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Determining the Spread: Potential Biomarkers and Treatment for Seizure-Induced-Spreading Depolarization in a Mouse Model of Genetic Epilepsy

A Hyperthermic Seizure Unleashes a Surge of Spreading Depolarizations in *Scn1a*-Deficient Mice

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Spreading depolarization (SD) is a massive wave of cellular depolarization that slowly migrates across the brain gray matter. Cortical SD is frequently generated following brain injury, while less is understood about its potential contribution to genetic disorders of hyperexcitability, such as *SCN1A*-deficient epilepsy, in which febrile seizure often contributes to disease initiation. Here we report that spontaneous SD waves are predominant EEG abnormalities in the *Scn1a*-deficient mouse ($Scn1a^{+/R1407X}$) and undergo sustained intensification following a single hyperthermic seizure. Chronic DC-band EEG recording detected spontaneous SDs, seizures, and seizure-SD complexes in $Scn1a^{+/R1407X}$ mice but not WT littermates. The SD events were infrequent, while a single hyperthermia-induced seizure robustly increased SD frequency over 4-fold during the initial postictal week. This prolonged neurological aftermath could be suppressed by memantine administration. Video, electromyogram, and EEG spectral analysis revealed distinct neurobehavioral patterns; individual seizures were associated with increased motor activities, while SDs were generally associated with immobility. We also identified a stereotypic SD prodrome, detectable over a minute before the onset of the DC potential shift, characterized by increased motor activity and bilateral EEG frequency changes. Our study suggests that cortical SD is a pathological manifestation in *SCN1A*-deficient epileptic encephalopathy.

Commentary

Dravet syndrome (DS) is a developmental epileptic encephalopathy associated with a high rate of sudden unexpected death in epilepsy (SUDEP).[1] While mechanisms for SUDEP are only beginning to be understood, there is evidence that, in many cases, seizures negatively influence neural circuits and networks to lead to death by dysregulating breathing, cardiac and autonomic function, or arousal mechanisms.[2] One way that seizures may impact these distant network nodes is via spreading depolarization (SD); a wave of cellular depolarization that moves outward from the injury or seizure focus.[3] Direct current (DC) recordings are required to detect SD. Direct current recordings are not routinely made in patients thus SD is not commonly reported following seizures in patients. Models of genetic epilepsies, which differentially affect ion channels and other regulators of excitation-inhibition balance, afford the opportunity to probe the molecular, circuit, and network underpinnings of SD. The $Scn1a^{+/R1407X}$ ($Scn1a^{+/RX}$) mutant mouse is a well-studied model harboring a knock-in mutation in the SCN1A gene found in patients with DS. This mouse recapitulates many features of DS in patients, including manifestation early in life, spontaneous seizures, susceptibility to heat-induced seizures, and high mortality rate.[4] This mouse also demonstrates seizure-induced respiratory arrest that precedes cardiac dysregulation, a fairly consistent phenotype emerging in patients[5] and many animal models.[6]

Aiba and colleagues[7] examined the spontaneous incidence and related semiology of SD in adult $Scn1a^{+/RX}$ mice. $Scn1a^{+/RX}$ and WT littermates were monitored using DC-band chronic EEG and infrared video. After 7 days of baseline recording, each animal had a single hyperthermic seizure-induced via a heat lamp and were then monitored for an additional 10 days.

The authors characterized 3 types of abnormal spontaneous events in the $Scn1a^{+/RX}$ animals that occurred at baseline—isolated seizures, isolated SD, and seizure+SD complexes where SD emerged within several minutes following a seizure. Notably, there were mice that exhibited only seizures with no accompanying SD or seizure+SD complexes. Following the hyperthermic seizure, the majority of $Scn1a^{+/RX}$ mice experienced an increase in SD frequency which lasted up to a week. The authors also noted a weak diurnal trend in the frequency of SD, with its incidence peaking during the light–dark cycle transitions. The presence of SD following a seizure was not

related to the incidence or duration of postictal generalized EEG suppression. However, both SD and seizures were significantly shorter when they occurred as part of a seizure+SD complex as opposed to individually. It is intriguing that a single hyperthermic seizure was sufficient to set off spontaneously occurring SDs that continued to recur for up to a week. This suggests there may be a prolonged period of altered excitability after a hyperthermic seizure in susceptible individuals. They noted an increase in high-frequency EEG activity that preceded SD by about a minute and identified complex EEG spikes at the onset of SD which may be reliable biomarkers of SD.

Next, the authors sought to reduce the incidence of spontaneous events following hyperthermic seizure induction using the N-methyl-D-aspartate receptor antagonist memantine (10 mg/kg), which has been found to reduce SD generation in rats.[8] They utilized a pretreatment paradigm, a posttreatment paradigm, and a combination of both the pre- and posttreatment paradigm. Posttreatment eliminated the increase in spontaneous events compared to baseline. Pre + posttreatment with memantine was the most successful, with significantly lowered incidence of seizures and SD-related events compared to baseline.

To identify physiological changes that may accompany SD, the authors analyzed EMG and locomotor activity. Prior to SD, they observed minutes-long increases in motor activity, followed by a protracted period of suppression. Following seizure+SD complexes, there was a prolonged suppression of EMG tone and locomotor activity, whereas seizures alone were followed by spikes in motor activity.

Lastly, the authors analyzed EEG characteristics preceding cortical detection of SD and seizure+SD complexes. They identified a distinct EEG signature preceding cortical SD—bilateral, simultaneous reduction in low frequency (0-30 Hz) band activity, and an increase in high-frequency (30-120 Hz) activity. A similar pattern was observed in seizure + SD complexes; however, high-frequency activity was not affected on the side ipsilateral to the SD.

The presence or absence of SD or seizure+SD complexes did not affect the rate of seizure-induced death. Nor was there any difference in mortality rate in the different phenotypes seen with the *Scn1a*$^{+/RX}$ mice (ie, seizure only vs experienced SD). In this study, SD was measured only cortically. As it has been documented that seizure spread to the amygdala and brainstem can affect breathing and contribute to seizure mortality,[9-11] measuring SD in these regions may have yielded a connection to seizure-induced death. Indeed, SD has been shown to reach the brainstem in other models.[10] Similarly, an optimal age-group for these experiments may not have been utilized. They may have seen more seizures associated with death had they employed a slightly younger age-group. These mice start dying immediately after weaning and most that will die do so by about P60.[4] Thus, a fair amount of their cohort was outside of this range and may represent a sampling of less susceptible individuals.

Notably, the authors observed a consistent rise in high-frequency EEG activity preceding SD development and EEG spikes that coincided with the onset of SD. These EEG changes may serve as a biomarker to identify impending SD and thus an increased risk for SUDEP. As more is learned about specific SD mechanisms in different epilepsies, measures may be developed that could be deployed in individuals with these EEG signatures to prevent SD and death. More work will be needed to identify other epilepsy-specific mechanisms for SD and additional potential biomarkers. This may represent a good opportunity to employ machine learning methods to identify consistent subtle changes that may portend increased risk.

It is not clear yet if a common final element that is conserved across all incidents of SUDEP will be identified. It may be that specific epilepsies (ie, specific etiologies, channelopathies, etc) will require specific "personalized" preventive measures. There certainly seem to be many ways that seizures may invoke associated morbidity, including death. Distant effects can be due to SD but could also be due to specific circuit activation/inactivation via synaptic mechanisms. Seizure-induced dysfunction of respiratory system components plays a major role in SUDEP pathophysiology, but cardiac, autonomic, and arousal control centers may also be involved. The effects of SD may not be directed to these sites specifically, but rather they may involve a common intermediary such as the amygdala.[9] More will be needed to fully understand the mechanisms for SD, but this paper certainly identifies important possibilities for how SD contributes to seizure-related morbidity.

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Declaration of Conflicting Interests

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