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From the Subiculum to the Anterior Nuclei of the Thalamus: The Key to Hippocampal Seizure Generalization?

Discrete Subicular Circuits Control Generalization of Hippocampal Seizures

Fei F, Wang X, Xu C, Shi J, Gong Y, Cheng H, Lai N, Ruan Y, Ding Y, Wang S, Chen Z, Wang Y. *Nat Commun*. 2022;13(1):5010. doi:10.1038/s41467-022-32742-x

Epilepsy is considered a circuit-level dysfunction associated with imbalanced excitation-inhibition, it is therapeutically necessary to identify key brain regions and related circuits in epilepsy. The subiculum is an essential participant in epileptic seizures, but the circuit mechanism underlying its role remains largely elusive. Here we deconstruct the diversity of subicular circuits in a mouse model of epilepsy. We find that excitatory subicular pyramidal neurons heterogeneously control the generalization of hippocampal seizures by projecting to different downstream regions. Notably, anterior thalamus projecting subicular neurons bidirectionally mediate seizures, while entorhinal cortex-projecting subicular neurons act oppositely in seizure modulation. These two subpopulations are structurally and functionally dissociable. An intrinsically enhanced hyperpolarization-activated current and robust bursting intensity in anterior thalamus-projecting neurons facilitate synaptic transmission, thus contributing to the generalization of hippocampal seizures. These results demonstrate that subicular circuits have diverse roles in epilepsy, suggesting the necessity to precisely target specific subicular circuits for effective treatment of epilepsy.

Commentary

Temporal lobe epilepsy (TLE) is the most common type of human epilepsy.[1] Seizures in TLE typically initiate in the hippocampus.[1] Seizures constrained within the hippocampus for their full duration are classified as focal seizures (FS); if they propagate out of the hippocampus and recruit other brain regions, they are termed secondary generalized seizures (sGS). Secondary generalized seizures are typically more severe than FS and how frequently they occur is the most consistent risk factor for sudden unexplained death in epilepsy.[2] Understanding how epileptiform activity propagates out of the hippocampus is of obvious importance for understanding the process(es) of seizure generalization and for the design of more rational therapeutic interventions.

The subiculum is the first region downstream of the hippocampus. It receives the main output from CA1 pyramidal neurons and, in part, due to its neuroanatomical position, is thought to be important in the transition from FS to sGS.[3,4]

In their recent publication, Fei and colleagues provided new insights into how heterogeneity among subicular pyramidal neurons influences seizure generalization in TLE.[5] Their key findings were that burst-firing deep subicular pyramidal neurons, which are strongly recruited in FS and sGS, facilitate secondary generalization through their projections to the anterior thalamic nucleus (ANT). In contrast, superficial pyramidal neurons that project to the entorhinal cortex (EC) inhibit seizure generalization.

Firstly, they demonstrated the involvement of the subicular pyramidal neurons in FS and sGS in a hippocampal CA3 kindling model. Using the calcium indicator GCaMP6s and cFos staining, the authors showed an increase in pyramidal neuron excitability during FS and even more during sGS. They confirmed this result by showing an attenuation in the acquisition and severity of sGS after subicular lesion, indicating that the activity of subicular neurons is not merely associative but, to some degree, is necessary for the secondary generalization of seizures.[5]

Further probing the causality, Fei and colleagues modulated the activity of subicular pyramidal neurons with optogenetics. In the kindling model, activation of subicular pyramidal neurons with channelrhodopsin (ChR2) augmented the development of seizures, with fewer stimulations required to trigger a sGS. Inhibition of these neurons with Archaerhodopsin had the reverse effect, with sGS requiring more kindling stimulations to develop.

Importantly the authors showed heterogeneity in deep versus superficial subicular pyramidal layers that was relevant to the secondary generalization. Crucially, when the authors restricted ChR2-mediated activation to deep subicular pyramidal neurons, they observed a more potent proconvulsive effect

than stimulating both deep and superficial neurons.[5] This was a fundamental finding that set the stage for the second half of the study. Indeed, it suggested that (1) deep pyramidal neurons are more effective at causing sGS, and (2) that superficial pyramidal neurons might inhibit sGS.

This effect might be explained by the brain regions that deep and superficial subicular pyramidal neurons project to. Fei and colleagues delineated the regions that receive input from the subiculum using anterograde tracing and cFos staining after sGS. cFos levels increased in the nucleus accumbens, mammillary bodies, EC, and ANT. Then, using a retrograde tracer, they showed that deep subicular pyramidal cells project to the ANT while superficial subicular neurons project to the EC.[5]

To assess the impact of the deep subicular projections to the ANT versus superficial projections to the EC, the authors used an optogenetic strategy. Activation of the subicular afferents that terminate in the ANT produced a proseizure effect, and inhibition of these terminals had an anti-seizure effect. Interestingly and surprisingly, optogenetic inhibition of the subicular projections to the EC augmented seizures. These findings were paralleled with a chemogenetic strategy in a chronic intrahippocampal CA1 kainic acid model of TLE.[5]

Next, the authors explored whether the firing characteristics of deep versus superficial neurons also played a part. Using axonal calcium imaging they showed that the timing of the inputs to the ANT and EC differ. In FS, calcium signal intensity recorded from the subicular projections increased in the ANT and decreased in the EC, while in sGS, the intensity of the calcium signal exhibited a delayed increase in the ANT. Multiunit recording of spikes from the subiculum showed that deep neurons had a higher burst rate than the superficial neurons in vivo and patch-clamp recordings from deep neurons indicated that the burst-firing of deep neurons was facilitated by hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channel conductance. In their concluding experiments, Fei and colleagues showed, in the kindling model, using both pharmacological and genetic strategies, that HCN channel conductance in deep subicular pyramidal cells specifically is proconvulsive.[5]

Fei and colleagues' study elegantly showed the importance of the projections from the deep subicular pyramidal layer to the ANT for secondary seizure generalization, including a potential proepileptic role of HCN-1 channel expression in these neurons.[5] This study illustrates that there is functional heterogeneity in subicular pyramidal neurons, as has been previously reported in CA1[6,7] and CA3.[8] Targeting only the deep subicular pyramidal neurons projecting to ANT with a viral gene therapy could be a therapeutic strategy. The advantage of this approach is that it could, theoretically, attenuate sGS regardless of the precise site of seizure initiation in patients with TLE.

However, targeting only deep subicular pyramidal neurons in the clinic would be challenging. Even if a synthetic promoter selective for these neurons was developed, it would not enable discrimination of their projections to downstream regions. Is direct genetic inhibition of the ANT a better way forward for TLE patients? To assess this, a further experiment in the same models using chemo/optogenetics to decrease neuronal excitability in the ANT needs to be performed. Indeed, ANT deep brain stimulation is an effective treatment for drug-resistant epilepsy.[9] However, this option still leaves the hippocampal FS, which might still affect cognition.

Another challenge in selectively targeting brain (sub)regions to limit secondary generalization is interspecies differences in local and long-range connectivity. Adding further complexity are the circuit rewirings that take place in TLE, which vary between different species, models, and patients.[10] Thus, it will be necessary to investigate whether the subiculum-ANT connections play a similar role in different chronic models and, if possible, in TLE patients.

This study might inspire similar strategies for preventing secondary seizure generalization in neocortical focal epilepsies. However, inter-patient variability due to the different cortical regions affected is higher than for TLE.

In conclusion, Fei and colleagues demonstrated that there is heterogeneity among subicular pyramidal neurons, in their projections and firing characteristics, and opened the way for the design of rational therapeutic approaches to stop seizure generalization in TLE targeting subicular projections.

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Declaration of Conflicting Interests

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