



# The Devil's in the Details: How to Harness Inhibition for Seizure Control

Epilepsy Currents  
2020, Vol. 20(2) 99-101  
© The Author(s) 2020  
Article reuse guidelines:  
sagepub.com/journals-permissions  
DOI: 10.1177/1535759720902078  
journals.sagepub.com/home/epi



## Paradoxical Effects of Optogenetic Stimulation in Mesial Temporal Lobe Epilepsy

Levesque M, Chen LY, Etter G, et al. *Ann Neurol.* 2019;86(5):714-728. doi:10.1002/ana.25572.

**Objective:** To establish the effects induced by long-term, unilateral stimulation of parvalbumin (PV)-positive interneurons on seizures, interictal spikes, and high-frequency oscillations (80-500 Hz) occurring after pilocarpine-induced status epilepticus (SE)—a proven model of mesial temporal lobe epilepsy (MTLE)—in transgenic mice expressing or not expressing ChR2. **Methods:** Both PV-ChR2 ( $n = 6$ ) and PV-Cre ( $n = 6$ ) mice were treated with pilocarpine to induce SE. Three hours after SE onset, unilateral optogenetic stimulation (450 nm, 25 mW, 20 ms pulses delivered at 8 Hz for 30 seconds every 2 minutes) of CA3 PV-positive interneurons was implemented for 14 continuous days in both groups. **Results:** Rates of seizures ( $P < .01$ ), interictal spikes ( $P < .001$ ), and interictal spikes with fast ripples (250-500 Hz;  $P < .001$ ) were lower in PV-ChR2 than in PV-Cre mice. Ripples (80-200 Hz) occurring outside of interictal spikes had higher rates in the PV-ChR2 group ( $P < .01$ ), whereas isolated fast ripples had lower rates ( $P < .01$ ). However, seizure probability was higher during optogenetic stimulation in PV-ChR2 compared to PV-Cre animals ( $P < .05$ ). **Interpretation:** Our findings show that the unilateral activation of CA3 PV-positive interneurons exerts anti-ictogenic effects associated with decreased rates of interictal spikes and fast ripples in this MTLE model. However, PV-positive interneuron stimulation can paradoxically trigger seizures in epileptic animals, supporting the notion that  $\gamma$ -aminobutyric acid type A signaling can also initiate ictogenesis.

## Commentary

Albert Einstein is quoted as having noted that “Everything should be made as simple as possible, but not simpler.” The role of parvalbumin (PV)-expressing inhibitory neurons in seizures has been shown to be anything but simple.

The role of GABAergic signaling itself is complicated and can depend on a number of factors including potentially the reversal potential of chloride at any given time or location. This in turn can impact the effect of activation of inhibitory neurons on seizures. For example, photoactivation of inhibitory neurons in CA3<sup>1</sup> or dentate gyrus<sup>2</sup> has been shown to inhibit seizures, while photoactivation of inhibitory neurons in the subiculum actually prolonged seizures,<sup>1</sup> and photoactivation of inhibitory neurons in the entorhinal cortex (in the dorsal intrahippocampal kainate model of temporal lobe epilepsy) had no effect,<sup>2</sup> presumably due to its relationship to the seizure focus.

Increasing the specificity of intervention to exciting just PV-expressing interneurons does little to reduce the complexity. On-demand intervention, with light delivery to the hippocampus ipsilateral or contralateral to the seizure focus in mice expressing channelrhodopsin in PV neurons (PV-ChR2 mice), can inhibit spontaneous seizures months after

intrahippocampal kainate injection.<sup>3</sup> Similarly, others have shown that chemogenetic activation of hippocampal PV neurons can inhibit seizures.<sup>4</sup> However, a study examining acute seizures (focal 4AP application to the cortex) found mixed effects depending on the relative timing of the optogenetic activation of PV cells, with light delivery inhibiting seizures when applied during the ictal phase, but interictal light delivery actually having a strong ictogenic effect.<sup>5</sup> Work examining effects on epileptiform activity in slices has shown divergent effects of stimulation of PV cells,<sup>6-11</sup> again with suggestions that the timing<sup>7</sup> and/or relative location of light delivery<sup>9</sup> are key factors. An additional important consideration is the heterogeneity of PV cells themselves.<sup>12</sup>

A recent study by Lévesque and colleagues adds additional complexity to the effects of PV cell activation on seizures. Lévesque et al<sup>13</sup> examined the impact of periodic optogenetic stimulation of CA3 PV neurons for 2 weeks starting 3 hours after intraperitoneal pilocarpine-induced SE in mice. Stimulation consisted of 20 milliseconds light pulses at 8 Hz, for 30 seconds, every 2 minutes. While the power of light used was fairly high (25 mW), opsin-negative animals were used as a control, relieving concerns of off-target effects of the light.



Creative Commons Non Commercial No Deriv CC BY-NC-ND: This article is distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDeriv 4.0 License (<https://creativecommons.org/licenses/by-nc-nd/4.0/>) which permits non-commercial use, reproduction and distribution of the work as published without adaptation or alteration, without further permission provided the original work is attributed as specified on the SAGE and Open Access pages (<https://us.sagepub.com/en-us/nam/open-access-at-sage>).



The authors report no change in latent period or average seizure duration but did find a significant (and sizeable) reduction in seizure rate with light delivery in PV-ChR2 compared to PV-Cre (opsin-negative control) animals. This was accompanied by a reduction in interictal spiking in PV-ChR2 animals, and an increase in isolated ripples. The reduction in seizure frequency is of obvious clinical relevance, but so too is the reduction in interictal spiking and increase in isolated ripples, as it may improve cognitive outcomes.<sup>14,15</sup> Together, these findings suggest a strong inhibition of epileptiform activity through activation of PV cells.

However, things got more complicated when Lévesque et al took a closer look at their data. Given the decrease in both interictal spiking and seizure rate in animals with periodic activation of PV cells, a particularly surprising finding was that light-on periods had a relatively higher rate of seizures than light-off periods in PV-ChR2 mice. This suggests both a seizure inhibiting effect (PV-ChR2 vs PV-Cre) and a seizure promoting effect (light-on vs light-off) of activation of PV cells. What could be driving these differing results?

Experimentally, the comparisons are different. One possibility is that activation of PV neurons (in this location and model) increases the probability of seizures, and the reason that it appears to reduce seizure frequency when comparing PV-ChR2 and PV-Cre mice is actually a strain susceptibility difference. Specifically, the seizure phenotype—regardless of activation of PV cells—might be stronger in PV-Cre mice. However, the authors separately examined the frequency of seizures in PV-ChR2 mice not treated with light; the seizure frequency in nontreated PV-ChR2 mice appeared to be higher than in either PV-Cre or light-treated PV-ChR2 mice. Therefore, it appears that the dichotomous effects of light are real—activation of PV cells produces a global decrease in seizure frequency, but, at the specific time of light delivery, the animals are relatively susceptible to seizures.

How could this be happening? One possibility is that activation of PV cells creates a pro-ictal state, but that immediately following light, an (apparently stronger) anti-ictogenic state is entered. This potential explanation is somewhat difficult to reconcile with previous literature but is a tempting interpretation of the cumulative histograms Levesque and colleagues provide. Visual inspection of those histograms suggests a strong reduction in seizure probability immediately following light delivery.

Another possibility, discussed briefly above, is that the timing of the intervention is critical. For example, if the animal is just starting to have a seizure, light delivery may be able to quickly prevent that seizure. In contrast, light delivery at other times could help create a pro-ictal state. This may be especially true with pilocarpine<sup>16</sup> and given the strong impact Levesque and colleagues noted of the chosen 8 Hz light pattern on (non-ictal) hippocampal oscillations. Such an interpretation is in strong agreement with previous work using an acute seizure model in the cortex, in which PV activation during

electrographic seizures terminated seizures, while PV activation during interictal phases actually had a pro-ictal effect.<sup>5</sup> Overall, in the work by Levesque and colleagues, the decrease in seizure likelihood appears to outweigh the occasional increase, resulting in a net decrease in seizure frequency. This could explain why a chemogenetic approach to activation of PV cells (which would also lack temporal specificity) would also show a (net) decrease in seizure frequency.<sup>4</sup> Finally, a timing explanation additionally fits squarely with previous work showing that on-demand (ie., where light delivery is timed to match seizures) excitation of PV cells inhibits seizures.<sup>3</sup> If the timing interpretation is true, it would provide a strong argument for using on-demand interventions, which would allow the benefits of intervention without the drawbacks.

One thing is clear—the impact of PV cells on seizure activity is not simple. However, detangling the complexity may be a fruitful endeavor; harnessed the correct way, PV inhibitory neurons could provide significant benefits for outcomes in epilepsies.

By Esther Krook-Magnuson 

## ORCID iD

Esther Krook-Magnuson  <https://orcid.org/0000-0002-6119-0165>

## References

1. Wang Y, Xu C, Xu Z, et al. Depolarized GABAergic signaling in subicular microcircuits mediates generalized seizure in temporal lobe epilepsy. *Neuron*. 2017;95(5):1221.
2. Lu Y, Zhong C, Wang L, et al. Optogenetic dissection of ictal propagation in the hippocampal-entorhinal cortex structures. *Nat Commun*. 2016;7:10962.
3. Krook-Magnuson E, Armstrong C, Oijala M, Soltesz I. On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy. *Nat Commun*. 2013;4:1376.
4. Wang Y, Liang J, Chen L, et al. Pharmacogenetic therapeutics targeting parvalbumin neurons attenuate temporal lobe epilepsy. *Neurobiol Dis*. 2018;117:149-160.
5. Assaf F, Schiller Y. The antiepileptic and ictogenic effects of optogenetic neurostimulation of PV-expressing interneurons. *J Neurophysiol*. 2016;116(4):1694-1704.
6. Ledri M, Madsen MG, Nikitidou L, Kirik D, Kokaia M. Global optogenetic activation of inhibitory interneurons during epileptiform activity. *J Neurosci*. 2014;34(9):3364-3377.
7. Ellender TJ, Raimondo JV, Irkle A, Lamsa KP, Akerman CJ. Excitatory effects of parvalbumin-expressing interneurons maintain hippocampal epileptiform activity via synchronous after discharges. *J Neurosci*. 2014;34(46):15208-15222.
8. Yekhlief L, Breschi GL, Lagostena L, Russo G, Taverna S. Selective activation of parvalbumin- or somatostatin-expressing interneurons triggers epileptic seizurelike activity in mouse medial entorhinal cortex. *J Neurophysiol*. 2015;113(5):1616-1630.



9. Sessolo M, Marcon I, Bovetti S, et al. Carmignoto, parvalbumin-positive inhibitory interneurons oppose propagation but favor generation of focal epileptiform activity. *J Neurosci.* 2015; 35(26):9544-9557.
10. Shiri Z, Manseau F, Lévesque M, Williams S, Avoli M. Interneuron activity leads to initiation of low-voltage fast-onset seizures. *Ann Neurol.* 2015;77(3):541-546.
11. Shiri Z, Manseau F, Lévesque M, Williams S, Avoli M. Activation of specific neuronal networks leads to different seizure onset types. *Ann Neurol.* 2016;79(3):354-365.
12. Christenson Wick Z, Krook-Magnuson E. Seizing sequencing data to consider cell and circuit complexity. *Epilepsy Curr.* 2019;19(2):124-125.
13. Lévesque M, Chen LY, Etter G, et al. Paradoxical effects of optogenetic stimulation in mesial temporal lobe epilepsy. *Ann Neurol.* 2019;86(5):714-728.
14. Gelinas JN, Khodagholy D, Thesen T, Devinsky O, Buzsáki G. Interictal epileptiform discharges induce hippocampal-cortical coupling in temporal lobe epilepsy. *Nat Med.* 2016;22(6):641-648.
15. Kleen JK, Scott RC, Holmes GL, et al. Hippocampal interictal epileptiform activity disrupts cognition in humans. *Neurology.* 2013;81(1):18-24.
16. Yi F, DeCan E, Stoll K, Marceau E, Deisseroth K, Lawrence JJ. Muscarinic excitation of parvalbumin-positive interneurons contributes to the severity of pilocarpine-induced seizures. *Epilepsia.* 2015;56(2):297-309.