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The Heated Relationship Between Neural Activity and Seizures

Interneuron Desynchronization Precedes Seizures in a Mouse Model of Dravet Syndrome

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Recurrent seizures, which define epilepsy, are transient abnormalities in the electrical activity of the brain. The mechanistic basis of seizure initiation, and the contribution of defined neuronal subtypes to seizure pathophysiology, remains poorly understood. We performed in vivo 2-photon calcium imaging in neocortex during temperature-induced seizures in male and female Dravet syndrome (*Scn1a*$^{+/-}$) mice, a neurodevelopmental disorder with prominent temperature-sensitive epilepsy. Mean activity of both putative principal cells and parvalbumin-positive interneurons (PV-INs) was higher in *Scn1a*$^{+/-}$ relative to wild-type controls during quiet wakefulness at baseline and at elevated core body temperature. However, wild-type PV-INs showed a progressive synchronization in response to temperature elevation that was absent in PV-INs from *Scn1a*$^{+/-}$ mice. Hence, PV-IN activity remains intact interictally in *Scn1a*$^{+/-}$ mice, yet exhibits decreased synchrony immediately before seizure onset. We suggest that impaired PV-IN synchronization may contribute to the transition to the ictal state during temperature-induced seizures in Dravet syndrome.

Commentary

An enduring quest in epilepsy research is to explain how altered firing patterns of neurons lead to the occurrence of spontaneous seizures. This is both an intellectually satisfying and practical pursuit, as a clearer picture of neural activity prior to and during seizures will help define both treatment targets and how brain activity needs to be modified to reduce the occurrence of seizures and mitigate related disease symptoms. Obtaining this information, however, is no small task. The heterogeneity of seizures, the sheer number of neurons that participate in seizures, their spatial distribution, physiological heterogeneity, and uncertain relationship to seizure topology are enough to make even the most daring of scientists sweat and drive them to existential self-doubt in the form of questions such as, "What *is* a seizure, anyway?"

Still, the brave and/or foolish among us have persisted over the years, and, in line with the unconstrained and complex nature of the problem, have arrived at various conclusions. Highlights include findings that excitatory neurons (Ex) generally increase their activity levels minutes before seizures begin,[1] although this activity before and even during seizures is not uniformly high and synchronous,[1,2] and that the activity of inhibitory (In) neurons plays a large role in shaping the dynamics of ictal events.[3] However, each of these conclusions is likely highly dependent on the type of seizure being studied and the location of the observed neurons relative to seizure topology, among other variables. This reinforces the problem's complexity and makes the use of clever constraints or new technologies all the more necessary to arrive at rigorous conclusions.

Recent technological advances in large-scale imaging of neural activity have brought new hope. Multicellular calcium imaging (MCI), primarily via 2-photon (2P) microscopes, has been applied to study the activity of neurons and its relationship to seizures.[4] In contrast to single-unit electrophysiological recordings, which still retain certain, especially temporal, advantages, this approach can often monitor the activity of a larger number of neurons at once, and more precisely identify the types of neurons being monitored, both of which increase the strength of the conclusions. To date, however, these studies, at least those that have been performed in intact animals and not brain slices, have mostly imaged ictal activity generated by application of chemoconvulsants such as 4-aminopyridine (4-AP, but see the study by Meyer et al[5]).

A recent paper by Tran et al used 2P MCI to monitor neural activity in Ex neurons and parvalbumin-positive (PV^{+}) In neurons leading up to seizures in *Scn1a*$^{+/-}$ mice, which model the human disease Dravet Syndrome,[6] involving loss of function of the voltage-gated sodium channel Na(v) 1.1. This design is innovative because it places further constraints on the issue by (1) using a mouse genetic model of epilepsy in which the cellular effects of the causative gene variant are relatively well understood, (2) imaging a genetically defined cell population

that is known to be vulnerable to *Scn1a* loss, and (3) taking advantage of the fact that these mice have temperature-dependent seizures. Imaging neural activity while increasing the body temperature of the mice allowed them to compare pre-seizure activity in $Scn1a^{+/-}$ mice to temperature-matched wild-type (WT) mice, and to image naturalistic seizure events.

So, what does this buy them? First, one may expect that the PV^+ In neurons in the $Scn1a^{+/-}$ mice would be less active than those in WT mice, both at baseline and preceding seizures, while Ex neurons would be more active. This expectation relies on several previous studies that have found reduced membrane excitability and AP firing in PV^+ neurons caused by $Scn1a^{+/-}$ loss, which may impair their ability to inhibit Ex neuron activity.[7] The authors did not observe this. Instead, they found that both putative Ex neuron and PV^+ neuron populations were more active, as measured by fluorescence burst amplitude and rate, in $Scn1a^{+/-}$ mouse brains, both at baseline and preceding naturalistic seizures. Why did this occur? First, a previous study in vivo study did not detect decreases in PV^+ neuron firing in $Scn1a^{+/-}$ mice,[8] although others have after *Scn1a* knockdown in vivo,[9] suggesting that the effect of reduced cellular excitability of $Scn1a^{+/-}$ PV^+ neurons may not easily translate to reduced firing in vivo. This may be due to increased overall activity levels or synaptic rearrangements that result in increased excitatory drive to PV^+ neurons or limitations in the MCI's ability to report activity in PV^+ neurons, although the latter does not explain why their activity would be increased. Alternatively, these mice may have been imaged at a time point at which the strongest alterations in PV^+ neuron excitability have resolved, but overall brain activity is still increased. PV^+ neurons notwithstanding, this study agrees with previous findings of widespread increases in neural activity before seizures. Although notably, one of the very few previous studies that imaged in vivo spontaneous seizures in a genetic model found widespread decreases in neural activity during ictal activity, albeit absence seizures.[5] Altogether, this highlights some of the challenges in relating ex vivo and in vivo cellular activity in the same model, as well as in vivo activity between models.

A second major finding of the article was the changes in the intra- and interneuron synchronicity between PV^+ and Ex neurons, as measured by cross-correlation analysis of the deconvolved fluorescence traces, both between genotypes and between baseline and preceding seizures. Synchrony among PV^+ neurons and between PV^+ and Ex neurons was increased in $Scn1a^{+/-}$ mice compared to WT mice at baseline. However, whereas these measurements increased in WT mice as the their temperature increased, they did not increase in $Scn1a^{+/-}$ mice in the lead up to seizure, suggesting that the failure to synchronize between excitatory and inhibitory populations in $Scn1a^{+/-}$ mice relative to WT may relate to transition to seizure.

The study adds further doubt to the hypothesis that reduced AP firing of PV^+ In neurons directly leads to seizure generation in $Scn1a^{+/-}$ mice, at least in older animals. Instead, it suggests that developmental, synaptic, or population-level alterations such as synchrony may be more proximal to seizure activity. It also highlights many of the strengths and weaknesses of in vivo imaging of the relationship between neural activity and seizures. One difficulty is the relative rarity of seizures and the difficulty of collecting the relevant data. This study was able to work around the rarity issue by exploiting the temperature-sensitive seizures in $Scn1a^{+/-}$ mice, but still unable to image repeated seizures in the same mouse due to the extreme stress of the protocol for $Scn1a^{+/-}$ mice, which was lethal in $>30\%$ of seizures. A second common difficulty is knowing the relationship of the imaged neural activity to the spatial and temporal dynamics of the seizure. This study did perform electroencephalogram recordings proximal to the imaging, which gives confidence that the imaged tissue is participating in the seizure, but whether this neural activity is representative of the type of pathological neural activity that generates seizures or reflective of passive spread is unknown. Despite this, we do get a truly different angle on the problem here, due to the application of a relatively new technology and the strengths in experimental design mentioned above. Continued advances such as this should help us overcome our existential doubts and get to the essence of seizure generation.

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