Implicating Interneurons: Optogenetic Studies Suggest That Interneurons Are Guilty of Contributing to Epileptiform Activity

Excitatory Effects of Parvalbumin-Expressing Interneurons Maintain Hippocampal Epileptiform Activity via Synchronous Afterdischarges.

Ellender TJ, Raimondo JV, Irkle A, Lamsa KP, Akerman CJ. *J Neurosci* 2014;34(46):15208–15222.

Epileptic seizures are characterized by periods of hypersynchronous, hyperexcitability within brain networks. Most seizures involve two stages: an initial tonic phase, followed by a longer clonic phase that is characterized by rhythmic bouts of synchronized network activity called afterdischarges (ADs). Here we investigate the cellular and network mechanisms underlying hippocampal ADs in an effort to understand how they maintain seizure activity. Using *in vitro* hippocampal slice models from rats and mice, we performed electrophysiological recordings from CA3 pyramidal neurons to monitor network activity and changes in GABAergic signaling during epileptiform activity. First, we show that the highest synchrony occurs during clonic ADs, consistent with the idea that specific circuit dynamics underlie this phase of the epileptiform activity. We then show that ADs require intact GABAergic synaptic transmission, which becomes excitatory as a result of a transient collapse in the chloride (Cl^-) reversal potential. The depolarizing effects of GABA are strongest at the soma of pyramidal neurons, which implicates somatic-targeting interneurons in AD activity. To test this, we used optogenetic techniques to selectively control the activity of somatic-targeting parvalbumin-expressing (PV^+) interneurons. Channelrhodopsin-2-mediated activation of PV^+ interneurons during the clonic phase generated excitatory GABAergic responses in pyramidal neurons, which were sufficient to elicit and entrain synchronous AD activity across the network. Finally, archaerhodopsin-mediated selective silencing of PV^+ interneurons reduced the occurrence of ADs during the clonic phase. Therefore, we propose that activity-dependent Cl^- accumulation subverts the actions of PV^+ interneurons to perpetuate rather than terminate pathological network hyperexcitability during the clonic phase of seizures.

Commentary

Epilepsy is a network disorder that requires specificity in the manipulation of these networks for optimal treatment. Current pharmacologic approaches lack specificity, affecting virtually all cells expressing their target of action, often resulting in unwanted and even offsetting effects, which can impact unaffected networks. The development of optogenetics, which offers spatial, temporal, and cell-type specific control, has generated enthusiasm for the use of this technology in the treatment of the epilepsies (1).

Optogenetics refers to the approach of combining genetics with optical technology for the ability to specifically control target cells. Different opsins have been developed to specifically activate (e.g., channelrhodopsin-2 [ChR2]) or inhibit (e.g., halorhodopsin [NpHR] and archaerhodopsin [Arch]) cells. These opsins can be genetically targeted to specific subsets of neurons, such as interneuron subtypes, allowing control over their activity with light. Thus, optogenetics represents a useful tool to investigate the role of specific populations of neurons in the generation and progression of seizure activity. However, basic science investigations into the antiseizure potential of optogenetics have raised some issues to consider regarding the translational potential of optogenetics.

Employing optogenetics to silence excitatory neurons is effective at suppressing seizures (1). However, attempts to suppress seizures via activation of interneurons have led to interesting observations regarding the role of interneurons in seizure progression, which is the focus of this commentary. Here, we highlight a recent study by Ellender et al. demonstrating a role for parvalbumin (PV)-positive interneurons in contributing to the generation of a specific type of epileptiform activity, specifically rhythmic bouts of synchronized network activity termed afterdischarges. These findings are supported by more recent reports demonstrating that optogenetic activation of interneurons exacerbates epileptiform activity in vitro (Table 1) (2, 3).

Ellender et al. demonstrated that excitatory actions of GABA during seizure progression, due to a collapse in the

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TABLE 1. ***Optogenetic Manipulation of Interneurons on Epileptiform Activity****

Epilepsy Model	opsin	driver	stimulation paradigm	timing of optogenetic stimulation	effect on seizure activity	Citation
in vitro: 4-AP (100 μM)	ChR2	VGAT	5 ms, 1 Hz stimulation	stimulated in "hippocampal slices exhibiting epileptiform activity", exact timing not reported	altered the dynamics (power and frequency) of epileptiform activity; entrains activity	Ladas, 2015
in vitro: 4-AP (100 μM)	ChR2	VGAT	5 ms, 1 Hz stimulation	N/A	GABA drives pyramidal cell excitation	Ladas, 2015
in vitro: 4-AP (150 μM)	ChR2	PV	1/2 s, 0.2 Hz for 30 s (130 interval between trains)	After the induction of epileptiform activity, exact timing not reported	increased epileptiform activity, induced ictal-like events, alters the dynamice of epileptiform activity (drives low voltage fast ictal discharges)	Shiri, 2015
in vitro: 4-AP (100–200 μM)	ChR2	PV	Four 300-ms pulses,15 s aparat. Repeated 10 times with a time interval of 35–60 s	prior to initiation of epileptiform activity	induced epileptiform activity	Yekhlef, 2015
in vitro: 4-AP (100–200 μM)	ChR2	SOM	Four 300-ms pulses,15 s aparat. Repeated 10 times with a time interval of 35–60 s	prior to initiation of epileptiform activity	induced epileptiform activity	Yekhlef, 2015
in vitro: 4-AP (100–200 μM)	NphR2/HR	PV	Four 300-ms pulses,15 s aparat. Repeated 10 times with a time interval of 35–60 s	prior to initiation of epileptiform activity	epileptiform activity unaffected	Yekhlef, 2015
in vitro: 4-AP (100–200 μM)	ChR2	PV	Four 300-ms pulses,15 s aparat. Repeated 10 times with a time interval of 35–60 s	4s after preictal spike	epileptiform activity unaffected	Yekhlef, 2015
in vitro: 4-AP (100–200 μM)	ChR2	SOM	Four 300-ms pulses,15 s aparat. Repeated 10 times with a time interval of 35–60 s	4s after preictal spike	epileptiform activity unaffected	Yekhlef, 2015
in vitro: 0 mM Mg^{2+}, 5 mM K^+	ChR2	PV	1 ms laser pulses every 10 s	After the induction of epileptiform activity	GABA signaling became depolarizing during the epileptiform activity; synchronizes afterdischarges	Ellender, 2014
in vitro: 0 mM Mg^{2+}, 5 mM K^+	Arch	PV	10 s	during the afterdischarge phase	decrease in afterdischarge frequency	Ellender, 2014
in vitro: 4-AP (50 μM)	ChR2	Gad2	5 ms, 20/50 Hz or constant illumination for 5/10 s	After the induction of epileptiform activity, exact timing not reported	altered the dynamics of epileptiform activity	Ledri, 2014
in vitro: 4-AP (50 μM)	ChR2	PV	5 ms, 20/50 Hz or constant illumination for 5/10 s	After the induction of epileptiform activity, exact timing not reported	altered the dynamics of epileptiform activity	Ledri, 2014
in vitro: 4-AP (50 μM)	ChR2	SST	5 ms, 20/50 Hz or constant illumination for 5/10 s	After the induction of epileptiform activity, exact timing not reported	altered the dynamics of epileptiform activity	Ledri, 2014
in vivo: intrahippocampal kainic acid (50-100 nL, 20 mM)	ChR2	PV	Intervention consisted of 3 s of pulsed light delivery. Both short light pulses (50 ms on, 100 ms off) and longer pulses (1000 s on, 50 ms off) were investigated.	closed loop system, exact timing not reported	reduced seizure duration	Krook-Magnuson, 2014
in vivo: intrahippocampal kainic acid (50-100 nL, 20 mM)	NphR2/HR	PV	Intervention consisted of 3 s of pulsed light delivery. Both short light pulses (50 ms on, 100 ms off) and longer pulses (1000 ms on, 50 ms off) were investigated.	closed loop system, exact timing not reported	reduced seizure duration	Krook-Magnuson, 2014
in vivo: intrahippocampal kainic acid (50-100 nL, 20 mM)	ChR2	PV	Intervention consisted of 30-60 s of pulsed light delivery. Both short light pulses (50 ms on, 100 ms off) and longer pulses (2000 ms on, 50 ms off) were investigated.	closed loop system, exact timing not reported	reduced seizure duration, reduction in behavioral seizures	Krook-Magnuson, 2013

*This table summarizes the peer-reviewed publications on the impact of activation or inhibition of interneurons using optogenetics on epileptiform activity in vitro and in vivo. These studies highlight the inconsistencies in the findings regarding the effect of activating or silencing interneurons on epileptiform activity.

chloride gradient, drive afterdischarges. Optogenetic activation of PV-interneurons entrained and increased the duration of afterdischarges; whereas, silencing PV-interneurons reduced afterdischarges in an in vitro epilepsy model. The involvement of excitatory actions of GABA is supported by recent findings demonstrating that activation of GABAergic interneurons induces pyramidal cell excitation in the 4-aminopyridine (4-AP) model (4, 5). A single in vitro study claimed that optogenetic activation of interneurons suppressed epileptiform activity (5). Indeed, activation of interneurons suppressed ictal-like activity, but closer examination demonstrates that activation of interneurons may alter the nature of epileptiform activity, similar to other reports (Table 1) (2, 4), driving what appears to be lower frequency epilepti-

form activity (5). The majority of these studies utilized the 4-AP model of epilepsy. The highlighted study was critical in demonstrating that interneuron activation contributed to the generation of epileptiform activity in a different in vitro model (O-Mg^{2+}), suggesting that these findings are not model specific. In patients with epilepsy, large-scale recordings from the neocortex demonstrate that fast-spiking interneurons fire throughout the tonic phase of a seizure then cease firing about halfway through a seizure event (6). The cessation of fast-spiking interneuron firing is associated with an increase in spike-and-wave activity, suggesting that the activity of interneurons may modulate the type of epileptiform activity, mediating the transition from tonic and clonic phases of seizure activity (6). Of interest, these data suggest that GABAergic inhibition may become ineffective or even detrimental throughout seizure progression, contributing to the generation of epileptiform activity and raising questions as to the therapeutic potential of optogenetically activating these neurons for seizure control.

In contrast to the highly reproducible findings reviewed above demonstrating a role of interneurons in driving epileptiform activity in vitro, the same does not appear to be the case in vivo. The two studies that have investigated the ability of optogenetic activation of interneurons to control seizure activity in vivo have demonstrated antiseizure effects (7, 8). Using a closed-loop system, optogenetic activation of PV-interneurons at the onset of seizure activity reduced the duration of both electrographic and behavioral seizures (7, 8). These data support an inhibitory role for interneurons throughout seizure progression and suggest that activation of interneurons may be beneficial for seizure control. Of interest, this group observed similar antiseizure effects by optogenetically inhibiting PV-interneurons (7), suggesting that disruption of the epileptic network, either by activating or inhibiting PV-interneurons, is sufficient to reduce seizure activity.

It is clear that the neuronal networks in vivo are more complex than those studied in vitro. However, there may be additional differences in the experimental design that contribute to conflicting observations regarding the impact of interneurons on epileptiform activity. It is possible that the timing of interneuron activation during seizure progression is essential to their role in seizure activity. For example, in the in vivo studies, interneurons were activated early in the onset of electrographic seizure activity, prior to the manifestation of behavioral seizures (8). In contrast, the in vitro studies largely focused on the activation of interneurons once epileptiform activity had already developed (Table 1). It remains to be determined whether the role of interneurons in modulating epileptiform activity differs at the onset of epileptiform activity versus during prolonged epileptiform activity. Prolonged epileptiform activity may be necessary to cause a collapse in the chloride gradient, resulting in excitatory actions of GABA, contributing to the progression of epileptiform activity. It is also possible that the pattern of stimulation may be important for promoting synchrony and entraining principal neurons to drive epileptiform activity. However, Krook-Magnuson et al. (7, 8) used different stimulation patterns that were both capable of reducing electrographic and behavioral seizures. Further, the location of interneuron activation in the epileptic network may be important. Temporally controlled focal seizure induction enables investigation into the role of timing and localization of interneuron activation on epileptiform activity (9, 10). Optogenetic activation of PV-interneurons within the epileptogenic focus prior to seizure induction exacerbated epileptiform activity (9, 10). Of interest, activation of interneurons within the epileptogenic focus rapidly induced and synchronized afterdischarges (11), similar to the observations in the currently highlighted study. However, activation of interneurons distal from the epileptogenic focus restricted seizure propagation (9). Krook-Magnuson et al. (7) demonstrated that activation of interneurons in the cerebellum is sufficient to reduce epileptiform activity in a temporal lobe model of epilepsy. These findings highlight that epilepsy is a network disorder and that interactions between brain regions must be considered.

Another important issue to consider is that the interneuron networks may be altered in some epilepsies. As mentioned above, GABAergic signaling may become dysfunctional during seizure progression. Alterations in the connectivity of interneurons have been demonstrated in experimental models of epilepsy (11), and stimulation of these aberrant inhibitory networks may have adverse consequences. However, there is evidence that optogenetic activation of grafted GABAergic interneurons enhances inhibition (12) and may be an effective strategy for seizure control.

These incongruent findings regarding the role of interneurons in seizure generation and progression demonstrate that it is imperative that we develop a better understanding of the role of interneurons in different brain regions in modulating seizure activity before attempting to manipulate them for seizure control.

by Jamie L. Maguire, PhD

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