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Et tu, CA2: CA2 Is Hyperexcitable and Controls Seizures in a Mouse Model of Temporal Lobe Epilepsy

Enhanced Excitability of the Hippocampal CA2 Region and Its Contribution to Seizure Activity in a Mouse Model of Temporal Lobe Epilepsy

Whitebirch AC, LaFrancois JJ, Jain S, Leary P, Santoro B, Siegelbaum SA, Scharfman HE. *Neuron*. 2022;110(19):3121-3138. doi:10.1016/j.neuron.2022.07.020. PMID: 35987207

The hippocampal CA2 region, an area important for social memory, has been suspected to play a role in temporal lobe epilepsy (TLE) because of its resistance to degeneration observed in neighboring CA1 and CA3 regions in both humans and rodent models of TLE. However, little is known about whether alterations in CA2 properties promote seizure generation or propagation. Here, we addressed the role of CA2 using the pilocarpine-induced status epilepticus model of TLE. Ex vivo electrophysiological recordings from acute hippocampal slices revealed a set of coordinated changes that enhance CA2 PC intrinsic excitability, reduce CA2 inhibitory input, and increase CA2 excitatory output to its major CA1 synaptic target. Moreover, selective chemogenetic silencing of CA2 pyramidal cells caused a significant decrease in the frequency of spontaneous seizures measured *in vivo*. These findings provide the first evidence that CA2 actively contributes to TLE seizure activity and may thus be a promising therapeutic target.

Commentary

Temporal lobe epilepsy is a focal epilepsy disorder that is predominantly characterized by seizures that are initiated in the hippocampus or surrounding temporal lobe. Despite intense interest in understanding the specific cell types, subregions, and circuit projections that drive temporal lobe epilepsy, there is still little consensus about the specific neural changes that initiate and sustain seizures. Within the hippocampus, most studies of temporal lobe epilepsy have focused on the canonical trisynaptic pathway that projects from the dentate gyrus onto CA3 and then CA1, in part because of extensive cell death observed in these subregions. On the other hand, the CA2 subregion has been almost entirely overlooked and is relatively spared from cell death in epilepsy. This led to the novel hypothesis that CA2 may play an outsized role in the development of chronic seizures. Indeed, in their recent work, Whitebirch and colleagues[1] used a rodent model of temporal lobe epilepsy and found that CA2 is hyperexcitable and can control the frequency of chronic seizures in epileptic mice. These compelling results suggest a novel circuit that is disrupted in epilepsy and may be a viable target to treat chronic epilepsy. These results are also important in the broader context of temporal lobe epilepsy, where there are brain-wide changes in connectivity and synchronization that contribute to seizures and comorbid changes in behavior. Indeed, several subregions and cell types in hippocampus and entorhinal cortex have also been shown to have altered excitability and to control seizures, and these regions likely work together to initiate and maintain chronic seizures. Thus, it is important for future work to determine how these hyperexcitable circuits interact to produce chronic seizures.

Whitebirch and colleagues[1] found clear and compelling evidence that CA2 becomes hyperexcitable and can control seizures in chronic epilepsy. The authors used a mouse model of temporal lobe epilepsy by injecting systemic pilocarpine, which causes a prolonged status epilepticus and, after a latent period, drives chronic recurrent seizures. They then used in vitro slice electrophysiology to examine the intrinsic properties and input-evoked synaptic currents in CA2 pyramidal cells. In pilocarpine-treated mice, they first found increased intrinsic excitability in CA2 pyramidal cells with increased current-evoked spiking and higher maximum firing rates. They then stimulated the primary axonal inputs onto CA2 pyramidal cells from CA3, DG, and recurrent CA2 connections and in all cases, they found reduced inhibitory currents, while excitatory inputs were mostly unchanged. This led to a reduction in the ratio of inhibition to excitation, and an overall increase in excitability. Together, these results suggest that both intrinsic and synaptic changes drive hyperexcitability of CA2 in pilocarpine-treated mice. Critically, the authors further showed that chronically

inhibiting CA2 can reduce seizure frequency in pilocarpine-treated epileptic mice. Using chemogenetics, they selectively inhibited CA2 neurons for 2 to 3 weeks and found that this inhibition reduced the frequency of spontaneous chronic seizures. Interestingly, this manipulation did not impact seizure susceptibility in naïve (nonepileptic) mice, suggesting that the ability of CA2 to control seizures was associated with its hyperexcitable state and further reinforces its likely contribution to driving chronic seizures in epilepsy.

Identifying CA2 as a subregion in hippocampus that is hyperexcitable and controls chronic seizures is a critical step toward understanding and treating temporal lobe epilepsy. Most importantly, this work suggests that specific modulation of CA2 pyramidal neurons can control seizure susceptibility in chronic epilepsy and may be a viable target for new therapeutic interventions. While targeting these specific neurons in patients is not yet feasible, substantial ongoing work is aimed at targeting precise brain regions and cell types with viral chemogenetics, direct electrical stimulation, or noninvasive neuromodulation. The results from Whitebirch and colleagues[1] suggest that targeting CA2 with these techniques should be a priority for ongoing investigations. For viral expression, CA2 is well positioned for selective targeting because it has a relatively unique transcriptional profile[2] that can be leveraged with novel promoters and enhancers. In addition, while deep brain stimulation and responsive neurostimulation have become successful therapeutics in some cases of temporal lobe epilepsy, there has been very little optimization of where to place stimulation electrodes or ideal stimulation parameters. Thus, future studies should examine how electrode position relative to hippocampal subregions influences the efficacy of electrical stimulation and investigate whether targeting near CA2 may enhance its seizure-suppressing effects.

Temporal lobe epilepsy drives extensive cell death and reorganization of both excitatory and inhibitory neurons in the hippocampus. While most studies have focused on areas that degenerate in epilepsy, Whitebirch and colleagues[1] hypothesized that cell death, particularly in CA3, may leave CA2 as the predominant influence on hippocampal signaling and thus may strongly contribute to seizures. Indeed, this work clearly implicates CA2 as an important mediator of chronic seizures in epilepsy. Yet it is also important to note that the hippocampus is a highly connected brain region and brain-wide changes in excitability, connectivity, and synchronization have been observed in temporal lobe epilepsy.[3] With so many changes across the brain, it is difficult to identify which are the most important for seizure generation and much ongoing work has sought to identify a singular mechanism that specifically initiates seizures. However, these brain-wide changes suggest that multiple cell types and brain regions likely work together to initiate and propagate seizure activity. Indeed, emerging theories of seizure initiation suggest that network dynamics across brain regions may create hypersynchronous loops that drive the progression of seizure states.[4] This notion is further supported by the current study, where chemogenetic inhibition of CA2 in naïve mice did not impact acute seizure susceptibility, suggesting that the changes in CA2 likely interact with additional changes in other brain regions to control seizures. While these distributed changes complicate efforts to localize a single seizure-driving mechanism, it also suggests that modulating different brain regions can each impact seizures. Indeed, in models of temporal lobe epilepsy, several brain regions have been identified as hyperexcitable and that can control epileptic activity including the dentate gyrus, medial entorhinal cortex, CA1, and even cerebellum.[5-7] Therapeutically, this is quite advantageous as these regions may each be able to independently control seizures.

One important implication of disrupted CA2 excitability in epilepsy is that this region is highly implicated in social memory[8] and has an emerging role in learning and memory processes.[9] Thus, changes in CA2 processing could directly contribute to altered social behavior and cognition. Indeed, social and cognitive deficits are 2 of the most common comorbidities of epilepsy, and epilepsy shares many behavioral, genetic, and circuit abnormalities with autism spectrum disorder (ASD).[10] These comorbid symptoms severely impact the quality of life for people with epilepsy and have limited treatment options. Thus, understanding how these changes may drive altered behavior and identifying new interventions that impact both seizures and comorbidities could have a tremendous benefit for people with epilepsy.

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