



# Imaging Interneuron Impairment in Ictogenesis: A Spatiotemporal Sweet Spot of Seizure Sampling in Scn1a+/-

Epilepsy Currents

2021, Vol. 21(5) 376–378

© The Author(s) 2021

Article reuse guidelines:

[sagepub.com/journals-permissions](https://sagepub.com/journals-permissions)

DOI: 10.1177/15357597211032045

[journals.sagepub.com/home/epi](https://journals.sagepub.com/home/epi)

## Two-Photon Calcium Imaging of Seizures in Awake, Head-Fixed Mice

Somarowthu A, Goff KM, Goldberg EM. *Cell Calcium*. 2021;96: 102380. doi:10.1016/j.ceca.2021.102380

Epilepsy is a severe neurological disorder defined by spontaneous seizures. Current treatment options fail in a large proportion of patients, while questions as to the basic mechanisms of seizure initiation and propagation remain. Advances in imaging of seizures in experimental model systems could lead to a better understanding of mechanisms of seizures and epilepsy. Recent studies have used two-photon calcium imaging (2 P imaging) in awake, behaving mice in head-fixed preparations to image seizures *in vivo* at high speed and cellular-level resolution to identify key seizure-related cell classes. Here, we discuss such advances and present 2 P imaging data of excitatory neurons and defined subsets of cerebral cortex GABAergic inhibitory interneurons during naturalistic seizures in a mouse model of Dravet syndrome (Scn1a+/- mice) along with other behavioral measures. Results demonstrate differential recruitment of discrete interneuron subclasses, which could inform mechanisms of seizure generation and propagation in Dravet syndrome and other epilepsies.

## Commentary

Many of the ~30% of epileptic patients who experience breakthrough seizures have relatively long stretches of time in which everything is “normal”. Seizures interrupt these periods of “normalcy” with bouts of convulsions, unconsciousness and temporary loss of cognitive function. The unpredictability of seizures is especially disruptive as it turns everyday situations such as driving or even bathing into potential safety hazards. What happens when a brain transitions from a state of healthy cognition to a state of seizure? Very little is known about the nature of ictogenesis, in part because the rarity of spontaneous seizures makes them experimentally challenging to study.

Acute brain slices do not seize spontaneously; *in vitro* recordings of ictogenesis in acute brain slices rely on application of chemical convulsants. While such preparations recapitulate the electrophysiological phenotype of seizures and are compatible with intracellular electrophysiological recordings and large-scale calcium imaging, the mechanisms of ictogenesis may depend heavily on the target of the applied convulsant and thus differ from spontaneous seizures. More recently, organotypic hippocampal slice cultures have been observed to produce spontaneous recurrent seizures after ~1 week *in vitro*.<sup>1,2</sup> However, they remain an accelerated and reduced model of seizures in an intact brain. Recordings of ictogenesis *in vivo* are complicated by technical limitations in spatiotemporal scale and resolution. *In vivo*, animal models of acquired epilepsy commonly begin with convulsant-induced status epilepticus or traumatic brain injury. Spontaneous recurrent seizures vary with model but typically occur weeks-months later at a frequency of

~1/day. Thus, capturing a seizure requires long-term, chronic recordings, which currently are feasible only with electrophysiology. Microelectrode arrays enable high temporal resolution and stable recordings during baseline activity, but have a limited ability to identify cell types or track individual neurons throughout ictus.<sup>3</sup> *In vivo* calcium imaging is a powerful tool that enables robust identification of activity in neurochemically defined cells through targeted expression of genetically encoded calcium indicators. However, most imaging modalities are limited to relatively short recording epochs. As such, *in vivo* calcium imaging of epileptiform activity has largely been confined to interictal spikes<sup>4</sup> or seizures evoked by chemical<sup>5</sup> or optogenetic<sup>6</sup> stimulation. Electrophysiological recordings of ictogenesis in humans have dramatically improved in recent years,<sup>7,8</sup> but are limited in identification of cell type and are also challenged by the relatively low frequency of spontaneous seizures. To gather sufficient data for pre-surgical planning, patients are often weaned from antiepileptic drugs to increase ictal frequency, which may alter seizure kinetics.

In the highlighted manuscript, Somarowthu et al<sup>9</sup> continue to advance the ongoing quest to record novel data during physiologically relevant seizures *in vivo*. Towards this end, they performed two-photon calcium imaging in awake, head-fixed Scn1a+/- mice, a model of the severe, genetic epilepsy known as Dravet Syndrome. As with models of acquired epilepsies, Scn1a+/- mice have spontaneous seizures at a frequency that is incompatible with head-fixed imaging: approximately 1 seizure per 2 days. However, conveniently, Scn1a+/- mimic the Dravet Syndrome phenotype of hyperthermia-induced seizures. The





authors took advantage of this to induce physiologically relevant seizures within an experimentally tractable imaging time window. Specifically, they used this experimental paradigm to track pre-ictal and ictal activity in neurochemically defined populations of neurons, recordings which are currently only possible with fluorescence imaging. To accomplish this, a synapsin-driven adeno-associated virus vector was used to non-selectively transduce neurons with a green genetically encoded calcium indicator, while cre-lox targeting was used to label with a red fluorescent protein 3 different classes of interneurons: parvalbumin (PV), somatostatin (SST), and vasoactive intestinal peptide (VIP).

Following a baseline recording during which the head-fixed animal was resting or running on a spherical treadmill, body temperature was gradually increased until ictogenesis was identified using 4 monitored parameters: movement, pupil diameter, whisking, and calcium dynamics. The calcium traces, simultaneously recorded from labelled interneuron subtypes and presumed principal cells, revealed 2 key findings. First, SST+ interneurons activated significantly earlier than principal cells, with a mean recruitment time that preceded seizure onset by >3 s. Second, VIP+ interneurons activated with a mean amplitude that was significantly lower; ~62% of that observed in presumed principal cells.

These findings support a model wherein VIP+ interneuron activity appears to be pathologically decreased in Scn1a+/- mice. Since VIP+ cells prominently inhibit SST cells, this decrease in VIP activity may effectively disinhibit SST cells, supporting the early SST activation observed. It is, however, difficult to know whether the amplitudes of ictal VIP cell calcium transients are truly pathological in Scn1a+/- mice since wild type (WT) control mice have no seizures, hence no ictal calcium transients. Simply comparing baseline activity in Scn1a+/- vs control mice may not be sufficient to detect firing deficiencies that only appear during bouts of elevated activity. However, if baseline VIP cell activity appears normal in Scn1a+/- mice, one might infer that there is another mechanism that drives Scn1a+/- VIP cells into the range wherein they fail. A ubiquitous challenge in discovering the network pathology that leads to seizure onset is determining whether observed pathologies are the “chicken” or the “egg” in ictogenesis. Other controls in future work to identify whether VIP cells are the salient population that drives ictogenesis might include cre targeting of Scn1a mutations using recently developed conditional mouse models of Dravet Syndrome, imaging cell-type specific activity in WT mice during convulsant-evoked seizures to test whether the observed calcium kinetics result from the Scn1a+/- mutation, and, if so, optogenetic manipulations to mimic VIP deficits in WT mice.

As detailed above, the strength of this model is that it elegantly exists at the narrow intersection of physiological relevance and experimental tractability. Of course, no experimental paradigm is perfect. As noted in previous work, the mortality rate for hyperthermia-induced seizures is approximately 30%,<sup>10</sup> which prevented characterization of intra-animal seizure variability and presumably led to the low number of animals reported in the highlighted study. Also, as with animal models of acquired

epilepsy, it is unknown whether the region being imaged is the focal onset zone. Thus, as discussed in the highlighted work, the activation patterns observed likely represent propagation of seizure, rather than ictogenesis per se.

Together, these results are exciting in that they identify, for the first time, cell-type specific activation patterns during propagation of seizures that are directly homologous to hyperthermic seizures in Dravet Syndrome. The findings support a VIP interneuron action potential generation deficit previously identified in acute slices prepared from Scn1a+/- mice and further identify a putative secondary pre-ictal effect in SST interneurons. In addition to identifying a putative mechanism for seizure propagation in a model of Dravet Syndrome, the work provides a framework for imaging *in vivo* cell type specific activity in any model of febrile seizures. One could further extrapolate techniques described to image cell type specific activity in models of epilepsy with frequent spontaneous non-convulsive seizures, such as the intrahippocampal kainate model. Gaining knowledge of cell-type specific activity during epileptiform activity will undoubtedly provide insight into novel and specific targets for antiepileptic therapies. The highlighted study by Somarowthu et al represents an important step toward making such recordings during physiologically relevant seizures.<sup>9</sup>

By Kyle P. Lillis 

## Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by National Institute of Neurological Disorders and Stroke (grant no. R01NS112538).

## ORCID iD

Kyle P. Lillis  <https://orcid.org/0000-0003-0219-8113>

## References

1. Dyhrfeld-Johnsen J, Berdichevsky Y, Swiercz W, Sabolek H, Staley KJ. Interictal spikes precede ictal discharges in an organotypic hippocampal slice culture model of epileptogenesis. *J Clin Neurophysiol.* 2010;27:418-424. doi:10.1097/WNP.0b013e3181fe0709
2. Magalhães DM, Pereira N, Rombo DM, Beltrão-Cavacas C, Sebastião AM, Valente CA. Ex vivo model of epilepsy in organotypic slices—a new tool for drug screening. *J Neuroinflammation.* 2018; 15:203. doi:10.1186/s12974-018-1225-2
3. Merricks EM, Smith EH, McKhann GM, et al. Single unit action potentials in humans and the effect of seizure activity. *Brain.* 2015; 138:2891-2906. doi:10.1093/brain/awv208
4. Muldoon SF, Villette V, Tressard T, et al. GABAergic inhibition shapes interictal dynamics in awake epileptic mice. *Brain.* 2015; 138:2875-2890. doi:10.1093/brain/awv227
5. Wenzel M, Hamm JP, Peterka S, Yuste R. Two-photon imaging reveals the population dynamics of spatiotemporally compartmentalized ictal networks *In vivo*. Presented at the Society for



- Neuroscience; October 2015; Chicago, IL; Society for Neuroscience; 2015.
6. Khoshkhoo S, Vogt D, Sohal VS. Dynamic, cell-type-specific roles for GABAergic interneurons in a mouse model of optogenetically inducible seizures. *Neuron*. 2017;93:291-298. doi:[10.1016/j.neuron.2016.11.043](https://doi.org/10.1016/j.neuron.2016.11.043)
  7. Schevon CA, Weiss SA, McKhann G, et al. Evidence of an inhibitory restraint of seizure activity in humans. *Nat Commun*. 2012; 3:1060. doi:[10.1038/ncomms2056](https://doi.org/10.1038/ncomms2056)
  8. Truccolo W, Ahmed OJ, Harrison MT, et al. Neuronal ensemble synchrony during human focal seizures. *J Neurosci*. 2014;34: 9927-9944. doi:[10.1523/JNEUROSCI.4567-13.2014](https://doi.org/10.1523/JNEUROSCI.4567-13.2014)
  9. Somarowthu A, Goff KM, Goldberg EM. Two-photon calcium imaging of seizures in awake, head-fixed mice. *Cell Calcium*. 2021;96:102380. doi:[10.1016/j.ceca.2021.102380](https://doi.org/10.1016/j.ceca.2021.102380)
  10. Tran CH, Vaiana M, Nakuci J, et al. Interneuron desynchronization precedes seizures in a mouse model of dravet syndrome. *J Neurosci*. 2020;40:2764-2775. doi:[10.1523/JNEUROSCI.2370-19.2020](https://doi.org/10.1523/JNEUROSCI.2370-19.2020)