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Suppress Globally or Seize Locally: Cortical Network Activity Explains Seizure Diversity Among *Kcnt1* Mutants

Distinct Features of Interictal Activity Predict Seizure Localization and Burden in a Mouse Model of Childhood Epilepsy

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The epileptic brain is distinguished by spontaneous seizures and interictal epileptiform discharges (IEDs). Basic patterns of mesoscale brain activity outside of seizures and IEDs are also frequently disrupted in the epileptic brain and likely influence disease symptoms, but are poorly understood. We aimed to quantify how interictal brain activity differs from that in healthy individuals, and identify what features of interictal activity influence seizure occurrence in a genetic mouse model of childhood epilepsy. Neural activity across the majority of the dorsal cortex was monitored with widefield Ca^{2+} imaging in mice of both sexes expressing a human *Kcnt1* variant (*Kcnt1*$^{m/m}$) and wild-type controls (WT). Ca^{2+} signals during seizures and interictal periods were classified according to their spatiotemporal features. We identified 52 spontaneous seizures, which emerged and propagated within a consistent set of susceptible cortical areas, and were predicted by a concentration of total cortical activity within the emergence zone. Outside of seizures and IEDs, similar events were detected in *Kcnt1*$^{m/m}$ and WT mice, suggesting that the spatial structure of interictal activity is similar. However, the rate of events whose spatial profile overlapped with where seizures and IEDs emerged was increased, and the characteristic global intensity of cortical activity in individual *Kcnt1*$^{m/m}$ mice predicted their epileptic activity burden. This suggests that cortical areas with excessive interictal activity are vulnerable to seizures, but epilepsy is not an inevitable outcome. Global scaling of the intensity of cortical activity below levels found in the healthy brain may provide a natural mechanism of seizure protection.

Commentary

Epilepsies attributed to single defective genes often present with an astounding variety of symptoms and severities. In the case of the Y796H variant of *KCNT1*, a gene that encodes a subunit of the Na^{+}-activated K^{+} channel, the range of symptoms is bewildering: some individuals are nonverbal and have severe sleep-related hypermotor epilepsy whereas others appear completely unimpaired.[1] Some of this phenotypic diversity could be attributable to aberrant activity within various discrete networks rather than global, brain-wide dysfunction. But where should we begin our search for these potential sensitive networks? Taking an unbiased "bird's-eye view" to observe activity broadly across the cortex in epileptic mice is a good place to start. Identifying uniquely sensitive brain regions and networks will guide the development of targeted therapies and more granular research. Tobin and Weston did just this by recording cortical activity across the entire dorsal cortex in mice harboring the Y796H mutation (i.e., *Kcnt1*$^{m/m}$).[2] By doing so, they characterize a few key features of seizure initiation and spread. They also identify prominent distinctions between epileptic and nonepileptic Y796H mutant mice, which provide new perspectives on why seizure phenotype differs among individuals with the same mutation.

The "bird's-eye view" in this experiment was achieved using mesoscopic Ca^{2+}-imaging. To this end, *Kcnt1*$^{m/m}$ mice were crossed with mice expressing GCaMP in neurons (*Snap25*-GCaMP6s). Mice were then imaged with a tandem-lens macroscope while head-fixed on a treadwheel. This approach is well-suited for characterizing circuit-level activity patterns because it captures a large field of view with much greater sensitivity than a conventional microscope objective.[3] While a more traditional approach using a gridded electrode array to record electroencephalogram (EEG) or electrocorticogram (ECoG) clearly provides superior temporal resolution, mesoscopic Ca^{2+} imaging provides higher spatial resolution across a wide field. The 2 methods also measure fundamentally different events: GCaMP measures intracellular Ca^{2+} activity, which at the mesoscopic scale, primarily reflects action potentials of neurons integrated across space and time. EEG/ECoG, on the other hand, captures field potentials which are primarily composed of summated synaptic potentials.[4] Thus, concurrent Ca^{2+} and EEG/EcoG measurements would likely provide

additional confidence that the study's conclusions do not depend on recording method. That shortfall notwithstanding, mesoscopic Ca^{2+} imaging is a novel and useful approach for studying circuit-wide activity, and this marks its first application to a genetic model of epilepsy.

In all experiments, fluorescence intensities across the entire dorsal surface of the cortex were aligned to the Allen Mouse Common Coordinate Framework and segmented into discrete areas (eg, primary visual cortex, secondary motor cortex, etc). Three distinct categories of cortical activity were evaluated seizures, interictal epileptic discharges (IEDs), and interictal "events," which were defined as significant deviations of cortical activity from baseline that did not reach the threshold defined for seizures or IEDs. Regarding seizures, the authors identified 2 cortical areas uniquely vulnerable in *Kcnt1*$^{m/m}$ mice. One area comprised the "posterior emergence zone," a patch of tissue at the convergence of retrosplenial and higher order visual areas. The second seizure-prone area corresponded to the medial secondary motor cortex. These areas that activate earliest during seizures also show the largest increase in activity and maintain this activity for the longest duration over the course of a seizure. This indicates that the cortex of *Kcnt1*$^{m/m}$ mice is not uniformly susceptible to seizures. Instead, a distinct subnetwork of cortical areas seems especially sensitive to producing seizures.

By also evaluating the spatial propagation of seizures, the authors demonstrate that ictal activities adhere to cortical connectivity rules observed in healthy, nonepileptic brains. That is, highly connected areas, as defined by the Allen Connectivity Atlas, showed the highest degree of coactivity during seizures. Instances of "long-range" seizure propagation were well explained by synaptic connectivity strengths derived from wild type, nonepileptic mice. Thus, although some cortical areas are uniquely seizure prone, once initiated, seizures propagate along typical anatomical projections rather than along newly created or modified connections. This finding is consistent with observations made in mice with motor seizures.[5] Indeed, this conclusion may ultimately be true of most genetically acquired epilepsies; that is, profound circuit reorganizations are not to be found.

Surprisingly, some of the study's most novel insights come from the analysis of cortical activity that was deemed neither seizure nor IED (ie, "interictal events"). First, seizure susceptible cortices repeatedly activated for several seconds before activity crossed the threshold for seizure detection. This kind of persistent, repetitive activity occurred only in *Kcnt1*$^{m/m}$ mice, not controls. Also, cortical areas that had more frequent subthreshold events were most likely to be emergence zones. And finally, the intensity of these events covaried with epilepsy severity. This final observation regarding the intensity of the interictal events is particularly insightful. The authors created an index of severity called the "epileptic activity burden" that incorporates both the total seizure duration and IED rate. Mice with higher intensity interictal events showed a greater burden. Conversely, *Kcnt1*$^{m/m}$ mice with low event intensities had low burden scores. Moreover, 2 *Kcnt1*$^{m/m}$ mice that had no seizures had even lower event intensities than control mice. Thus, subthreshold events that are neither seizures nor IEDs nonetheless predict disease severity. This finding also suggests that nonepileptic brains with YH variants—despite having the same mutation—possess seizure-suppressive mechanisms that can dampen subthreshold activities below those observed in healthy mice.

The authors attribute the variability among subthreshold event intensities to a global scaling of activity across the cortex in *Kcnt1*$^{m/m}$ mice; mice with greater burdens had increased activity globally across the whole cortex and during interictal periods. Mice with low burdens had downscaled activity below that of control mice. Based on this observation, it seems that the cortex can only compensate for local pockets of hyperactivity by reducing cortical activity globally. This comes at the cost of pushing healthy areas below normal levels. It's an effective, albeit imprecise solution because it seems to prevent seizures but also causes hypoactivity, an outcome that might explain the associated negative symptoms of nonverbosity and nonambulation seen in some YH human patients. Despite collecting movement data from their mice, the authors missed an opportunity to evaluate the potential relationship between neural activity and ambulation. Such an analysis seems warranted given the symptoms in some human patients and the cortical hypoactivity observed in some *Kcnt1*$^{m/m}$ mice.

Overall, Tobin and Weston provide a significant contribution to our understanding of the circuit-level activity dysfunction of epileptic brains. Not only do they provide the first instance of mesoscopic imaging in a model of genetic epilepsy, they also bolster earlier reports suggesting seizures do not necessarily involve significantly altered circuitry. Moreover, the authors' holistic, quantitative approach provides new perspectives on the neural correlates of the phenotypic diversity associated with single gene mutations. And although more work is needed to elucidate the links between gene and circuit dysfunction at a more granular level, this study gives us vital information on where to start.

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Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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