



# When a Good Cop Turns Bad: The Pro-Ictal Action of Parvalbumin Expressing Interneurons During Seizures

## KCC2 Overexpression Prevents the Paradoxical Seizure-Promoting Action of Somatic Inhibition

Magloire, V., Cornford, J., Lieb, A., Kullmann, D. M., and Pavlov, I. *Nat. Commun.* 10, 1225. doi:10.1038/s41467-019-08933-4.

Although cortical interneurons are apparently well-placed to suppress seizures, several recent reports have highlighted a paradoxical role of perisomatic-targeting parvalbumin-positive (PV+) interneurons in ictogenesis. Here, we use an acute in vivo model of focal cortical seizures in awake behaving mice, together with closed-loop optogenetic manipulation of PV+ interneurons, to investigate their function during seizures. We show that photo-depolarization of PV+ interneurons rapidly switches from an anti-ictal to a pro-ictal effect within a few seconds of seizure initiation. The pro-ictal effect of delayed photostimulation of PV+ interneurons was not shared with dendrite-targeting somatostatin-positive (SOM+) interneurons. We also show that this switch can be prevented by overexpression of the neuronal potassium-chloride co-transporter KCC2 in principal cortical neurons. These results suggest that strategies aimed at improving the ability of principal neurons to maintain a trans-membrane chloride gradient in the face of excessive network activity can prevent interneurons from contributing to seizure perpetuation.

Epileptic seizures can occur when inhibitory systems within the brain fail to prevent the initiation and spread of aberrant neuronal excitation. From a therapeutic perspective, it is crucial that we understand what causes inhibitory system failure, how this can contribute to initiating and sustaining seizures, and whether we can “shore up” inhibition to stop seizures.

Fast synaptic inhibition in the brain is primarily mediated by the neurotransmitter GABA binding to postsynaptic type A GABA receptors (GABA<sub>A</sub>Rs). When activated, GABA<sub>A</sub>Rs become permeable to chloride ions. Under physiological circumstances, the concentration of chloride outside of neurons is far greater than that inside; this is largely due to the action of the membrane bound chloride transporter, KCC2, which extrudes chloride from mature neurons. Therefore, when activated, GABA<sub>A</sub>Rs result in an inward flux of chloride into cells. This inward flow of negative charge hyperpolarizes neurons and makes them less likely to fire action potentials, underlying the inhibitory effect of GABA. It is well known that blockade of GABA<sub>A</sub>Rs, by suppressing this action, readily elicits seizures in vitro and in vivo. Conversely, GABA<sub>A</sub>R enhancers (such as benzodiazepines) are widely used antiepileptic agents.

Parvalbumin expressing (PV+) interneurons, which predominantly form GABAergic synapses on the cell bodies of their targets, near the site of action potential generation, are thought of as one of the most powerful inhibitory cells in the brain. However, evidence has begun to emerge that the simple classification of

GABA<sub>A</sub>R signaling as strictly inhibitory is likely incorrect. For example, under conditions where there is a high intracellular chloride concentration within neurons, activation of GABA<sub>A</sub>Rs can lead to an efflux of negative charge, neuronal depolarization and paradoxically, action potential generation. This can occur when large amount of chloride influx occurs<sup>1</sup> or when chloride extrusion is compromised.<sup>2</sup> In addition, previous work has shown that during seizure-like events in vitro, profound chloride accumulation can occur, which renders GABAergic transmission excitatory.<sup>3,4</sup> This suggests that the timing of GABA<sub>A</sub>R activation relative to seizure-onset, might be crucial for determining the effects of GABAergic inhibition. For example, prior to, or early during a seizure, activating interneurons should inhibit ictal activity, while late activation should enhance ictal activity. However, to date, in vivo evidence for this phenomenon has been lacking. In addition, selective activation of inhibitory interneurons using optogenetics has recently been suggested as a possible strategy for dynamically treating seizures.<sup>5</sup> Therefore, determining the role of inhibitory interneurons during the evolution of seizure activity in vivo is an important goal for epilepsy research.

To address this, Vincent Magloire, together with colleagues at University College London Institute of Neurology, employed automated seizure onset detection and closed-loop optogenetic activation of different interneuron subtypes during cortical seizures in awake behaving mice. Remarkably, they found that the effect of selectively activating local PV+ interneurons switched





from being antiepileptic to pro-epileptic during the first few seconds following seizure onset. This pro-epileptic effect of PV+ cells could be abolished by increasing the expression of the chloride extruder KCC2 in pyramidal neurons. As a result, this study provides persuasive *in vivo* evidence for the hypothesis that an increase in intracellular chloride concentration, and a subversion in the action of PV+ interneurons, can contribute to the maintenance of seizure activity.

In this technically sophisticated study, the authors evoked seizure activity using acute application of the convulsant pilocarpine delivered via a cannula into the deep cortical layers of V1 in awake mice. Electrographic activity could then be recorded via an electrocorticogram electrode beneath the cannula connected to a subdermal wireless transmitter. Seizure events could then be detected and light delivered via an optical fibre through the same cannula at precise times relative to seizure onset. In mice where the light-activated cation channel (channelrhodopsin) was genetically targeted to PV+ interneurons, and light was delivered immediately following the onset of seizures, a significant reduction in seizure duration was observed. This indicated an inhibitory action of PV+ interneurons early in the ictal activity, which is in line with previous closed-loop studies utilizing optogenetic activation of these cells following seizure onset.<sup>5</sup> Interestingly, when these same cells were optogenetically activated after a delay of >2 seconds following the onset of seizures, they reliably increased seizure duration. Conversely, using instead an optogenetic silencer (Arch, a light-activated outward proton pump) expressed in PV+ cells, the authors observed that deferred light administration now reduced seizure duration. This clearly supports the counterintuitive conclusion that after a few seconds of seizure activity, PV+ interneurons promote the maintenance of seizure activity. Using the same experimental strategy to selectively activate somatostatin expressing (SOM+) interneurons, which target the dendrites of pyramidal cells (rather than the cell bodies), the authors found a slightly different picture. Like PV+ cells, SOM+ interneurons are inhibitory when activated early after seizure onset, but when activated after 2 seconds, they had no observable effect on seizure duration. This difference supports previous *in vitro* work which demonstrated more profound chloride accumulation at the cell bodies of neurons as compared to the dendrites during seizures.<sup>6</sup>

To confirm the hypothesis that the observed excitatory action of PV+ interneurons during seizures might be due chloride accumulation, Magloire et al used a viral strategy to enhance the expression of the potassium-chloride cotransporter KCC2 in cortical pyramidal cells. They demonstrated that this was effective in improving the Cl<sup>-</sup> extrusion capacity of the neurons. In mice where KCC2 was overexpressed, the optogenetic activation of PV+ interneurons a few seconds after seizure onset no longer prolonged the seizures. However, it should be noted that seizures were not reduced in duration either. Therefore, although the authors provide compelling evidence that a breakdown in the chloride gradient underlies the seizure-associated excitatory shift in the action of PV+ interneurons, their strategy to enhance Cl<sup>-</sup> extrusion was not sufficient to rescue the inhibitory action of these interneurons. This failure may be due to the inability of overexpressed KCC2 to traffic to

the cell membrane or a failure of PV cells to maintain their function during prolonged seizures. Future work could use emerging technologies to directly measure the spatial and temporal dynamics of chloride accumulation during seizures *in vivo*.<sup>7</sup> The development of novel strategies to extrude chloride from neurons may also serve to preserve the inhibitory capacity of interneurons in the face of progressive ictal activity.<sup>8</sup>

From a clinical perspective, the results generated by Magloire and colleagues have direct relevance for the treatment of patients in status epilepticus (SE), which represents a seizure which does not stop of its own accord. First-line therapy for SE currently constitutes the benzodiazepine class of drugs, which enhance GABA<sub>A</sub>R signaling. This work suggests that at least at the seizure focus, GABA<sub>A</sub>R signaling via PV+ interneurons may in fact exacerbate aberrant excitation. Benzodiazepine resistance is known to occur in SE. This study suggests that seizure-induced accumulation of intracellular Cl<sup>-</sup> may be a mechanism underlying this challenging clinical quandary. Therefore, the design of future strategies for arresting seizures would benefit from the level of mechanistic understanding provided by Magloire et al and focus on improving chloride extrusion, enhancing alternative inhibitory systems in the brain, or the more timely delivery of benzodiazepines and similar GABA-enhancing pharmacological therapies.

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## References

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