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Slow Down and Seize: Seizures Triggered by Slow Wave Oscillations in a GABAergic Model of Dravet Syndrome

Sleep Slow-Wave Oscillations Trigger Seizures in a Genetic Epilepsy Model of Dravet Syndrome

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Sleep is the preferential period when epileptic spike–wave discharges appear in human epileptic patients, including genetic epileptic seizures such as Dravet syndrome with multiple mutations including SCN1A mutation and $GABA_A$ receptor γ2 subunit $Gabrg2^{Q390X}$ mutation in patients, which presents more severe epileptic symptoms in female patients than male patients. However, the seizure onset mechanism during sleep still remains unknown. Our previous work has shown that the sleep-like state-dependent homeostatic synaptic potentiation can trigger epileptic spike–wave discharges in one transgenic heterozygous $Gabrg2^{+/Q390X}$ knock-in mouse model. Here, using this heterozygous knock-in mouse model, we hypothesized that slow-wave oscillations themselves *in vivo* could trigger epileptic seizures. We found that epileptic spike–wave discharges in heterozygous $Gabrg2^{+/Q390X}$ knock-in mice exhibited preferential incidence during non-rapid eye movement sleep period, accompanied by motor immobility/facial myoclonus/vibrissal twitching and more frequent spike–wave discharge incidence appeared in female heterozygous knock-in mice than male heterozygous knock-in mice. Optogenetically induced slow-wave oscillations *in vivo* significantly increased epileptic spike–wave discharge incidence in heterozygous $Gabrg2^{+/Q390X}$ knock-in mice with longer duration of non-rapid eye movement sleep or quiet–wakeful states. Furthermore, suppression of slow-wave oscillation-related homeostatic synaptic potentiation by 4-(diethylamino)-benzaldehyde injection (*i.p.*) greatly attenuated spike–wave discharge incidence in heterozygous knock-in mice, suggesting that slow-wave oscillations *in vivo* did trigger seizure activity in heterozygous knock-in mice. Meanwhile, sleep spindle generation in wild-type littermates and heterozygous $Gabrg2^{+/Q390X}$ knock-in mice involved the slow-wave oscillation-related homeostatic synaptic potentiation that also contributed to epileptic spike–wave discharge generation in heterozygous $Gabrg2^{+/Q390X}$ knock-in mice. In addition, EEG spectral power of delta frequency (0.1–4 Hz) during non-rapid eye movement sleep was significantly larger in female heterozygous $Gabrg2^{+/Q390X}$ knock-in mice than that in male heterozygous $Gabrg2^{+/Q390X}$ knock-in mice, which likely contributes to the gender difference in seizure incidence during non-rapid eye movement sleep/quiet–wake states of human patients. Overall, all these results indicate that slow-wave oscillations *in vivo* trigger the seizure onset in heterozygous $Gabrg2^{+/Q390X}$ knock-in mice, preferentially during non-rapid eye movement sleep period and likely generate the sex difference in seizure incidence between male and female heterozygous $Gabrg2^{+/Q390X}$ knock-in mice.

Commentary

Vigilance state is well known to significantly modulate epileptiform discharges and seizure occurrence.[1] Vigilance state can also dictate whether seizures will remain localized or spread more widely.[2] Specifically, seizures and epileptiform discharges occur more commonly during the slow wave sleep stages of nonrapid eye movement (NREM) sleep.[2] While this has been appreciated for over half a century, specific mechanisms for why this is the case are not well understood. It has been postulated that the increased amplitude and synchrony of the delta waves in NREM make it more likely for electrical activity in the brain to eclipse the seizure threshold; whereas this is less likely in states with lower amplitude electroencephalogram (EEG) activity (e.g., wake and rapid eye movement [REM] sleep).[3] The naturally occurring mechanisms that permit or restrict epileptiform discharges and seizures may constitute a mechanism to target in seizure prevention. This is especially important since nearly a third of patients with epilepsy are resistant to treatment, suffer considerable morbidity, and are at risk for seizure-associated mortality, despite the existence of dozens of therapeutic options.[4]

Using the $Gabrg2^{+/Q390X}$ knock-in mouse model, Catron and colleagues evaluated slow wave mechanisms for seizures.[5] This mutation has been identified in a subset of patients with

the epileptic encephalopathy, Dravet syndrome. While most Dravet syndrome cases involve one of many mutations in *Scn1a*, the gene encoding the voltage-gated sodium channel $Na_V1.1$, other mutations have been identified, including this one in *Gabrg2*, the gene for the gamma subunit of the $GABA_A$ receptor.[6,7]

First, *Gabrg2*$^{+/Q390X}$ mice and wild type (WT) littermates were instrumented for EEG recording. They demonstrated more abundant epileptiform discharges and seizures during slow wave sleep in *Gabrg2*$^{+/Q390X}$ mice. The *Gabrg2*$^{+/Q390X}$ mice demonstrated exceedingly more spike wave discharges (SWDs), in general, than their WT counterparts which had sparingly few. Both genotypes spent the largest proportion of time in NREM, followed by wake, and REM, with considerably more time in NREM than in wake or REM. While *Gabrg2*$^{+/Q390X}$ mice spent comparable total amounts of time in each vigilance state over 24 hours compared to WT littermates, sleep was generally more fragmented with a greater number of shorter bouts of each state. Whether this was due to the $GABA_A$ receptor mutation per se or due to the plentiful SWDs in these mice was not differentiated. They also did not evaluate whether there was any difference in the vigilance state distribution of SWDs between sexes.

Next, they showed that optogenetically driving slow waves could increase the likelihood of seizures. One major difference between NREM compared to wake and REM is the abundance of slow, rhythmic delta waves that define slow wave sleep. It has been proposed that these slow oscillations drive the seizure-permissive nature of NREM.[3] They employed a method they developed to induce slow wave oscillations (SWOs).[8] Optical fibers and stimulating electrodes were implanted into the somatosensory cortex of WT and *Gabrg2*$^{+/Q390X}$ mice expressing the inhibitory opsin halorhodopsin in cortical neurons. Neurons were optogenetically stimulated at low frequency (0.5 Hz) and simultaneously electrically stimulated to increase SWOs. Increasing SWOs in this manner increased the amount of time in NREM sleep, with a concomitant reduction in REM and wake. Increasing SWOs also triggered SWDs.

Given previous work demonstrating that SWOs are associated with homeostatic synaptic potentiation,[8] they examined the effects of suppressing this potentiation by blocking aldehyde dehydrogenase with systemically administered 4-(diethylamino)-benzaldehyde (DEAB) to reduce retinoic acid synthesis and found that this decreased the incidence and duration of SWDs in *Gabrg2*$^{+/Q390X}$ mice. DEAB treatment had no effect on sleep-wake architecture in either genotype.

Since sleep spindles share thalamocortical mechanistic components with SWDs and indeed can evolve into SWDs under pathological conditions,[9] the authors examined whether sleep spindles were similarly affected and modulated in *Gabrg2*$^{+/Q390X}$ mice. They found that, just like SWDs, sleep spindles were increased with SWO induction and decreased with suppression of homeostatic synaptic potentiation with DEAB.

Finally, and somewhat surprisingly, they identified some sex differences in that delta power during NREM sleep in female *Gabrg2*$^{+/Q390X}$ mice was significantly larger than in male counterparts. Sex differences in seizure susceptibility during NREM sleep have been reported. This finding may begin to explain these differences.

This is an intriguing study that sheds light on the role of NREM sleep and especially SWOs in facilitating seizure occurrence. They propose that SWOs in NREM lead to unchecked excitatory synaptic potentiation that promotes seizures. Though there are some inherent challenges in manipulating the rhythms that are used to define the vigilance state (i.e., if the rhythm is experimentally altered, is it still the same state?) that triggering SWOs or blocking the synaptic potentiation caused by SWOs differentially regulates SWD occurrence suggests that the SWOs themselves are the inciting event.

While the preponderance of evidence through the years suggests seizures occur much less frequently in REM sleep,[2] there is evidence to suggest that the more synchronous theta components of REM sleep can also be conducive to seizures in certain circumstances.[10] It would be interesting to know if this model could also be useful in testing seizure-facilitatory effects of other rhythms (e.g., by experimentally driving rhythmic theta) and whether comparable underlying mechanisms are at play. Another interesting point to consider is that interictal epileptiform discharges have been shown to impair memory consolidation. Memory consolidation is known to occur predominantly during sleep and is associated with sleep spindles.[11] Understanding mechanisms to reduce interictal epileptiform discharges during sleep may improve memory consolidation. This alone could have profound impact on functioning and quality of life in patients with epilepsy. But also reducing the effects of SWOs on spindles themselves could improve memory consolidation. In addition, nocturnal seizures are considered a risk factor for sudden unexpected death in epilepsy, or SUDEP, the leading cause of death in patients with refractory epilepsy.[10] Perhaps using these findings to devise ways to reduce seizures during nighttime sleep would eliminate a subset of SUDEP cases.

This study, along with this group's prior work, supplies plausible mechanistic underpinnings to the long-known propensity for seizures in NREM sleep. At present, with the methodologies employed here, it would be challenging to translate these findings to the clinic. However, further unraveling these mechanisms and learning to manipulate them in patients promises a new avenue for development of novel therapies for seizure prevention. This may also have important implications for memory consolidation and reducing mortality from seizures, thus minimizing effects of comorbidities if not overtly eliminating seizures.

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Declaration of Conflicting Interests

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

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