Epilepsy is a disease in which those affected have unpredictable spontaneous seizures. Seizures occur for a myriad of reasons. Much of the time, isolated seizures do not signify epilepsy and often will not recur. However, in some cases, seizures beget seizures. Kindling is a process by which initially insignificant stimulations presented repeatedly eventually lead to emergence of local afterdischarges (AD), focal seizures, and ultimately spread to generalized seizures. This experimental approach has been exploited to determine that long-term changes in neuronal excitability occur. However, determining how these changes occurred is not well understood. Kindling is a process by which repeated seizures lead to circuit reorganization that facilitates subsequent seizures to occur more readily and have more wide-reaching effects, how these changes occur is not well understood. Kindling is a process by which repeated seizures lead to circuit reorganization that facilitates subsequent seizures to occur more readily and have more wide-reaching effects, how these changes occur is not well understood.

In the present study, the authors take advantage of the precise cell-type specific stimulation afforded by optogenetics to specifically stimulate excitatory neurons in the ventral hippocampus and establish an optogenetic kindling model for the first time. Then, they examine the resulting brain-wide circuit changes using functional magnetic resonance imaging (fMRI). Sprague Dawley rats received an injection of an adeno-associated viral (AAV) vector into the ventral hippocampus, allowing expression of the excitatory opsins, channelrhodopsin (hChR2), in excitatory neurons under the control of the calcium/calmodulin-dependent protein kinase II (CaMKIIa) promoter. Rats also received an injection site, and a carbon fiber electrode/optical fiber (optrode) just superior to the injection site, and a carbon fiber electrode into the medial prefrontal cortex. All animals underwent baseline resting fMRI imaging at least 1 week prior to kindling. One group of rats underwent AD threshold determination and was then subjected to 40 Hz blue light (473 nm) stimulation at the individual rat’s AD threshold every 15 min up to 12 times or until the rat reached displayed a generalized motor seizure. A second group (non-kindled) of rats was instrumented and handled identically to the kindling group, except they did not undergo AD threshold determination with 40 Hz blue light stimulation. Ten of 12 rats in the kindling group were kindled by day 7, requiring an average

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of 53.6 stimulations. All rats were kindled by day 11. Consistent with other kindling models, there was minimal anatomical damage in either ventral hippocampus. The hyperexcitability was permanent, persisting at 3 and 12 weeks post-kindling. Similar stimulation in non-kindled rats had no effect.

To examine brain activation following optogenetic stimulation in kindled vs non-kindled rats they coupled optogenetics with fMRI (ofMRI). fMRI has previously shown changes associated with focal and generalized seizures, but it could not easily discern causality. Making ofMRI measurements during optogenetic stimulation in cells of known type in a known location allows cause-and-effect to be readily differentiated, and does not simply observe associations without directionality. 12 weeks after kindling, rats underwent repeat fMRI, but this time coupled with nonAD-generating 10 Hz blue light stimulation (six 5-second stimulations, 1 minute apart). Non-kindled animals had local ipsilateral activation primarily in hippocampus, amygdala, medial prefrontal cortex, and septum. Kindled animals had activation in these ipsilateral regions, but also more widely ipsilaterally and to some analogous contralateral structures. NonAD-generating stimulations led to ipsilateral functional connectivity. Given behavioral comorbidities of epilepsy, the authors performed 3 behavioral assays, seeing no difference in sucrose preference (anhedonia) or forced swim (despair) in kindled rats compared to non-kindled rats, but observing that kindled rats spent less time in the center in open field testing compared to non-kindled rats, suggesting an anxiety phenotype.

About 14 weeks after kindling, 5 kindled and 7 non-kindled rats underwent longer optogenetic stimulation to induce a seizure or AD. In kindled animals, this resulted in gradual propagation to bilateral cortex, but in non-kindled animals, activation occurred only locally. Seizures in the kindled group were also associated with widespread functional connectivity. Interestingly, stimulation in both kindled (15/17 seizures in 5/5 rats) and non-kindled (8/20 AEs in 4/7 rats) animals caused a slow-migrating core of activity rising from the hippocampus, moving from the ventral to dorsal hippocampus at similar rates in both groups. In kindled animals, it continued to propagate to the contralateral side. The authors propose that this may be a novel mechanism to target to control seizures.

Thus, the authors demonstrate that repeated optogenetic stimulation of ventral hippocampus leads to kindling and reliably provokable seizures. Using ofMRI they were able to show that seizures spread locally from the site of stimulation to the rest of the hippocampus, and then to the contralateral hippocampus and to the bilateral cortex. This pattern of spread suggests synaptic propagation and not simply moving to contiguous loci.

While this is a powerful demonstration of the propagation pattern of a focal seizure originating from a known locus, a limitation of this study is the lack of spontaneous seizures generated from the kindling model. Translating these findings in an attempt to understand what happens following a spontaneous seizure will have similar limitations to previous attempts. However, now the data can be viewed with the knowledge of how the stimulated seizure engaged networks. It will be interesting to see if the slow propagating migrating core through the hippocampus can be captured with spontaneous seizures. In future studies, it may be possible, as with other kindling models, to cause animals to have spontaneous seizures with continued daily optogenetic stimulations.

It will further be informative to learn whether preventing this core of activity prevents more widespread seizure propagation and possibly epileptogenesis. This could revolutionize the study and preventive management of epilepsy. Similarly, it would also be interesting to know if this wave occurs in other models of epileptogenesis and not just kindling, for example, post-status epilepticus models. Further study will be needed to understand the mechanism of the migrating core to understand what it means, and why it can lead to contralateral spread following kindling. For management of patients with epilepsy, the question arises of when to intervene, such as after status epilepticus, after a first seizure, or after an insult that could lead to epilepsy such as traumatic brain injury, infection, stroke, intracranial hemorrhage, etc. Optogenetic kindling coupled with fMRI should prove to be a useful and important tool to continue to understand mechanisms for seizure propagation and possibly epileptogenesis, which would facilitate development of novel treatments for seizures and epilepsy.

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