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Rhythmic 3–4 Hz discharge is insufficient to produce cortical BOLD fMRI decreases in generalized seizures

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ABSTRACT

Absence seizures are transient episodes of impaired consciousness accompanied by 3–4 Hz spike–wave discharge on electroencephalography (EEG). Human functional magnetic resonance imaging (fMRI) studies have demonstrated widespread cortical decreases in the blood oxygen-level dependent (BOLD) signal that may play an important role in the pathophysiology of these seizures. Animal models could provide an opportunity to investigate the fundamental mechanisms of these changes, however they have so far failed to consistently replicate the cortical fMRI decreases observed in human patients. This may be due to important differences between human seizures and animal models, including a lack of cortical development in rodents or differences in the frequencies of rodent (7–8 Hz) and human (3–4 Hz) spike–wave discharges. To examine the possible contributions of these differences, we developed a ferret model that exhibits 3–4 Hz spike–wave seizures in the presence of a sulcated cortex. Measurements of BOLD fMRI and simultaneous EEG demonstrated cortical fMRI increases during and following spike–wave seizures in ferrets. However unlike human patients, significant fMRI decreases were not observed. The lack of fMRI decreases was consistent across seizures of different durations, discharge frequencies, and anesthetic regimes, and using fMRI analysis models similar to human patients. In contrast, generalized tonic–clonic seizures under the same conditions elicited sustained postictal fMRI decreases, verifying that the lack of fMRI decreases with spike–wave was not due to technical factors. These findings demonstrate that 3–4 Hz spike–wave discharge in a sulcated animal model does not necessarily produce fMRI decreases, leaving the mechanism for this phenomenon open for further investigation.

Introduction

Absence seizures are a form of generalized epilepsy that present as brief staring spells marked by an abrupt 3–4 Hz spike-and-wave discharge on electroencephalography (EEG). These events can occur up to hundreds of times per day and may disrupt normal cognitive and psychosocial development in children (Camfield and Camfield, 2002; Crunelli and Leresche, 2002; Wirrell et al., 1996, 1997). Although effective medical treatments are available, this is not a benign condition because a substantial proportion of patients have persistent deficits in attention even when seizures are not occurring, and about 30% will not outgrow their seizures (Camfield and Camfield, 2002; Wirrell et al., 1997). To improve treatment of absence seizures, it is necessary to understand the fundamental pathophysiology of associated changes in brain networks. This effort has been greatly advanced in recent years by simultaneous EEG–functional magnetic imaging (fMRI), which has provided maps of brain activity changes associated with absence seizures. Interestingly, the most consistent cortical change observed in these studies has been blood oxygenation level-dependent (BOLD) fMRI decreases in widespread regions, including the default mode network (Aghakhani et al., 2004; Archer et al., 2003; Salek-Haddadi et al., 2003). The fMRI decreases persist for over 20 s after seizures end and do not fit the expected hemodynamic response function for the brief EEG discharges (Bai et al., 2010; Carney et al., 2010). Thus, although fMRI maps of brain activity in human absence seizures are available, the most prominent and consistent cortical changes remain unexplained.

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Because fMRI signals are only indirectly related to neuronal activity and in some cases can be misleading (Mishra et al., 2011; Schridde et al., 2008), it is crucial to investigate the relationship between fMRI changes in absence seizures and underlying neurophysiology to correctly interpret BOLD fMRI mapping. Animal models have provided some insights into these relationships. For example, direct multi-unit and local field potential recordings in rodent models have shown that cortical and thalamic fMRI increases are associated with transient increases in neuronal action potential firing and synaptic activity during spike–wave seizures, as expected (Mishra et al., 2011; Nersesyan et al., 2004a, 2004b). However, animal models have yet to shed light on the mechanism of cortical fMRI decreases that predominate in human studies. This may be because previous rodent and non-human primate models have only inconsistently shown cortical fMRI decreases, and they are typically minor compared to the widespread decreases observed in humans (Brevard et al., 2006; David et al., 2008; Mishra et al., 2013, 2011; Nersesyan et al., 2004a, 2004b; Tenney et al., 2003; Tenney et al., 2004a, 2004b).

There are a number of possible explanations for why animal models have so far failed to replicate the widespread cortical fMRI decreases seen in human studies. One important factor that could influence neuronal activity and cortical fMRI signals is that the spike–wave discharge frequency in human absence seizures is typically 3–4 Hz, whereas it is 7–8 Hz in most rodent models (Depaulis and van Luijtelaar, 2005; Motelow and Blumenfeld, 2009; Sitnikova and van Luijtelaar, 2007). Other interspecies differences from humans such as the lack of sulci and gyri in rodents and marmosets, and the lack of intrinsic inhibitory neurons in the ventroposterior thalamic nuclei of rats (Barbaresi et al., 1986) may also play a role.

To address the potential contributions of these factors to the lack of fMRI decreases we sought to develop a new animal model with 3–4 Hz spike–wave seizures more closely resembling human patients. Studies in non-lisencephalic animals such as felines (Prince and Farrell, 1969) have been more successful in replicating the 3–4 Hz electrographic activity observed in humans. Ferret thalamic slices have also been shown to spontaneously generate 3–4 Hz spike-and-wave discharges when exposed to gamma-aminobutyric acid A ($GABA_A$) antagonists *in vitro* (Blumenfeld and McCormick, 2000; Lee et al., 2005; von Krosigk et al., 1993). We therefore decided to administer a $GABA_A$ antagonist to ferrets *in vivo* to develop a new model of spike–wave seizures that more closely resembles human patients. This approach enabled us to investigate a number of possible contributing factors to fMRI decreases, including typical 3–4 Hz discharges, presence of cortical sulci and gyri, various anesthetic regimens, duration and amplitude of seizures, comparison to larger tonic–clonic seizures, and use of model-based and timecourse analysis methods similar to human studies.

Methods

All experimental procedures were approved by the Yale University Institutional Animal Care and Use Committee and are in agreement with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Neuroimaging experiments were performed on adult female ferrets (*Mustela putorius furo*) obtained from Marshall BioResources and housed for a minimum of 3 days prior to use. EEG–fMRI data were obtained during seizures from 17 animals, and data were selected for analysis from 13 animals (425 g ± 70) based on appropriate blood gas and systemic parameters in the physiological range as described previously (Englot et al., 2008; Schridde et al., 2008) .

Animal preparation and surgery

All animals underwent initial sedation in an induction chamber ventilated with 3–4% isoflurane. Following tracheostomy and initiation of artificial ventilation (70% N_2O, 30% O_2), anesthesia was continued with either 0.5–3.0% isoflurane or intramuscular injections of ketamine/xylazine (30/1 mg/kg). In one experiment, isoflurane was supplemented with intravenous injections of 2% α-chloralose solution. Depth of anesthesia was monitored using heart rate, blood pressure, and scalp electroencephalogram (EEG). To reduce movement artifact during neuroimaging experiments, paralysis was induced using intravenous injections of D-tubocurarine chloride (0.5 mg/kg).

An intravenous femoral line was installed for anesthetic and experimental injections. Continuous monitoring of arterial blood pressure and heart rate was facilitated through cannulation of a femoral artery. This line was also used to collect blood samples for periodic evaluation with an ABL 5 blood gas analyzer (Radiometer Analytical, Lyon, France). Physiological values (pO_2, pCO_2, and pH) were maintained as in prior studies (Mishra et al., 2011; Schridde et al., 2008) through adjustment of respiration rate and intravenous injections of sodium bicarbonate solution. Body temperature was recorded with a digital rectal probe and maintained at 37 °C using a warm-water heating pad.

Experimental procedures

Bench electrophysiology experiments were performed to develop spike–wave and tonic–clonic seizure models using ferrets. These methods were replicated during subsequent neuroimaging experiments. Carbon-filament electrodes (1 mm) were installed subcutaneously for the measurement of scalp EEG as described previously (Mishra et al., 2011). The electrodes were oriented in the coronal plane over frontal and occipital regions. Electrographic signals were acquired in differential mode, amplified (×100) and filtered (1–30 Hz) using a Model 79D Data Recording System (Grass Instruments Co., Quincy, MA). A CED Micro 1401 with Spike 2 software (Cambridge Electronic Design, Cambridge, UK) was used to digitize EEG data at a 1000 Hz sampling rate for later analysis.

A custom-designed plastic holder was used for head fixation at the center of the surface coil during fMRI recordings. Each experimental session included multiple seizure episodes. Seizures were induced by intravenous injection of the $GABA_A$ antagonist bicuculline (0.12 mg/kg ± 0.01 for spike–wave seizures or 0.27 mg/kg ± 0.05 for tonic–clonic seizures). All MR data were obtained on a modified 9.4 T system with a Varian (Agilent Technologies, Santa Clara, CA) spectrometer using a custom-built ^1H radiofrequency surface coil (5 by 3 cm coil diameter). During fMRI recordings, the ferret was positioned prone in a specially designed plastic holder in such a manner that the modified surface coil would be at the top of the ferret's head. The magnetic field homogeneity was optimized by localized shimming to yield a typical water spectrum line-width of less than 20 Hz across the slices. High resolution anatomical scans were acquired using fast spin-echo contrast multi-slice sequences (FSEMS). For each animal 10, 15, or 20 slices were captured in the coronal plane with the following parameters: repetition time (TR) = 4 s, echo time (TE) = 20 ms, field of view (FOV) = 32 × 32 mm, matrix size = 128 × 128, for an in-plane resolution of 500 × 500 μm and slice thickness = 1000 or 2000 μm (no gap).

The custom surface head coil used for these experiments allowed excellent signal/noise ratio fMRI data acquisition for the cortex (SNR > 30) but not of deeper subcortical structures. BOLD signal was acquired using either spin-echo echo-planar imaging (8 animals) or gradient-echo echo-planar imaging (4 animals). Functional MRI measurements were performed with 10, 15 or 20 coronal slices in the same plane as anatomical images, using the following parameters: TR = 2000 ms, TE = 34 ms, FOV = 32 × 32 mm, 64 × 64 matrix size resulting in an in-plane resolution of 500 × 500 μm and slice thickness = 1000 or 2000 μm (no gap). The slices were acquired over 2000 ms, followed by a 1 or 2 s pause before the next image onset so that EEG could readily be interpreted during data acquisition as described previously (Mishra et al., 2011; Nersesyan et al., 2004a, 2004b); time between onset of consecutive image acquisitions was therefore 3 or 4 s. Between 300 and 900 images were acquired per