



# Slower, Fewer Hippocampal Ripples in Loss-of-Function Model of Dravet Syndrome

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## Impairment of Sharp-Wave Ripples in a Murine Model of Dravet Syndrome

Cheah CS, Lundstrom BN, Catterall WA, Oakley JC. *J Neurosci*. 2019;39(46):9251-9260. doi:10.1523/JNEUROSCI.0890-19

Dravet syndrome (DS) is a severe early-onset epilepsy associated with heterozygous loss-of-function mutations in *SCN1A*. Animal models of DS with global *Scn1a* haploinsufficiency recapitulate the DS phenotype, including seizures, premature death, and impaired spatial memory performance. Spatial memory requires hippocampal sharp-wave ripples (SPW-Rs), which consist of high-frequency field potential oscillations (ripples, 100-260 Hz) superimposed on a slower SPW. Published *in vitro* electrophysiologic recordings in DS mice demonstrate reduced firing of GABAergic inhibitory neurons, which are essential for the formation of SPW-R complexes. Here, *in vivo* electrophysiologic recordings of hippocampal local field potential in both male and female mice demonstrate that *Scn1a* haploinsufficiency slows intrinsic ripple frequency and reduces the rate of SPW-R occurrence. In DS mice, peak ripple-band power is shifted to lower frequencies, average intertrough intervals of individually detected ripples are slower, and the rate of SPW-R generation is reduced, while SPW amplitude remains unaffected. These alterations in SPW-R properties, in combination with published reductions in interneuron function in DS, suggest a direct link between reduced inhibitory neuron excitability and impaired SPW-R function. A simple interconnected, conductance-based *in silico* interneuron network model was used to determine whether reduced sodium conductance is sufficient to slow ripple frequency, and stimulation with a modeled SPW demonstrates that reduced sodium conductance alone is sufficient to slow oscillatory frequencies. These findings forge a potential mechanistic link between impaired SPW-R generation and *Scn1a* mutation in DS mice, expanding the set of disorders in which SPW-R dysfunction contributes to impaired memory.

## Commentary

Dravet syndrome (DS), originally named “severe myoclonic epilepsy in infancy” by Charlotte Dravet, is an epileptic encephalopathy characterized by refractory seizures and developmental impairments. Dravet syndrome is most commonly associated with *de novo* loss-of-function mutations of the *SCN1A* gene encoding the  $\text{Na}_v1.1$  voltage-gated sodium channel.<sup>1</sup> Consistently, haploinsufficient *Scn1a* mutant mice with reduced  $\text{Na}_v1.1$  protein levels have served as an excellent model system in which to study the mechanisms of DS-related seizures.<sup>1</sup> However, patients with DS also show cognitive and memory impairments,<sup>2</sup> stressing the importance of understanding memory-related circuits in models of DS. A recent study by Cheah et al utilizes the haploinsufficient *Scn1a* mutant mouse model to analyze how hippocampal sharp-wave ripples (SPW-Rs)—important neural signatures related to memory consolidation—are altered in DS.<sup>3</sup> As discussed below, their findings are clear and compelling and highlight the sharp differences between how ripples are altered in DS versus the ripple-related changes seen in temporal lobe epilepsy (TLE).

Converging evidence points to the key role played by the loss of  $\text{Na}_v1.1$  specifically in inhibitory neurons in DS. Inhibitory neurons normally have higher expression levels of  $\text{Na}_v1.1$  compared to excitatory neurons.<sup>4</sup> Given the importance of intact inhibitory neuronal firing in preventing epileptic activity, it is therefore unsurprising that cell-type-specific deletion of  $\text{Na}_v1.1$  selectively in inhibitory neurons leads to seizures and DS-like phenotypes.<sup>3</sup> Fast-spiking (FS) inhibitory neurons are the dominant source of inhibition onto excitatory pyramidal cells in the hippocampus and neocortex. Fast-spiking cells thus play a critical role in controlling and synchronizing the activity of pyramidal cells and are central to the generation of behavioral gamma rhythms that correlate with increased attention, faster reaction times, and improved sensory perception. Fast-spiking cells are also centrally involved in the generation of hippocampal CA1 ripples<sup>5,6</sup> seen during non-random-eye-movement (NREM) sleep and quiet wakefulness. Behavioral activity patterns, such as the sequential firing of hippocampal place cells during navigation, are replayed in a compressed neural sequence during ripples, helping to consolidate related memories. The



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frequency of healthy ripples is thought to be determined by the properties of FS-FS synapses within CA1.<sup>5,6</sup> Ripples are impaired in a multitude of epilepsies, likely contributing to memory and cognitive deficits in patients. In TLE, healthy ripples are often supplemented or replaced by pathological high-frequency activity that reflects a severely impaired excitation–inhibition balance<sup>5</sup> and can sometimes help to demarcate the seizure onset zone. Given the critical role of FS cells in ripple generation<sup>5,6</sup> and the impairment of FS cells in DS,<sup>7</sup> it logically follows that ripples should be expected to be impaired in mouse models of DS. However, how would this impairment manifest itself in DS: would it lead to faster, more frequent pathological high-frequency activity as in TLE, or would it lead to slower, less frequent ripples?


Cheah et al<sup>3</sup> answered this question by comparing the properties of hippocampal CA1 ripples in wild-type (WT) versus globally haploinsufficient *Scn1a* mutant (DS) mice. Their central finding was relatively unequivocal. No pathological high-frequency activity was observed in the hippocampus of DS mice. Instead, the frequency of both NREM and quiet wakefulness ripples was significantly and substantially decreased, from ~160 Hz in WT to ~130 Hz in DS mice. A computational model consisting of excitatory inputs to networks of synaptically coupled inhibitory neurons was able to successfully reproduce the *in vivo* observations: when the sodium conductance in inhibitory neurons in the model was reduced, the ripple frequency decreased. This is consistent with the hypothesis that selectively decreased  $Na_v1.1$  expression in inhibitory neurons extends the duration of each individual ripple cycle and hence reduces ripple frequency. The number of cycles within each ripple event was also significantly reduced in DS mice, with important functional implications. Behavioral sequences are replayed during ripples, and the more cycles included within a ripple event, the longer the sequence that can theoretically be replayed and consolidated. Indeed, there is now evidence that longer ripple durations, with more individual cycles per event, are associated with better memory consolidation.<sup>8</sup> Thus, the decrease in both ripple frequency and number of cycles per ripple event is likely to impair hippocampal memory consolidation in DS.

Is CA1 ripple frequency altered only because of sodium conductance changes in FS cells or could other factors play a role? Large volleys of excitatory inputs from CA3 onto CA1 cells lead to a sharp deflection in the CA1 local field potential. This large deflection is called a sharp-wave, and ripples are embedded within this sharp-wave. Together this complex is called the SPW-R. The size of the sharp-wave approximates the magnitude of the excitatory synaptic input arriving onto CA1 pyramidal cells from their CA3 counterparts. Cheah et al compared the size of sharp-waves in WT versus DS mice, but found no difference, suggesting that net synaptic input from CA3 onto CA1 cells is potentially unaltered. It should be noted, however, that previous work has shown that the synaptic excitation of cortical FS cells by local excitatory cells is diminished in this DS model.<sup>7</sup> If there is a similar impairment in terms of the excitatory CA3 synapses onto CA1 FS cells,


then this could represent an additional reason for reduced CA1 FS firing in DS mice, and further contribute to decreased ripple frequencies.

Cheah et al also found that the rate of occurrence of SPW-Rs was dramatically reduced in DS mice. Thus, not only are individual ripple events reduced in frequency but they also occur far less often, further impacting the memory consolidation abilities of this circuit in DS. But why would the rate of SPW-Rs decrease? Alterations in regions extrinsic to the hippocampus are likely to play a role. In particular, the medial septum is a key regulator of hippocampal circuits, and optogenetic activation of cholinergic neurons in the medial septum can reduce the rate of hippocampal SPW-Rs.<sup>9</sup> The medial septum also contains inhibitory neurons that project to the hippocampus directly but are also interconnected with the local septal cholinergic neurons. Reduced firing of septal inhibitory neurons may thus directly impact hippocampal circuits while also indirectly allowing greater cholinergic drive of the hippocampus by disinhibition of septal cholinergic neurons. These and other possibilities will need additional studies to mechanistically resolve. In addition, single unit recordings of both hippocampal and septal neurons in DS mouse models will be critical to understand the impact of altered  $Na_v1.1$  levels on the behavioral encoding of space by place cells<sup>10,11</sup> and the precise replay dynamics of place cell sequences during subsequent NREM ripples.

This study highlights the distinct ways in which hippocampal ripple-generating circuits can be impaired in different types of epilepsy. Selective, partial decreases in inhibitory neuronal firing, such as those expected in the haploinsufficient model of DS, can lead to decreased ripple frequencies, as demonstrated by Cheah et al. However, more pronounced loss of inhibition or perhaps depolarization block of FS cells, coupled with hyperexcitability of excitatory neurons may allow for runaway excitation that results in the pathological high-frequency activity<sup>5</sup> seen in TLE and other epilepsies. It will therefore be informative to study the changes in hippocampal ripple properties in mouse models with gain-of-function  $Na_v1.1$  mutations. With a higher probability of FS depolarization block following gain-of-function  $Na_v1.1$  mutations, are ripples likely to still be decreased in frequency, or would pathological high-frequency activity start to emerge? Perhaps most importantly, it will be beneficial to add ripple-related properties (rate, frequency, duration, power) as key metrics to track when evaluating the benefits of promising antisense oligonucleotide therapies in mouse models of DS.<sup>12,13</sup> The most impactful therapies will ideally result in decreased seizures as well as a restoration of hippocampal circuit signatures associated with functional learning and memory.

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