Optokindling, GABA Dwindling

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Chronic Loss of Inhibition in Piriform Cortex Following Brief, Daily Optogenetic Stimulation

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It is well established that seizures beget seizures, yet the cellular processes that underlie progressive epileptogenesis remain unclear. Here, we use optogenetics to briefly activate targeted populations of mouse piriform cortex (PCx) principal neurons in vivo. After just 3 or 4 days of stimulation, previously subconvulsive stimuli trigger massive, generalized seizures. Highly recurrent allocortices are especially prone to "optokindling." Optokindling upsets the balance of recurrent excitation and feedback inhibition. To understand how this balance is disrupted, we then selectively reactivate the same neurons in vitro. Surprisingly, we find no evidence of heterosynaptic potentiation; instead, we observe a marked, pathway-specific decrease in feedback inhibition. We find no loss of inhibitory interneurons; rather, decreased gamma-aminobutyric acid synthesis in feedback inhibitory neurons appears to underlie weakened inhibition. Optokindling will allow precise identification of the molecular processes by which brain activity patterns can progressively and pathologically disrupt the balance of cortical excitation and inhibition.

Commentary

Epilepsy can be a progressive disease. In humans and in animal models, symptoms commonly worsen over time, with seizures increasing in severity and/or frequency. This disease progression can be modeled in a very controlled way using the "kindling" model of epilepsy. In kindling, a brain area (typically the amygdala) is periodically stimulated with electrical current to induce epileptiform activity. A fixed stimulation protocol induces initially subconvulsive, then progressively more severe focal, and secondarily generalized seizures.[1] Although kindling has been widely used to study epileptiform activity in neural circuits for more than half a century, much remains unknown about the cellular and synaptic changes that underlie the decrease in seizure threshold.

Several factors complicate the study of electrical kindling at the cellular level. First, electrical stimulation with a limited electrode montage is non-specific; all cells and neurites in the vicinity of the stimulating electrode will be activated. Second, electrical kindling induces tissue damage that resembles hippocampal sclerosis,[2] though the reported damage threshold ranges greatly from 1 to 6000 seizures (see table 3 in Kotloski et al., 2002).[3] It is unclear whether this is a result of electrode placement, high current densities near the electrode, or repeated seizure activity induced by the stimulation. Thus, it is difficult to differentiate synaptic mechanisms of epileptogenesis from those caused by neuronal loss. Finally, a challenge that is not unique to kindling, obtaining intracellular recordings from cells involved in ictogenesis, is technically challenging. Recently developed strategies for optogenetic seizure induction[4] and optogenetic kindling[5] address the first two of these issues. Using this approach, the population of cells stimulated during kindling is limited to those expressing the channelrhodopsin (ChR2), which can be selected using gene promoters or cre-lox targeting. Optogenetic kindling has largely the same semiology as electrical kindling, with a progressive increase in seizure severity despite constant stimulation. It lacks, however, the neuronal loss and glial reactivity associated with electrical kindling, further reducing the potential mechanisms of epileptogenesis in this model.

In the highlighted study, Ryu et al (2021)[6] use optogenetic kindling of the piriform cortex to study synaptic changes downstream of the optically stimulated neurons. A robust kindling effect with daily optical stimulation was demonstrated both in the worsening of behavioral seizures and in the spatial extent of Fos-positive neurons, a marker of high-level neural activity. In animals sacrificed on day 1 of kindling, Fos positivity was restricted to the directly stimulated region of anterior piriform cortex, while animals sacrificed on day 6 of stimulation showed widespread Fos expression in non-stimulated cells of the anterior and (most prominently) posterior piriform cortices.

The authors next set out to determine whether the expansion of propagation of optically induced neural activity resulted from a shift in the ratio of excitatory to inhibitory (E/I) synaptic input to pyramidal cells post-synaptic to the stimulated cells. To accomplish this, acute slices were prepared from optically kindled and non-kindled control animals. Non-stimulated pyramidal cells were identified morphologically, by lack of expression of the

YFP fused to ChR2, and by lack of a depolarizing response to optical stimulation. Whole cell voltage clamp recordings were obtained from non-stimulated cells at holding potentials of −70 mV and +5 mV to isolate excitatory and inhibitory currents, respectively. Synaptic currents were measured while stimulating using either optical activation of ChR2+ neurons (as performed *in vivo* in kindled animals) or extracellular electrical stimulation of mitral cell axons in the lateral olfactory tract (LOT, non-kindled pathway). Of the four stimulation groups (optical vs electrical stimulation in slices from kindled vs control animals), only *optical* stimulation of slices from *kindled* animals represents stimulation of *"kindled neurons."* Interestingly, there was a significantly larger E/I ratio of post-synaptic currents for optical stimulation of ChR2+ neurons in kindled animals (compared to controls), primarily due to a decrease in inhibitory current amplitude. Conversely, electrical stimulation of the LOT pathway revealed no difference between slices from control and kindled animals. In other words, the synaptic pathway involving kindled neurons had an elevated E/I ratio, whereas the upstream LOT pathway, in the same animals, was unaffected.

A set of standard electrophysiological and pharmacological experiments confirmed that the excitatory synaptic parameters (release probability, receptor sensitivity, and recurrent excitation) were unaffected. Rather, a decrease in miniature IPSC amplitude and frequency pointed to a deficit in inhibition. Post-hoc staining for vesicular GABA transporter (vGAT), gamma-aminobutyric acid (GABA), and parvalbumin (PV) revealed that there was no change in the number of interneurons or density of GABA terminals, but there was a marked decrease in both GABA and PV following optical kindling, particularly in the posterior piriform cortex, where the largest spread of kindling-induced Fos expression was observed. Electrophysiological experiments involving stimulation of interneurons in the presence of glutamate receptor antagonists further demonstrated both decreased GABA concentration and slowed refilling of GABA vesicles in slices from optically kindled mice.

Together these results support a model wherein daily episodes of intense optical activation of pyramidal cells lead to a deficit in GABA synthesis, transport, and/or packaging in downstream interneurons. This deficit in turn produces a failure in disynaptic feedforward inhibition, causing feedforward excitation to spread pathologically. The lack of disruption to the excitatory synaptic pathway suggests that there is no phototoxicity or excitotoxicity caused directly by the optogenetic stimulation itself. The downstream effect on interneurons points to either a homeostatic alteration in GABA metabolism or an excitotoxic effect on interneurons. Homeostatic decreases in GABA metabolism would be counter-intuitive for a seizure-prone neural circuit, but it is possible that homeostatic processes respond more to the long-term post-ictal depression in activity than to the seizures themselves. The existence and importance of interneuron excitotoxicity in animal models of epilepsy remain a somewhat unclear picture.[7-9] Many such studies rely primarily on the counting of neurons in fixed tissue rather than evaluation of interneuron physiology per se. The approach of Ryu et al. enabled *in vitro* electrophysiological evaluation of interneuron pathology in the epileptic synaptic pathway. However, the nature of the pathology remains somewhat uncertain. One possibility is that the interneurons with reduced GABA and PV are in the process of dying. Future studies may look at markers of cell health and potentially evaluate interneurons at later timepoints following the onset of optokindling.

While neither optical nor electrical kindling produces spontaneous recurrent seizures, they do generate predictable seizure onset times, which may be helpful in imaging the spread of seizure-like activity (similar to the fiber photometry performed in Khoshkhoo et al., 2017[4]). Future studies involving optokindling will further benefit from its previously unexploited advantage highlighted here: the capacity for nearly co-registered *in vivo* and *in vitro* study of epileptic synaptic pathways.

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