these techniques are measures of blood flow and oxygenation, and thus indirectly linked to neuronal activity via neurovascular coupling (NVC). Inferring the locus of neuronal activity from perfusion-based measures will require a better understanding of neurovascular coupling.

Recently, it has become technically feasible in animal studies, to simultaneously record hemodynamic signals and electrical activity in large-scale neuronal populations to generate better models of neurovascular coupling. Here, we highlight two such studies, both of which make use of genetically encoded calcium indicators to image neuronal activity, while simultaneously optically measuring cerebral blood flow during focal cortical electrographic seizures induced by chemical convulsant 4-aminopyridine (4-AP). In the first study,[1] investigated the correlations among preictal and ictal changes in local field potential, blood flow (using laser Doppler flowmetry), vessel diameter (using 2-photon microscopy), and activity in individual neurons (using 2-photon imaging of genetically encoded calcium indicators targeted to either excitatory or inhibitory neurons). Preictally, they found that there is a dip in cerebral blood flow, a dip in baseline calcium in interneurons, an increase in interneuron synchronization, and a decrease in gamma power in the LFP signal. Ictally, there was an increase in blood flow, an increase in vessel diameter, and an increase in excitatory neuron synchronization. Furthermore, they found that more severe seizures correlated with a larger preictal dip in cerebral blood flow, lower preictal interneuron synchronization, and a lower preictal gamma power.

In the second study,[2] used widefield mesoscopic calcium imaging to capture the propagation of neuronal activity across the surface of the entire neocortex and intrinsic optical imaging to measure total hemoglobin (HbT), oxygenated hemoglobin (HbO), and deoxygenated hemoglobin (Hbr). While the experiments in the Lim et al study were all performed under anesthesia, Yang et al demonstrated that anesthesia had a substantial impact on ictal neurovascular coupling. Specifically, anesthetized mice exhibited a previously observed "epileptic dip" in HbO,[3] which was intriguing due to the relevance of HbO signal to the clinically available fMRI BOLD signal.[4] However, awake mice produced a more robust neurovascular response that maintained a well-oxygenated blood supply during the seizure, suggesting that the "epileptic dip" may be a nuance of experimental protocol. There was however a distinct, consistent increase in ictal HbT signal that correlated well with both the size and shape of the calcium activity in both awake and anesthetized animals, suggesting that measures of total blood flow may be good proxies for neuronal activity during seizure.

Experiments aimed at imaging seizures in head-fixed animals (as in the two studies highlighted here) are complicated by the fact that spontaneous recurrent seizures are rare in most animal models of epilepsy. Thus, to guarantee capturing a seizure during an acute imaging session, it was necessary to induce seizures using the chemical convulsant 4-AP. 4-AP is an A-type potassium channel blocker, which differentially affects interneurons,[5] as well as potentially affecting vascular smooth muscle cells directly, somewhat limiting the breadth of conclusions that can be drawn in both of the highlighted studies. For example, in the study by Lim et al, it was observed that interneuron synchronization preceded seizure onset. Since interneuron synchronization is a well-documented consequence of 4-AP application, even in the absence of seizures,[6] the authors were unable to conclude that preictal interneuron synchronization is related to spontaneous ictogenesis. They were, however, able to characterize the hemodynamic and field potential signatures associated with interneuron synchronization, which may provide mechanistic insight into experimental observations that can be made during spontaneous seizure. Yang et al faced a similar challenge interpreting differences in ictogenesis between anesthetized and awake animals, as both the convulsant (4-AP) and the anesthesia (isoflurane) preferentially alter GABAergic inhibition. Thus, it was difficult to know whether the anesthesia altered neurovascular coupling directly or interfered with the convulsant action of 4-AP to cause a differential alteration in cerebral blood flow. To control for this, the authors mimicked in awake animals the hypothesized anesthesia-induced disruption to neurovascular coupling using illumination of intravenous fluorescein dextran, which locally restricts vasodilation. They found that this opto-pharmacological manipulation mimics the ictal hemodynamic response observed in anesthetized animals.

Independent of mechanism of ictogenesis, both highlighted studies describe a dramatic increase in cerebral blood flow at the site of seizure initiation. Clinical tools for measuring CBF include single photon emission computed tomography (SPECT) and functional magnetic resonance imaging (fMRI). Yang et al demonstrated substantial variability in the ictal kinetics of blood oxygenation, suggesting that the blood-oxygen level dependent (BOLD) signal measured by using fMRI may be an unreliable indicator of seizure focus. Rather, measurements of total blood flow, such as ictal SPECT, are more valuable at localizing seizure foci. Lim et al demonstrated that blood vessel diameter is also directly correlated to total blow flow. Recent advances in arterial spin labeling (ASL) have made it feasible to measure blood vessel diameter using MRI, which is advantageous over ictal SPECT in that it requires no radioactive dyes and is high resolution. Indeed, a newly published retrospective study[7] and several recent papers[8-10] support the notion that ASL MRI is useful for establishing prognosis and localizing a focus in patients with epilepsy, although the predictive power of ASL depended on how soon after a seizure images were acquired.

Mesoscopic imaging of neuronal activity and hemodynamics in the study by Yang et al demonstrated that localized elevation in total blood flow tracks the propagation of neuronal activity associated with a seizure. Unfortunately, both ictal SPECT and ASL MRI have a limited ability to capture the time

window corresponding to ictogenesis. Ictal SPECT depends on well-timed injection of a radioactive tracer, after a seizure has been detected, to capture the pattern of blood flow during the window of time during which the tracer is present in the blood. ASL MRI cannot be performed during seizure and must be performed immediately post-ictally. Thus, ictal SPECT and ASL MRI can, at best, detect the hemodynamic changes associated with the entire propagation path of the seizure, with localization of the onset zone depending on the timing between seizure onset and dye injection (for SPECT), the interval between seizure and MRI (for ASL MRI), and the presence of secondary generalization.[11] This information can however be used to guide placement of invasive electrical recordings, which would be capable of refining the seizure onset zone using signatures of an epileptic focus such as those identified by Lim et al. (e.g., increased preictal interneuron synchronization and decreased preictal gamma power). Ongoing studies with simultaneous measurement of epileptiform neuronal activity and cerebral blood flow, like those highlighted here, will continue to inform the use of (also rapidly advancing) clinical neuroimaging modalities to improve outcomes of patients undergoing surgical resection for the treatment of epilepsy.

ORCID iD

Kyle P. Lillis https://orcid.org/0000-0003-0219-8113

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