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Seizing After Freezing: Layer-, Site-, and Cell-Type-Specific Rewiring Mediates Epileptogenesis Following Cortical Freeze Lesion in Mouse

Circuit Mechanisms Underlying Epileptogenesis in a Mouse Model of Focal Cortical Malformation

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The way in which aberrant neural circuits contribute to epilepsy remains unclear. To elucidate this question, we dissected the circuit mechanisms underlying epileptogenesis using a mouse model of focal cortical malformation with spontaneous epileptiform discharges. We found that spontaneous spike-wave discharges and optogenetically induced hyperexcitable bursts in vivo were present in a cortical region distal to (>0.7 mm) freeze-lesion-induced microgyrus, instead of near the microgyrus. Channelrhodopsin-2-assisted circuit mapping revealed ectopic interlaminar excitatory input from infragranular layers to layers 2/3 pyramidal neurons as the key component of hyperexcitable circuitry. This hyperactivity disrupted the balance between excitation and inhibition and was more prominent in the cortical region distal to the microgyrus. Consistently, the inhibition from both parvalbumin-positive interneurons (PV) and somatostatin-positive interneurons (SOM) to pyramidal neurons were altered in a layer- and site-specific fashion. Finally, closed-loop optogenetic stimulation of SOM, but not PV, terminated spontaneous spike-wave discharges. Together, these results demonstrate the occurrence of highly site- and cell-type-specific synaptic reorganization underlying epileptic cortical circuits and provide new insights into potential treatment strategies.

Commentary

Epileptogenesis can be triggered by a wide range of inciting events.[1] Some factors underlying the mechanisms for epileptogenesis are understood, but much about mechanisms of epileptogenesis is unknown. Thus, ways to prevent it have remained elusive. In some cases, the inciting event is readily apparent, such as in post-traumatic epilepsy, or poststroke epilepsy. In other cases, the inciting event is less clear, but thought to have occurred during fetal development.[2] Some studies suggest that the mechanisms for epileptogenesis may be similar in these different instances.[3] Often, refractory epilepsies of extratemporal origin are found to be associated with a focal cortical malformation on pathology. One such focal cortical malformation with a particularly high rate of refractory epilepsy is polymicrogyria (PMG).[4] Notably, PMG is also associated with a high surgical failure rate.[5] There exist many animal models that attempt to emulate the pathophysiological features seen in patients and can be used to investigate these mechanisms. Yang et al set out to better understand circuit aberrations resulting in epileptogenesis using a neonatal freeze lesion (FL) model of focal cortical malformations in mouse.[6] Lesions in this model result in a 4-layered cortex similar to that seen in PMG in patients,[7,8] as opposed to the normal 6-layered architecture; however, a general supragranular, granular, and infragranular organization is maintained.[8] Lesions in this model also cause spike-wave discharges and spontaneous seizures similar to patients with PMG and other focal cortical malformations.[8]

In their study, Yang et al combined a powerful set of techniques including channelrhodopsin-2 (ChR2)-assisted circuit mapping (CRACM) in cortical brain slices, in vitro patch-clamp electrophysiology, in vivo linear microelectrode array recordings, and in vivo automated closed-loop optogenetic stimulation in wild-type mice and mice expressing Cre recombinase in a layer-specific or interneuron (IN)-specific manner. Using patch-clamp recordings and CRACM, they first determined that there is a layer-specific change in excitatory–inhibitory (E/I) balance. For CRACM, they injected AAV-ChR2 into the region of the FL or sham lesioned somatosensory cortex to express ChR2 in excitatory and inhibitory neurons. They then made cortical slices and recorded from pyramidal cells (PCs) in layer 2/3 or layer 5B while optogenetically stimulating the surrounding area in a 16 × 12 grid (75 μm between adjacent stimulation points) thereby creating excitation–inhibition maps for given PCs. Comparing mice subjected to FL to sham-treated mice, they found the E/I balance in supragranular layers (layer 2/3), was pushed toward excitation, but E/I balance in

infragranular layers (layer 5B), was tipped toward inhibition. Taken together, this suggests layer-dependent rewiring in an epileptic mouse cortex.

Next, they wanted to determine where, with respect to the FL, cortical circuit reorganization occurred. For this they turned to in vivo linear microelectrode array recordings in groups of mice subjected to burst discharge induction either by anesthesia or by optogenetics. Again, they used wild-type mice that had undergone FL or sham lesion and received an AAV-ChR2 injection. In both approaches, they saw that hyperexcitability was greatest at an extralesional site (>0.7 mm) and that epileptogenesis likely originates in the deeper infragranular layer. They performed similar CRACM experiments as described above using Scnn1a-cre mice, in which Cre recombinase is expressed under the control of the Scnn1a promoter present in layer 4 spiny neurons. Mice received a Cre-dependent flexed AAV-ChR2 injection into the granular layer (layer 4). Thus, while the AAV transfected all cells in the region, the Cre-dependent flexed construct restricted expression to layer 4 spiny neurons. They found that connections between layer 4 and layer 2/3 contribute to the reorganization of interlaminar and intercolumnar circuits, and thus contribute to the increased likelihood of abnormal discharges to generalize.

They further determined that populations of inhibitory neurons, namely parvalbumin (PV)-expressing and somatostatin (SOM)-expressing INs were differentially affected. Parvalbumin and SOM INs both provide synaptic inhibition to PCs; however, PV INs target the perisomatic region, whereas SOM INs target distal dendrites. Inhibitory postsynaptic potentials originating from both PV and SOM INs were reduced distal to FL in layer 2/3, but not proximally. This suggests dysfunction of the inhibitory inputs and accounts for the hyperexcitability in layer 2/3. Channelrhodopsin-2-assisted circuit mapping in PV- or SOM-cre mice that had been subjected to FL or sham lesion and received flexed AAV-ChR2 injection into somatosensory cortex, and thus had ChR2 expression only in PV or SOM INs, respectively, revealed a reduced inhibitory receptive field size in layer 5B in PV-cre mice suggesting reduced inhibition from PV INs. Conversely, CRACM revealed increased inhibitory receptive field size in layer 5B in SOM-cre mice, suggesting a greater contribution from SOM INs. Taken together, this suggests that SOM INs, but not PV INs, are responsible for the hypoexcitability seen in layer 5B and thus driving the excitation–inhibition imbalance. The authors hypothesize that this increased inhibitory contribution from SOM INs is due to enhanced inhibitory innervation of distal dendrites through axonal sprouting or is caused by an increase in SOM IN number.

Finally, to further understand the potential to manipulate these IN subtypes as a means to inform treatment strategies, they used closed-loop optogenetic stimulation, driving selective stimulation of PV or SOM INs in response to automatically detected spike-wave discharges in FL mice. They determined that stimulating SOM neurons, but not PV neurons, could reduce spike-wave discharges and restore normal excitability. This suggests that the increased inhibition is due to enhanced inhibitory innervation of distal dendrites by SOM INs. The authors propose that this enhanced SOM IN activity in layer 5B counteracts layer 2/3 hyperexcitability to reduce spike-wave discharges when layer 5B SOM INs are stimulated in the closed-loop system.

This study sheds light on several important issues regarding epileptogenesis. First, they demonstrate rather elegantly layer-specific and cell-type-specific circuit reorganization causing hyperexcitability and increased likelihood that epileptiform discharges will propagate more broadly or generalize. Second, they demonstrate that in this FL model, the site of maximal aberration is distal to the site of the lesion. There is more work needed to determine whether the site they identified is the optimal distance from the lesion, or if the optimal site of aberration could be even more distal. More work will also be needed to determine where the exact site is in humans. Regardless, this provides a plausible explanation for why there is a high surgical failure rate in these patients—simply not enough tissue was taken. Finally, this study sheds some light on possible optogenetic-based treatment avenues. Although on the surface, this seems like it may be some ways off, at least one clinical trial using optogenetics in humans is underway[9]; however, this trial is using intravitreous injections to treat vision loss.[9] Given recent advances in other types of gene therapies such as the use of antisense oligonucleotides[10] and gene editing,[11] using tools like optogenetics in people may not be that far off.

Although epilepsy in PMG is often refractory, PMG comprises a small fraction of all refractory epilepsies. Additionally, PMG represents a fairly small fraction of focal cortical malformations.[2] Thus, it remains to be seen how well findings from this study can be translated to other focal cortical epilepsies, such as post-traumatic epilepsy, poststroke epilepsy, or other malformations of cortical development. Indeed, these may share some similar pathogenicity.[3] Also, while a 4-layered cortex is identified in this neonatal FL model, similar to that seen in tissue from patients with PMG, it is not known how closely these fine microcircuit changes recapitulate what happens in patients. Regardless, the insights gleaned from this study regarding circuit reorganization leading to epileptogenesis are exciting and may lead to modifications of surgical approaches for some patients and inform new therapeutic avenues.

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