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It Matters Who Starts the Fire in Mesial Temporal Lobe Epilepsy

CA3 Principal Cell Activation Triggers Hypersynchronous-Onset Seizures in a Mouse Model of Mesial Temporal Lobe Epilepsy

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Mesial temporal lobe epilepsy (MTLE) is the most common form of focal epilepsy, and it is characterized by seizures that are often refractory to medications. Seizures in MTLE have two main patterns of onset that have been termed hypersynchronous (HYP) and low-voltage fast (LVF) and are believed to mainly depend on the activity of excitatory principal cells and inhibitory interneurons, respectively. In this study, we investigated whether unilateral open-loop optogenetic activation of CaMKII-positive principal cells in the hippocampus CA3 region favors the generation of spontaneous HYP seizures in kainic acid-treated (KA) CaMKII-ChR2 mice. Optogenetic activation of CA3 principal cells (1 Hz, 180 s ON, 220 s OFF) was implemented for 15 days after KA-induced status epilepticus. We found that both LVF and HYP seizures occurred in nonstimulated CaMKII-ChR2 ($n = 6$) and stimulated CaMKII-Cre ($n = 5$) mice. In contrast, optogenetic activation of principal cells in CaMKII-ChR2 mice ($n = 5$) triggered only HYP seizures that were characterized by high fast ripple (250–500 Hz) rates during the pre-ictal and ictal periods. These results provide firm evidence that in MTLE spontaneous seizures with different onset patterns depend on distinct neuronal network mechanisms of generation. They also demonstrate that HYP seizures occurring in vivo along with their associated fast ripples depend on the activity of principal cells in the CA3 region. NEW & NOTEWORTHY Previous evidence suggested that different seizure onset patterns rely on the activity of distinct neuronal populations. In this study, we show for the first time that in vivo optogenetic stimulation of CaMKII principal cells in kainic acid-treated mice triggers hypersynchronous-onset seizures that are associated with fast ripples. Our findings indicate that in patients with predominant HYP-onset seizures, anticonvulsant treatments should be aimed at limiting the firing of principal neurons in the seizure onset zone.

Commentary

Our inability to control seizures in approximately 30% to 40% of the patients with mesial temporal lobe epilepsy (MTLE) remains a significant clinical challenge. Despite the introduction of new anti-seizure medications and deep brain stimulation paradigms, this core population of patients remains untreatable. The development of more effective therapeutic approaches is dependent on better identification of the abnormal cellular networks responsible for seizure development. Historically, the EEG has been the definitive tool for the diagnosis of epilepsy. Initially, the identification of pathologic electrographic activity was limited to interictal spikes and ictal activity. With improvements in sampling rates, resolution, and depth electrode recording 2 patterns of seizure onset have been identified in patients with MTLE[1,2] and in experimental models of epilepsy.[3,4] Hypersynchronous (HYP) onset seizures are characterized by repetitive high-amplitude low-frequency spikes ($\sim$2 Hz) lasting less than 5 seconds and are thought to be the result of HYP activity in excitatory principal neurons.[1-3,5,6] Low-voltage fast (LVF) onset seizures are characterized by a single spike followed by low-amplitude high-frequency activity in the gamma range with experimental evidence suggesting they result from HYP inhibitory neuron activity.[4-7] There is a strong correlation between the type of seizure onset and the pattern and distribution of neuronal loss in the accompanying anatomical lesion.[1,2] Hypersynchronous onset seizures tend to be focal in origin, associated with classic hippocampal sclerosis while LVF onset seizures tend to be associated with more diffuse and widespread cell loss, often involving extratemporal areas and more likely to spread to other structures.[1,2] The different types of seizure onset are also associated with different types of pathological high-frequency oscillations (HFOs).[3,4] Low-voltage fast seizures are associated with ripples (80-200 Hz) while HYP onset seizures are associated with fast ripples (250-500 Hz). This relationship between the type of seizure onset and type of pathological HFO is important since HFOs

have been hypothesized to be better biomarkers for the identification of the location of seizure onset than interictal spikes.[5] Collectively, these differences between the 2 different types of seizure onset suggest 2 different cellular networks are generating each type of seizure.[8] A better understanding of the pathological cellular networks responsible for the generation of each type of seizure onset has the potential to improve identification of the epileptogenic zone for more refined surgical resection and the development new pharmacologic therapies targeted to the individual patient's network defect.

The highlighted study[9] was well-designed to characterize the role of CA3 pyramidal neurons in the generation of seizures with the different types of ictal onset in vivo. It builds upon the results of previous experimental and clinical studies that suggest HYP onset seizures are the result of excitatory neuronal activity. Optogenetic stimulation combined with 24/7 video/EEG monitoring was performed in transgenic mice treated systemically with kainic acid to induce status epilepticus (SE). The transgenic mice were generated such that CaMKII-positive principal neurons expressed Cre activated channelrhodopsin-EYFP (CaMKII-ChR2) and control mice expressed CaMKII-Cre. Immunohistochemistry confirmed that staining for CaMKII-ChR-EYFP was negative in CaMKII-Cre mice and only expressed in CA3 pyramidal cells in CaMKII-ChR2 mice. No staining was observed in GAD-67 expressing GABAergic inhibitory neurons. Optogenetic activation of CA3 was initiated 3 hours after SE and continued for 15 days during the preictal period. Video/EEG recording was initiated before SE and continued for an additional 10 days after optogenetic stimulation was discontinued. Three experimental groups of mice were examined: (1) CaMKII-ChR2—optogenetically stimulated; (2) CaMKII-Cre-optogenetically stimulated; and (3) CaMKII-ChR2—no stimulation. All video/EEG recordings were analyzed manually to detect seizures with a duration greater than 5 seconds. Algorithms written in Matlab were used to calculate the occurrence of seizures in relation to optogenetic stimulation, circadian responses, and the occurrence of HFOs. Spontaneous seizures exhibiting both HYP and LVF types of ictal onset were observed in all 3 groups of mice with LVF seizures having a longer duration than HYP onset seizures. Surprisingly, seizures only occurred in the CaMKII-Cre mice during the 15 days of optogenetic stimulation despite not exhibiting channelrhodopsin. Optogenetic stimulation of CaMKII-ChR2 mice consistently only triggered seizures with a HYP ictal onset. These evoked seizures followed a circadian pattern with the frequency of HYP seizures higher during the 15-day stimulation period. These results clearly confirmed that seizures localized to the hippocampus with a HYP ictal onset were generated by activity of principal, excitatory neurons. This finding was supported by the observation that optogenetic stimulation of CaMKII-Cre mice never evoked a HYP onset seizure. The study also correlated seizures with the different types of ictal onset with types of HFOs. Consistent with earlier experimental[3,4] and clinical studies,[1,2] seizures with an LVF onset were associated with ripples, and seizures with a HYP ictal onset were associated with fast ripples. The strong correlation of fast ripples with the optogenetic activation of CA3 pyramidal neurons supports the hypothesis that fast ripples result from glutamatergic neuronal activity.

The observation that optogenetic stimulation of CA3 pyramidal neurons in CaMKII-ChR2 mice that had not undergone SE did not evoke a seizure suggests that a loss of feedback and feed forward inhibition, due to the loss of hippocampal inhibitory neurons after SE, contributes to an underlying increase in hyperexcitability necessary for seizure development and that optogenetic activation of excitatory neurons alone is insufficient to induce a seizure. It would be interesting to see if optogenetic activation of CA3 pyramidal neurons combined with inhibition of inhibitory neurons in CaMKII-ChR2 control mice would evoke HYP onset seizures.

If there is one weakness in the study, it is that the EEG analysis of interictal spike and HFO frequency and type was limited to a daily assessment of a 10 minutes EEG epoch, collected during the day while the animal was asleep. This limited sampling period may not have provided an accurate picture of the distribution and types of pathologic electrographic events. However, despite this limitation, the study provides definitive in vivo evidence that HYP onset seizures arise from excitatory neuronal activity.

In conclusion, this basic science study builds upon the results of previous experimental and clinical studies that correlated seizures with different types of ictal onset with patterns of neuronal loss and the expression of different types of pathological HFOs in the EEG. The present study provides definitive in vivo evidence that seizures with a HYP ictal onset result from the HYP activity of principal neurons and that this type of seizure correlates with an increase in pathological fast ripple HFOs. Moreover, the results of this study suggest that therapies for patients who exhibit seizures with a predominant HYP ictal onset should target excitatory neuronal activity and that the development of new therapeutic interventions be based on the individual patient's electrographic seizure onset patterns and pathological EEG signatures.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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