Ictogenesis? That’s Random…..

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In Vitro Ictogenesis is Stochastic at the Single Neuron Level
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Seizure initiation is the least understood and most disabling element of epilepsy. Studies of ictogenesis require high-speed recordings at cellular resolution in the area of seizure onset. However, in vivo seizure onset areas cannot be determined at the level of resolution necessary to enable such studies. To circumvent these challenges, we used novel GCaMP7-based calcium imaging in the organotypic hippocampal slice culture model of post-traumatic epilepsy in mice. Organotypic hippocampal slice cultures generate spontaneous, recurrent seizures in a preparation in which it is feasible to image the activity of the entire network (with no unseen inputs existing). Chronic calcium imaging of the entire hippocampal network, with paired electrophysiology, revealed 3 patterns of seizure onset: low amplitude fast activity, sentinel spike, and spike burst + low amplitude fast activity onset. These patterns recapitulate common features of human seizure onset, including low voltage fast activity and spike discharges. Week-long imaging of seizure activity showed a characteristic evolution in onset type and a refinement of the seizure onset zone. Longitudinal tracking of individual neurons revealed that seizure onset is stochastic at the single neuron level, suggesting that seizure initiation activates neurons in non-stereotyped sequences seizure to seizure. This study demonstrates for the first time that transitions to seizure are not initiated by a small number of neuronal “bad actors” (such as overly connected hub cells), but rather by network changes which enable the onset of pathology among a large populations of neurons.

Commentary

Understanding ictogenesis, the process of initiating a seizure, is one of the holy grails of epilepsy research. Seizure onset is unpredictable, occurring only at discrete times within the complex physical environment of the brain, making it extremely challenging to establish a cellular-level understanding of it. But preventing seizures at their earliest onset is the goal of multiple anti-seizure therapies including pharmacology, electrical stimulation, and even experimental optogenetic approaches, so establishing the cellular networks that initiate seizures is critically important. Computational, imaging, EEG, and electrophysiological approaches have all been used to attempt to establish the sequence of cellular events and brain regions that drive seizure initiation. The field has been successful in many ways in identifying seizure onset zones (SOZs), as evidenced by presurgical mapping and surgical resection of SOZs in patients with pharmaco-resistant epilepsy. Progress has been slower, however, in understanding ictogenesis with cellular resolution. In vivo single-unit recordings have implicated both excitatory and inhibitory neurotransmission in seizure onset and computational approaches suggest that there may be specific neurons that are hyper-connected, so called “hub neurons” that exert disproportionate influence over ictogenesis. Are there specific “bad actors” in seizure onset? Does a prescribed group of neurons repeatedly and predictably initiate seizures? A new study by Lau and colleagues uses chronic calcium imaging of organotypic brain cultures to demonstrate that in this preparation, ictogenesis is stochastic with different neurons randomly activated during the initiation of each seizure.

Lau et al use an established in vitro model of epileptogenesis to probe ictogenesis at the single-cell level. In the in vitro organotypical hippocampal slice model, hippocampal slices are prepared from neonatal mice and begin to spontaneously generate interictal spiking followed by seizure-like events (SLEs) after approximately 1 week in culture. The Staley lab and others have demonstrated that organotypic cultures serve as a model of epileptogenesis, maintain a significant portion of in vivo anatomical structures, and are responsive to standard-of-care anti-seizure drugs. Importantly,
this in vitro model allows imaging of neuronal activity in individual neurons with great temporal and spatial specificity while simultaneously recording electrical activity in the network for weeks at a time. In order to visualize ictal activity and identify seizure onset at the single-cell level, the authors used a combination of genetic and viral tools. They expressed the genetically encoded calcium sensor GCaMP7f in about \( \approx 20\% \) of all hippocampal neurons and the red fluorescent protein tdTomato in \( \approx 90\% \) of GABAergic interneurons. Approximately 35% of GCaMP7f-labeled neurons were GABAergic INs, allowing the authors to measure the activity of both inhibitory and excitatory neurons. These cultures were kept for weeks while collecting “movies” of calcium activity for 85 s once every 6 hr (35 Hz imaging speed). During these calcium “movies,” the authors regularly captured spontaneous seizures, allowing them to quantify ictogenesis at the single-cell level in a series of seizures from the same in vitro network over weeks of recording. This data set gave the authors unprecedented insight into how seizures initiate.

First, the authors found that SLEs in vitro can be characterized by 3 ictal onset patterns: low amplitude fast activity (LAF), sentinel spike (SS), and spike burst + low amplitude fast activity (SB+LAF). These patterns strikingly resemble seizure onset types in human EEG recordings of epilepsy. The interval between ictal events shortened over prolonged time in culture, and the SLE patterns evolved from LAF early in culture to SS and SB+LAF after 10 days in vitro. Next, SOZs became more resolvable the longer slices were kept in vitro, with SOZs largely identified in the dentate gyrus and hilus. At the single-cell level, there was no stereotyped sequence of neurons that always initiated an SLE; rather, ictogenesis seemed to occur stochastically, with random sets of neurons driving seizure onset in each individual seizure.

The authors used a series of imaging and analytical approaches to understand this variability in ictogenesis. First, they found that on average, GABAergic interneurons activate approximately 80 ms before principal cells in a seizure. This is consistent with data recorded from the human epileptic brain which suggests that GABAergic interneurons are robustly activated during seizure initiation. Second, the authors evaluated the order in which neurons were activated in an individual SLE. Although the SOZ was commonly found in the same region (dentate gyrus and hilus), the sequence of specific neurons that were activated during seizure initiation was highly variable. This variability in seizure onset included both excitatory and inhibitory neurons as well as spatial heterogeneity from seizure to seizure within the SOZ. Next, the authors used a cumulative distribution function (please see the paper for more information) that quantified the variability in the sequence of neurons activated at seizure onset. They found that variability in their data set was similar to what would be seen in a simulated seizure generated by completely random cell firing sequences. To drive home that this quantitative approach could detect “bad actor” neurons, should they exist, the authors tested their analytical methods on hypothetical seizure data in which 50 cells were artificially made into early activators (mimicking a situation in which there are hub cells that drive ictogenesis). Upon comparison of the experimental and “early activator” data sets, the authors found that the cumulative distribution function could detect “bad actors,” but that in experimental SLE data these “bad actors” did not exist. Finally, they looked at individual neuron activity 5 s before ictal onset and found most neurons are “quiet,” indicating there does not appear to be cells that are consistently active before ictal onset. Overall, this study strongly shows that in this in vitro preparation, SLEs occur randomly with no stereotyped pattern of cellular seizure initiation.

This is an impressive study from a technical, analytical, and biological standpoint. Achieving stable imaging of a network in culture over weeks to months is a significant challenge. Deciphering the sequence of neuronal activation across multiple seizures recorded weeks apart requires careful and precise isolation of neuronal firing. Showing that ictogenesis can occur in a random manner makes us reconsider a model in which a few cells trigger seizures and forces us to dedicate serious thought to statistical network theory that may be required to understand how circuits generate stochastic seizures. There are a few technical and biological caveats to this study. First, and most importantly, this work was performed in a small, cultured brain network. Organotypic cultures represent only a small section of a network (they are basically a single cell layer thick) and therefore any “bad actors” that drive ictogenesis by initiating activity in 3-dimensional space in vivo may be lost or may have truncated processes. In addition, this culture system lacks some changes known to occur in complex brain tissue including sclerosis and spatially constricted potassium and neurotransmitter diffusion in the extracellular space. Therefore, the possibility that hub cells and more stereotyped ictogenesis occurs in vivo cannot be fully excluded. Second, the sampling approach used to monitor neuronal activity may be missing hub cells for a few reasons. Only about 20% of neurons are labeled with GCaMP7f, meaning unlabeled “bad actors” may exist. In addition, because approximately 28 ms of activity is integrated in each frame of the “movie,” there may be neuronal ensembles that cannot be sub-dissected because they activate rapidly within the collection time of a single frame. It is unlikely that either of these technical caveats could account for missing hub cells if they were regularly driving ictogenesis, but they should be considered. The last caveat to consider is that there may be natural evolution of ictogenesis that occurs in this culture system and variability reported in the study may be partially driven by the ever-evolving nature of seizure networks. These caveats aside, this is an elegant study which acknowledges it limitations and shows very convincingly that in this in vitro network, SLEs occur stochastically without “bad actor” neurons initiating each seizure consistently.

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References

1. Jacob T, Lillis KP, Wang Z, Swiercz W, Rahmati N, Staley KJ. A proposed mechanism for spontaneous transitions between interictal and ictal activity. *The Journal of Neuroscience*. 2019;39(3):557-575.

2. Morgan RJ, Soltesz I. Nonrandom connectivity of the epileptic dentate gyrus predicts a major role for neuronal hubs in seizures. *Proceedings of the National Academy of Sciences*. 2008;105(16):6179-6184.

3. Hadjiabadi D, Lovett-Barron M, Raikov IG, et al. Maximally selective single-cell target for circuit control in epilepsy models. *Neuron*. 2021;109(16):2556-2572.

4. Truccolo W, Donoghue JA, Hochberg LR, et al. Single-neuron dynamics in human focal epilepsy. *Nature Neuroscience*. 2011;14(5):635-641.

5. Jette N, Reid AY, Wiebe S. Surgical management of epilepsy. *Canadian Medical Association Journal*. 2014;186(13):997-1004.

6. Toyoda I, Fujita S, Thamattoor AK, Buckmaster PS. Unit activity of hippocampal interneurons before spontaneous seizures in an animal model of temporal lobe epilepsy. *Journal of Neuroscience*. 2015;35(16):6600-6618.

7. Elahian B, Lado NE, Mankin E, et al. Low-voltage fast seizures in humans begin with increased interneuron firing. *Annals of Neurology*. 2018;84(4):588-600.

8. McBain CJ, Boden P, Hill RG. Rat hippocampal slices ’in vitro’ display spontaneous epileptiform activity following long-term organotypic culture. *Journal of Neuroscience Methods*. 1989;27(1):35-49.

9. Liu J, Saponjian Y, Mahoney MM, Staley KJ, Berdichevsky Y. Epileptogenesis in organotypic hippocampal cultures has limited dependence on culture medium composition. *PLoS One*. 2017;12(2):e0172677.

10. Li Q, Han X, Wang J. Organotypic hippocampal slices as models for stroke and traumatic brain injury. *Molecular Neurobiology*. 2016;53(6):4226-4237.