Glutamate’s Secret Interictal Life

Transitions between neocortical seizure and non-seizure-like states and their association with presynaptic glutamate release
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The transition between seizure and non-seizure states in neocortical epileptic networks is governed by distinct underlying dynamical processes. Based on the gamma distribution of seizure and inter-seizure durations, over time, seizures are highly likely to self-terminate, whereas inter-seizure durations have a low chance of transitioning back into a seizure state. Yet, the chance of a state transition could be formed by multiple overlapping, unknown synaptic mechanisms. To identify the relationship between the underlying synaptic mechanisms and the chance of seizure state transitions, we analyzed the skewed histograms of seizure durations in human intracranial EEG and seizure-like events (SLEs) in local field potential activity from mouse neocortical slices, using an objective method for seizure state classification. While seizures and SLE durations were demonstrated to have a unimodal distribution (gamma distribution shape parameter >1), suggesting a high likelihood of terminating, inter-SLE intervals were shown to have an asymptotic exponential distribution (gamma distribution shape parameter <1), suggesting lower probability of cessation. Then to test cellular mechanisms for these distributions, we studied the modulation of synaptic neurotransmission during, and between, the in vitro SLEs. Using simultaneous local field potential and whole-cell voltage clamp recordings, we found a suppression of presynaptic glutamate release at SLE termination, as demonstrated by electrically and optogenetically evoked excitatory postsynaptic currents (EPSCs), and focal hypertonic sucrose application. Adenosine A1 receptor blockade interfered with the suppression of this release, changing the inter-SLE shape parameter from asymptotic exponential to unimodal, altering the chance of state transition occurrence with time. These findings reveal a critical role for presynaptic glutamate release in determining the chance of neocortical seizure state transitions.

Commentary

Understanding the synaptic dynamics of transitions between seizure and non-seizure states is crucial for making sense of the epileptic brain. Although a variety of mechanisms have been suggested to regulate ictal-interictal transitions, including non-synaptic initiation and termination, there is strong evidence that synaptic vesicle pool depletion and refilling play a key role in this process. Studies by Trevelyan and colleagues suggested that recruitment of a neuron into an ictal event could occur when inhibition onto that neuron fails due to depletion of available GABAergic vesicles. Staley and colleagues have likewise suggested that burst events terminate due to depletion of glutamatergic synaptic vesicle pools, and that the state of these pools governs the probability of seizure or interictal spike occurrence. New research from Breton and colleagues provides further evidence for the importance of glutamatergic synaptic vesicle pool depletion in regulating ictal to interictal transitions.

To investigate this, the authors performed electrophysiological recordings in acute cortical slices bathed in a Mg2+-free solution. As a starting point, however, they trained a 2-state Hidden Markov Model with delta (0.5 to 3 Hz) and gamma (30 to 80 Hz) spectral features collected from either patient EEG or local field potential recordings from their slices. This analysis showed that human seizures and neocortical seizure-like events (SLEs) exhibited comparable delta and gamma spectral dynamics and accurately identified seizure and SLE onset and termination. They then fit the histograms of seizure and SLE durations with a gamma distribution, which is a type of probability distribution that describes data with 2 parameters including a shape parameter. The shape parameters of both durations showed a unimodal distribution, indicating an increased chance of the ictal state terminating over time. This similarity also suggests that the onset and termination of the ex vivo SLEs may occur due to similar synaptic dynamics as the onset and termination of human seizures.

With their model validated, the authors performed a series of experiments to identify the synaptic dynamics involved in SLE onset and termination. An initial experiment demonstrated that, although blockade of GABAergic transmission shortened SLE and inter-SLE state duration, it did not change the shape parameter of the duration histogram, suggesting that GABAergic transmission does not play a primary role in the onset or termination of SLEs caused by low Mg2+. In contrast,
blockade of glutamatergic transmission eliminated SLEs, indicating that glutamatergic transmission plays a significant role in SLE onset. Further experiments demonstrated that the amplitude of optogenetically evoked excitatory postsynaptic currents (EPSCs) were reduced at the time of SLE termination and remained depressed for the post-ictal portion of the following inter-SLE state. This reduction in EPSC amplitude was attributed to a decrease in the size of the readily releasable pool (RRP) of glutamate, assayed by hypertonic sucrose application. Additionally, the duration of the following inter-SLE state was correlated with the recovery rate of the glutamate RRP size. This correlation indicates that glutamate RRP size has a role in modulating the transition from an inter-SLE to an SLE state. Put simply, SLEs deplete glutamatergic neurons of synaptic vesicles to release, and another SLE is not likely to begin until a sufficient number of these vesicles are replenished.

The role of glutamate RRP size in ictal state transition was further interrogated by blocking adenosine A1 receptors with an antagonist. Activation of adenosine A1 receptors has been previously shown to suppress the glutamate RRP size, and adenosine activity plays a complex role in epilepsy. Blockade of adenosine A1 receptors restored glutamate RRP size to baseline levels, establishing these receptors as a mechanism underlying the observed decrease in glutamate RRP size. Adenosine A1 receptor blockade also modified the probability of state transition from an inter-SLE to an SLE state. The duration of inter-SLE states was shortened, and the shape parameter of duration histogram was altered. Specifically, the shape parameter increased from <1 to >1, indicating that while ordinarily the probability of state transition decreases as the duration of the inter-SLE state increases, adenosine A1 receptor blockade causes the probability of state transition to increase as inter-SLE duration increases. This change implies a role for glutamatergic signaling and its modulation by adenosine A1 receptors in the state transition from inter-SLE to SLE, and thus from an interictal state to seizure.

This novel mechanism of state transitions in an epileptic network offers an explanation for the success of drugs that block glutamate release in treating seizure subtypes in epilepsy models. Additionally, these results provide evidence that the increase in delta wave frequency that has been previously observed in seizure states is related to glutamate dynamics driving seizure, rather than a passive consequence of it. Although targeting adenosine receptors for therapeutic purposes may be challenging due to their ubiquity throughout the body, many of the proteins that regulate RRP dynamics are neuron type-specific, which may provide an opportunity to more specifically manipulate the synaptic vesicle dynamics and seizure state transitions. Indeed, another recent study found altered synaptic vesicle dynamics that were inhibitory or excitatory synapse-specific in the tetanus toxin epilepsy mouse model. This, combined with the increasing number of genetic variants that cause epilepsy found in synaptic vesicle associated proteins (e.g., *DNM1*, *STXBP1*, and *SNAP25*) makes it clear that the relevance of presynaptic vesicle dynamics to seizure occurrence is a topic worthy of further inquiry.

A lingering question that follows from these results is whether the changes in RRP size and their modulation by adenosine signaling are causal or not. Adenosine has many potential effects on neuronal function outside of RRP size, including effects on Ca++ channels; thus, it would be good know whether modulation of the RRP through other means also modifies the inter-SLE durations. The authors suggest that blockade of adenosine A1 receptors either triggers an SLE onset transition state or that the interictal state is composed of multiple sub-states and adenosine A1 receptor blockade promotes a sub-state that is more likely to lead to an SLE. A natural extension of the latter possibility is that GABAergic neurons might modulate the internal dynamics of the inter-SLE state, and investigation of their role could further elucidate the mechanisms that underlie SLE onset.

Breton and colleagues performed an elegant series of experiments in a well-validated model system to identify a novel mechanism of seizure state transition. Further investigation of this mechanism is needed in order to determine whether it could be a substrate for treatment of epilepsy. Additionally, this study provides a conceptual framework for ensuring that preclinical models of epilepsy are representative of the aspects of human epilepsy that they aim to reproduce. Computational methods that connect manipulable epilepsy models to clinical epilepsy data, as exemplified here, could be part of the key to overcoming the gap between preclinical success and lackluster clinical performance of novel epilepsy drugs.

By Erin R. Cullen and Matthew C. Weston

**ORCID iDs**

Matthew Weston https://orcid.org/0000-0001-5558-7070

Erin Cullen https://orcid.org/0000-0002-0134-8717

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