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Focus on the Focus

Acute Focal Seizures Start as Local Synchronizations of Neuronal Ensembles

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Understanding seizure formation and spread remains a critical goal of epilepsy research. We used fast in vivo 2-photon calcium imaging in male mouse neocortex to reconstruct, with single-cell resolution, the dynamics of acute (4-aminopyridine) focal cortical seizures as they originate within a spatially confined seizure initiation site (intrafocal region) and subsequently propagate into neighboring cortical areas (extrafocal region). We find that seizures originate as local neuronal ensembles within the initiation site. This abnormal hyperactivity engages increasingly larger areas in a saltatory fashion until it breaks into neighboring cortex, where it proceeds smoothly and is then detected electrophysiologically (local field potential). Interestingly, PV inhibitory interneurons have spatially heterogeneous activity in intra- and extrafocal territories, ruling out a simple role of inhibition in seizure formation and spread. We propose a 2-step model for the progression of focal seizures, where neuronal ensembles activate first, generating a microseizure, followed by widespread neural activation in a travelling wave through neighboring cortex during macroseizures.

Significance Statement

We have used calcium imaging in mouse sensory cortex in vivo to reconstruct the onset of focal seizures, elicited by local injection of the chemoconvulsant 4-aminopyridine. We demonstrate at cellular resolution that acute focal seizures originate as increasingly synchronized local neuronal ensembles. Owing to its spatial confinement, this process may at first be undetectable even by nearby local field potential electrodes. Further, we establish spatial footprints of local neural subtype activity which correspond to consecutive steps of seizure microprogression. Such footprints could facilitate determining the recording location (eg, inside/outside an epileptogenic focus) in high-resolution studies, even in the absence of a priori knowledge about where exactly a seizure started.

Commentary

As we strive to understand neural dynamics during seizures, a looming problem in the field is that heterogeneous data are often lumped together. An often-overlooked source of variability is that for any seizure, there is a site where a seizure initiates and other areas to which it propagates.[1] Even focal seizures appear to have compartmentalized zones when observed at the microcircuit level.[2] Are neural dynamics the same between initiation processes and propagation processes? This is a difficult question to answer empirically because of the challenges in defining the seizure initiation site. The problem becomes multifold more difficult at the microcircuit level as finer resolutions requires an even more precise definition of the initiation site.

In this study, Wenzel et al tackle this difficult question of whether dynamics differ between seizure initiation and propagation. As with previous studies,[3] they employ in vivo 2-photon imaging, here monitoring calcium dynamics of cortical pyramidal cells and parvalbumin-positive interneurons (PVs) at the seizure initiation site and at neighboring propagation areas following focal application of 4-aminopyridine (4-AP). Consistent with their previous study, they found that in the propagation area neural activity could be described as one continuous wave-like recruitment of neurons. In this study, however, in contrast, they observe at the initiation site bursts of recruitment of smaller discrete populations before activation engulfs the entire population. They describe this as synchronous activations of smaller ensembles (relative to the size of the seizure ensemble) but provide no analysis to rigorously define synchrony. It would have been interesting to know whether the size of these ensembles increased as a function of time, which would be reminiscent of the "buildup" seen in hippocampal region CA3 population bursts.[4] It is worth noting that small populations of synchronous activity in the seizure onset zone have also been recorded in human patients, so-called "microseizures,"[5]—however, the scale in Wenzel's

experiments is considerably smaller and, as discussed below, may not be observable with microelectrodes.

At the initiation site, Wenzel et al observe discord between the local field potential (LFP) and population calcium activity—whereas within propagation areas, there seems to be better temporal alignment between the 2 signals. The LFP measurements "miss" early phases of initiation dynamics. It is important to consider how 4-AP may be impacting these results. For these experiments, the 4-AP model is a double-edged sword. The strength is in pinpointing the site of seizure initiation, that is, the site of 4-AP application. The weakness is that it is unclear how 4-AP directly influences dynamics at that initiation site. Arguably, the action of 4-AP (blocking K+ channels) is an artificial situation, making it unclear how strongly it alters the dynamics of seizure initiation. However, there are several epilepsies associated with loss-of-function mutations in K+ channels,[6] so 4-AP blockade may roughly mimic conditions in those epilepsies. Turning back to the observation that early neuron recruitment is not detected by LFP recording; it is possible that local ensembles are not being driven synaptically (ie, the 4-AP is directly causing depolarization). Without strong synaptic synchrony, neural activation may not drive measurable field voltage changes unless neuron firing was synchronized with millisecond precision allowing action potential summation. If there is a synaptic mechanism at play (if direct depolarization by 4-AP is not sufficient to cause spiking but has reduced the synchrony requirements for synaptic excitation to push neurons suprathreshold), then it is possible that activated ensembles comprise too few synapses (thus small voltages) or else are in spatial geometries that produce interfering voltages. More experiments need to be done, preferably in a more realistic model for spontaneous seizure initiation to test whether initiation dynamics would be hidden from classical LFP measurements. It is certainly interesting to consider that for nonsynaptic mechanisms, for example, misregulated K+ buffering,[7] LFP electrodes may not detect activity of small ensembles.

To further understand the dynamic landscape leading up to the moment pathological activity "breakthrough," Wenzel et al perform principal component analysis. At the initiation site, leading up to the seizure, there are large excursions in parameter space. These excursions overlap to some extent, suggesting stereotyped patterns of neural activity leading up to the seizure. It is difficult to imagine how 4-AP would promote such repeatable stereotypy, suggesting that this is a feature of how the microcircuit processes activity and thus seems likely to apply to other epilepsy types/models. During the seizure event itself, the dynamics wander away in parameter space from the preseizure excursions, although this could simply reflect a larger number of active neurons in the seizure than the build-up activity. The propagation areas, in contrast, show a fundamental difference in that preseizure excursions are severely stunted compared to seizure trajectory. The authors speculate that this is because neural activity is suppressed in the propagation area prior to seizure invasion—indeed population calcium activity in the propagation area prior to seizure breakthrough is a fraction of baseline activity in the same area. In summary, principal component analysis supports the conclusion that in the focus, neural activity builds up in stereotyped patterns, whereas in propagation areas, neural activity is suppressed prior to seizure invasion. The most likely candidate for mediating these differences are inhibitory interneurons.

In the propagation area in the time prior to the seizure, they found that inhibitory PV interneurons were more strongly recruited than non-PVs. In contrast, in the seizure initiation site, PVs were recruited to the same extent as non-PVs. It is tempting to think that in the propagation area, the local suppression of pyramidal cells is mediated by the local strongly activated PVs—however, it is likely that the suppression is equally (if not more) dependent on activation of PVs in the neighboring (already seizure invaded) cortex, which then mediate feedforward/surround inhibition onto the local pyramidal cells. This would explain why the PV activation within the initiation site is insufficient to suppress local activity—in the case of seizure initiation, neighboring cortex is not experiencing hyperactivation and therefore is not engaging "hyper" feedforward/surround inhibition. If true, the prediction would be that seizure initiation would be most effectively blocked by activating PVs around the focus (as the inhibitory interneurons within the focus are already engaged—and possible undergoing depolarization block[8]). These results suggest that intervention strategies, for example, optogenetic activation of PVs,[9] should differ depending on whether the target is the focus or propagation area.

Importantly, this study reveals mechanistic differences between seizure initiation and propagation. Given the ambiguity surrounding 4-AP, we should be cautious about considering these initiation dynamics as fundamental to all seizures—but this work clearly motivates and sets the stage for future studies focused on the focus.

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