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Coupling between gamma-band power and cerebral blood volume during recurrent acute neocortical seizures

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ABSTRACT

Characterization of neural and hemodynamic biomarkers of epileptic activity that can be measured using non-invasive techniques is fundamental to the accurate identification of the epileptogenic zone (EZ) in the clinical setting. Recently, oscillations at gamma-band frequencies and above (>30 Hz) have been suggested to provide valuable localizing information of the EZ and track cortical activation associated with epileptogenic processes. Although a tight coupling between gamma-band activity and hemodynamic-based signals has been consistently demonstrated in non-pathological conditions, very little is known about whether such a relationship is maintained in epilepsy and the laminar etiology of these signals. Confirmation of this relationship may elucidate the underpinnings of perfusion-based signals in epilepsy and the potential value of localizing the EZ using hemodynamic correlates of pathological rhythms. Here, we use concurrent multi-depth electrophysiology and 2-dimensional optical imaging spectroscopy to examine the coupling between multi-band neural activity and cerebral blood volume (CBV) during recurrent acute focal neocortical seizures in the urethane-anesthetized rat. We show a powerful correlation between gamma-band power (25–90 Hz) and CBV across cortical laminae, in particular layer 5, and a close association between gamma measures and multi-unit activity (MUA). Our findings provide insights into the laminar electrophysiological basis of perfusion-based imaging signals in the epileptic state and may have implications for further research using non-invasive multi-modal techniques to localize epileptogenic tissue.

Introduction

Understanding the effects of epilepsy on the neurovascular unit is fundamental to elucidating the pathophysiology of the disease and for predicting, identifying and localizing epileptic activity. In medically intractable focal epilepsies, the surgical removal of epileptogenic tissue remains the most promising form of treatment. However, successful post-operative outcomes rely on an accurate delineation of the epileptogenic zone (EZ), defined as "the minimum amount of cortex that must be resected (inactivated or completely disconnected) to produce seizure freedom" (Luders et al., 2006). As a result, there has been a great deal of interest in characterizing potential biomarkers of epileptogenic networks, particularly those that may be measured using non-invasive techniques in order for there to be an appreciable clinical application. Recent research, due in part to the advent of powerful digital broad-band electroencephalogram (EEG) systems, has suggested that pathological neural oscillations at gamma-frequencies and above (>~30 Hz) are a valuable indicator of epileptogenic tissue in both neocortical and mesiotemporal regions (Andrade-Valenca et al., 2011; Bragin et al., 1999; Jirsch et al., 2006; Medvedev et al., 2011; Worrell et al., 2004; Zijlmans et al., 2012). Furthermore, the clinical amenability of blood-oxygenation level dependent (BOLD) functional magnetic resonance imaging (fMRI) has also led to it being combined with EEG to localize hemodynamic correlates of electrophysiological epileptic events and aid identification of the EZ (Gotman et al., 2006; Salek-Haddadi et al., 2006; Thornton et al., 2010). However, faithful interpretation of fMRI data in terms of underlying neural activation relies on a detailed understanding of neurovascular coupling, which can vary spatially across laminae (Goense et al., 2012) and brain-regions (Devonshire et al., 2012). A typical assumption that is made to facilitate analysis and interpretation of neuroimaging data is that neurovascular coupling is invariant across health and disease. Yet, since pathological brain states such as epilepsy may be associated with altered neurovascular coupling characteristics, the validity of this assumption has been the subject of much investigation with varying methodologies and results (Hamandi et al., 2008; Harris et al., 2013; Ma et al., 2012; Mirsattari et al., 2006; Stefanovic et al., 2005; Voges et al., 2012; Zhao et al., 2009). Further

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research is therefore needed to elucidate the extent to which neurovascular coupling characteristics are preserved in the epileptic state, in order to improve interpretation of neuroimaging data in the disorder and ensure the legitimacy of routine assumptions which make such techniques more practicable. Under normal conditions, local field potential (LFP) activity, and in particular the gamma-band component of the LFP, is thought to be a more reliable predictor of perfusion-based signals than multi-unit spiking activity (MUA), although the neurophysiological basis for this remains a topic of intense research (Goense and Logothetis, 2008; Logothetis et al., 2001; Niessing et al., 2005; Nir et al., 2007; Sumiyoshi et al., 2012). These reports underscore the potential value for non-invasive perfusion-based neuroimaging studies to probe cognitive processes. However, while there are considerable reports of pathological gamma activity in clinical (Doesburg et al., 2013; Fisher et al., 1992; Herrmann and Demiralp, 2005; Wu et al., 2008) and experimental (Köhling et al., 2000; Medvedev, 2002; Traub et al., 2005) epilepsy, whether pathological gamma activity is preferentially coupled with hemodynamic signals in the epileptic state is untested. Confirmation of this relationship would suggest a common neural driver of perfusion-related signals in health and epilepsy and, since gamma-band neural measures are strongly co-localized to the EZ, highlight the potential for EEG-neuroimaging paradigms to further delineate the EZ through localization of hemodynamic correlates of pathological gamma activity.

With the above in mind, we sought to examine the laminar electrophysiological underpinnings of seizure-related hemodynamic signals during recurrent ictal discharges in the urethane-anesthetized rat using the well-established 4-aminopyridine (4-AP) acute model of focal neocortical epilepsy. This model provides an ideal framework to examine neurovascular coupling in epilepsy, since seizures recur spontaneously and evolve through similar stages as spontaneous events in the human brain (Harris et al., 2013; Ma et al., 2012; Zhao et al., 2009). Using simultaneous high resolution two-dimensional optical imaging spectroscopy (2D-OIS), we show a powerful linear correlation between cerebral blood volume (CBV) and gamma-band power across all cortical laminae, which was most pronounced in layer 5. Furthermore we show that seizure-related gamma-band activity was most closely coupled to multi-unit activity in deeper laminae nearest the presumed EZ. Our findings provide insights into the laminar evolution of neural measures during recurrent seizures and perfusion-based imaging of seizure events for clinical purposes.

Materials and methods

All procedures described were approved by the UK Home Office under the Animals (Scientific procedures) Act of 1986. Female hooded Lister rats (total $N = 8$ weighing 260–400 g) were kept in a 12-hr dark/light cycle environment at a temperature of 22 °C, with food and water provided ad libitum. The animals were anesthetized with urethane (1.25 g/kg) intraperitoneally, with atropine being administered subcutaneously (0.4 mg/kg) to reduce mucous secretions during surgery. Depth of anesthesia was monitored throughout and supplementary doses of urethane (0.1 ml) were administered if necessary. We chose to use urethane anesthesia (ethyl carbamate) as it preserves excitatory/inhibitory synaptic transmission, unlike many general anesthetics (Sceniak and MacIver, 2006) and provides a persistent and steady depth of surgical anesthesia, reminiscent of natural sleep (Pagliardini et al., 2013). Moreover, neurovascular coupling is preserved under urethane anesthesia, not only insofar that a single whisker deflection elicits a hemodynamic response in the rat somatosensory cortex (Berwick et al., 2008) but also during CO_2 challenge (Kennerley et al., 2011), which has led to it being a common choice in neuroimaging studies in rat and neurovascular coupling characteristics to be well-documented during both task-related events (e.g. Berwick et al., 2008; Devor et al., 2005; Harris et al., 2013; Huttunen et al., 2008; Kennerley et al., 2011) and resting-state fluctuations (Bruyns-Haylett et al., 2013). It has also been shown that neither the spatial–temporal pattern of the evoked hemodynamic response (Devor et al., 2005), nor the relationship between neural activity and BOLD fMRI responses (Huttunen et al., 2008), differs between urethane and alpha-chloralose, another anesthetic routinely used in fMRI studies and whose neurovascular coupling characteristics in turn are comparable to a number of other agents (Franceschini et al., 2010).

A homoeothermic blanket (Harvard Apparatus) and rectal probe were used to maintain core body temperature at 37 °C. The animals were tracheotomized to allow artificial ventilation with pressurized room air and monitoring of end-tidal CO_2. Blood-gas and end-tidal CO_2 measurements were used to adjust ventilator parameters and maintain the animal within normal physiological limits (average values: $pO_2 = 92$ mm Hg $\pm$ 9.2, $pCO_2 = 31$ mm Hg $\pm$ 5.3). The left femoral artery and vein were cannulated to allow the measurement of arterial blood pressure and phenylephrine infusion (0.13 to 0.26 mg/h to maintain normotension between 100 and 110 mm Hg), respectively. The animal was secured in a stereotaxic frame (throughout experimentation), and the skull overlying coordinates 2 mm anterior to lambda to 2 mm anterior of bregma, and from 1 to 6 mm from midline, was thinned to translucency, in order to expose the somatosensory cortex. A circular plastic 'well' was located over the cranial window and filled with saline to reduce optical specularities from the brain surface during imaging.

The potassium channel blocker 4-aminopyridine (4-AP, Sigma, 15 mM, 1 μl) was used to elicit focal seizure-like discharges (Ma et al., 2012; Zhao et al., 2009) in the right vibrissal cortex (RVC). After a 30 s baseline recording period, 4-AP was infused at a depth of 1500 μm (i.e. layer 6) via a fluidic port on the multi-channel microelectrode (Neuronexus Technologies, Ann Arbor, MI, USA) over a 5 minute period (0.2 μl/min) using a 10 μl Hamilton syringe and syringe pump (World Precision Instruments Inc., FL, USA). Recordings were made for 50 min following regional injection of 4-AP.

Two-dimensional optical imaging spectroscopy (2D-OIS) was employed to produce 2D images over time of total hemoglobin concentration (Hbt). Under the reasonable assumption of a constant hematocrit, Hbt can be further interpreted as cerebral blood volume (CBV) and will therefore be referred to as the latter in ensuing text (with the exception of when reporting micro-molar concentrations of Hbt). This technique has been described in detail previously (Berwick et al., 2008). Briefly, illumination of the cortex was conducted at four different wavelengths (495 $\pm$ 31 nm, 559 $\pm$ 16 nm, 575 $\pm$ 14 nm and 587 $\pm$ 9 nm FWHM) using a Lamda DG-4 high speed filter changer (Sutter Instrument Company, Novata, CA, USA). Image data were recorded using a Dalsa 1M30P camera (Billerica, MA, USA, each pixel representing ~75 μm^2), synchronized to the filter switching (effective frame rate of 8 Hz/wavelength). These were then subjected to spectral analysis consisting of a path length scaling algorithm (PLSA) employing a modified Beer–Lambert law in conjunction with a path-length correction factor for each wavelength used, based on Monte Carlo simulations of light transport through tissue. After each experiment, a 'dark baseline' image data-set was obtained, in which the cortex was not illuminated, and later subtracted from 2D-OIS data in order to account for electrical noise arising from the camera system.

In order to localize the region of the somatosensory 'barrel' cortex and guide implantation of the multi-channel electrode into the said area, a preparatory 2D-OIS experiment was conducted in each animal. This technique has also been described in detail previously (Berwick et al., 2008). Briefly, the left mystacial pad was electrically stimulated using subcutaneous electrodes (30 trials, 2 s, 5 Hz, 1.2 mA intensity and 0.3 ms pulse width) and recorded image data subjected to the aforementioned spectral analysis. Spatiotemporal changes in Hbt were analyzed using statistical parametric mapping (SPM) in which each pixel's timeseries was regressed against a design matrix representing a direct current (DC) offset, ramp, and 'boxcar' function of the same duration as the stimulation. This produced a z-score activation map in which pixels within 50% of the maximum z-score were used to identify

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