2P or not 2P: The Question of Seizure Initiation

Layer- and Cell-Specific Recruitment Dynamics During Epileptic Seizures In Vivo

Aeed F, Shnitzer T, Talmon R, Schiller Y. *Ann Neurol.* 2020;87(1):97-115. doi:10.1002/ana.25628

Objective: To investigate the network dynamics mechanisms underlying differential initiation of epileptic interictal spikes and seizures. Methods: We performed combined in vivo 2-photon calcium imaging from different targeted neuronal subpopulations and extracellular electrophysiological recordings during 4-aminopyridine-induced neocortical spikes and seizures. Results: Both spikes and seizures were associated with intense synchronized activation of excitatory layer 2/3 pyramidal neurons (PNs) and to a lesser degree layer 4 neurons, as well as inhibitory parvalbumin-expressing interneurons (INs). In sharp contrast, layer 5 PNs and somatostatin-expressing INs were gradually and asynchronously recruited into the ictal activity during the course of seizures. Within layer 2/3, the main difference between onset of spikes and seizures lay in the relative recruitment dynamics of excitatory PNs compared to parvalbumin- and somatostatin-expressing inhibitory INs. Whereas spikes exhibited balanced recruitment of PNs and parvalbumin-expressing INs, during seizures IN responses were reduced and less synchronized than in layer 2/3 PNs. Similar imbalance was not observed in layers 4 or 5 of the neocortex. Machine learning-based algorithms we developed were able to distinguish spikes from seizures based solely on activation dynamics of layer 2/3 PNs at discharge onset. Interpretation: During onset of seizures, the recruitment dynamics markedly differed between neuronal subpopulations, with rapid synchronous recruitment of layer 2/3 PNs, layer 4 neurons, and parvalbumin-expressing INs and gradual asynchronous recruitment of layer 5 PNs and somatostatin-expressing INs. Seizures initiated in layer 2/3 due to a dynamic mismatch between local PNs and inhibitory INs, and only later spread to layer 5 by gradually and asynchronously recruiting PNs in this layer.

Commentary

Basic cellular and circuit mechanisms underlying seizure initiation and propagation remain poorly understood. Yet, advances in this domain may be vital for the development of new treatments for epilepsy. Further progress will benefit from detailed investigation of large-scale neural activity at high speed and across brain states including interictal, preictal, ictal, and postictal phases of seizures. New methods that facilitate such large scale recording with increasing spatial and temporal resolution are now being used and applied to the study of seizures and epilepsy in experimental model systems *in vivo*.

Electrophysiology (including single unit and local field potential [LFP] recording, electroencephalogram, etc) is the standard tool for monitoring neural activity in epilepsy research. Electrical recording can be limited by spatial resolution, scale, and an inability to definitively identify cell type of interest, although it should be noted that imaging cannot (yet) approach the temporal resolution of electrical recording. And of course electrophysiological methods also continue to advance, including optogenetic tagging of neuronal populations to identify specific cell types[1] and novel probes that facilitate recording of on the order of a thousand single units.[2] Recent studies[3-6] have used an optical imaging method, 2-photon laser scanning microscopy (2P imaging) combined with use of genetically encoded calcium indicators (2P calcium imaging) to record seizures. This technique uses a high-powered, pulsed infrared laser that facilitates imaging at depth in light scattering environments such as the intact brain. Calcium indicators, including genetically encoded indicators that can be delivered via viral vectors or expressed using transgenic mice, provide an indirect, low-pass filtered readout of electrical activity across hundreds or even thousands of cells with single-cell resolution, and can be expressed in genetically/molecularly defined cell types.

An exciting recent paper, Aeed et al, 2020,[7] combined 2-photon calcium imaging and electrophysiological recording to investigate the mechanism of initiation of seizure-like events induced by application of chemoconvulsive agents 4-aminopyridine (4-AP) or picrotoxin to the neocortex in a rodent model. The authors performed experiments in

transgenic mice expressing Cre recombinase under the control of promoter elements specific for key seizure-related cell types. In addition, the authors further combined this with advanced optical techniques such as use of a prism, to image at a 90° angle to the axis of the microscope objective which facilitates simultaneous recording of neuronal activity along the depth of the neocortex. Seizure-like events were induced mainly in anesthetized mice and activity was characterized separately in various cell types: excitatory neurons in layer 2/3, 4, and 5, and inhibitory parvalbumin-expressing (PV) GABAergic interneurons (PV-INs) and somatostatin-expressing (SST) GABAergic INs (SST-INs). Electrophysiological recordings were used to define the onsets of spikes and seizures. Relative to the identified onset of interictal spikes and seizures, the authors calculated cell–cell synchrony using cross-correlation between cells, and connectivity analysis was implemented to derive coactivation patterns based on temporal similarity. A machine learning algorithm, support vector machine, was implemented and applied to low dimensional diffusion maps extracted from the connectivity matrices to classify the 2-photon calcium imaging data.

The authors designed a very clever and technically challenging experiment to establish the key finding that seizure-like events initiate in layer 2/3, irrespective of where 4-AP is administered. The authors imaged layer 2/3, 4, or layer 5, with simultaneous recording of the LFP, and then applied 4-AP either to the surface of the neocortex, or locally. Interictal spikes were confined to layer 2/3 and 4, whereas seizures initiated in layer 2/3 and slowly invaded layer 5. Interestingly, pyramidal neurons in layer 2/3 were more prominently recruited by seizures (but not by interictal spikes) than PV-INs or SST-INs, whereas propagated seizures recruited different cell types similarly in layer 4. Application of the machine learning algorithm to layer 2/3 pyramidal neurons differentiated the onset of spikes and seizures with high accuracy. This data suggests that seizure initiation in this model requires and is driven by synchronous activation of layer 2/3 pyramidal neurons in the context of impaired recruitment of PV and SST-INs, whereas this "E/I mismatch" or imbalance does not occur in layer 4 or 5/6. This general pattern of synchronous recruitment of pyramidal neurons and PV-INs was also observed for interictal spikes; however, for spikes, recruitment of the two cell types in layer 2/3 remained balanced. This observation further suggests a potential mechanistic or causal relationship between the two types of events, with imbalance between pyramidal cell and PV-IN activation leading to transition from spike to seizure. This remains somewhat observational but potentially could be further tested via manipulation (activation or inhibition) of defined subsets of INs during interictal spikes and/or seizures, for example using chemo- or optogenetics.

Overall, the experimental design and data analysis were rigorous and provide support for the induction of differential cell type-specific neural activity by chemoconvulsant agents during spikes and seizure-like events. The combination of imaging and electrophysiology across layers and neuronal subclasses as well as simultaneous recording from layer 2/3 to 5 using a prism provides a novel glimpse into initiation and propagation of seizure-like events.

It should again be noted however that the seizure-like events were chemically induced in a healthy brain, and hence may not reflect the properties of initiation of spontaneous seizures in an epileptic brain. 4-aminopyridine, the agent used in the study, has many mechanisms of action, including blockade of Kv3 subfamily potassium channels at low concentrations which are well-known to facilitate high-frequency firing by fast-spiking GABAergic PV-INs in cerebral cortex. Hence, there may be some circularity in making inferences as to the mechanisms of seizure generation when such events are induced pharmacologically using agents that clearly act at least in part via impairment of inhibition. Would the same results obtain in a model in which PV-IN function was not impaired directly by 4-AP? Second, interictal spikes versus seizure-like events were recorded predominantly in anesthetized mice and classified based exclusively on LFP features, although the authors note that some events were accompanied by behavioral whisking movements. That said, 2P imaging of seizures, for example from experimental rodent models of acquired chronic temporal lobe epilepsy, has proven quite challenging for various reasons. However, naturalistic seizures have been studied using 2P imaging in mouse models of genetic epilepsies including absence seizures[3] and temperature-induced generalized tonic–clonic seizures in Dravet syndrome (Scn1a+/-mice).[4]

Despite these limitations, the highlighted study takes an important step forward both conceptually and methodologically toward greater understanding of seizure mechanisms. Two-photon calcium imaging and other large-scale imaging modalities, particularly when validated by or in combination with electrophysiology and combined with manipulation of activity, promise to be powerful tools for the studying of mechanisms of seizure initiation and propagation.

By Ala Somarowthu and Ethan M. Goldberg ⬤

ORCID iD

Ethan M. Goldberg ⬤ https://orcid.org/0000-0002-7404-735X

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