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You Snooze You Seize: GABAergic Potentiation of Genetic Generalized Seizures During NREM

Impaired State-Dependent Potentiation of GABAergic Synaptic Currents Triggers Seizures in a Genetic Generalized Epilepsy Model

Zhang C-Q, Catron MA, Ding L, Hanna CM, Gallagher MJ, Macdonald RL, Zhou C. *Cereb Cortex*. 2021;31(2):768-784. doi:10.1093/cercor/bhaa256. https://pubmed.ncbi.nlm.nih.gov/32930324/

Epileptic activity in genetic generalized epilepsy (GGE) patients preferentially appears during sleep and its mechanism remains unknown. Here, we found that sleep-like slow-wave oscillations (0.5 Hz SWOs) potentiated excitatory and inhibitory synaptic currents in layer V cortical pyramidal neurons from wild-type (wt) mouse brain slices. In contrast, SWOs potentiated excitatory, but not inhibitory, currents in cortical neurons from a heterozygous (het) knock-in (KI) Gabrg2+Q/390X model of Dravet epilepsy syndrome. This created an imbalance between evoked excitatory and inhibitory currents to effectively prompt neuronal action potential firings. Similarly, physiologically similar up-/down-state induction (present during slow-wave sleep) in cortical neurons also potentiated excitatory synaptic currents within brain slices from wt and het KI mice. Moreover, this state-dependent potentiation of excitatory synaptic currents entailed some signaling pathways of homeostatic synaptic plasticity. Consequently, in het KI mice, in vivo SWO induction (using optogenetic methods) triggered generalized epileptic spike-wave discharges (SWDs), being accompanied by sudden immobility, facial myoclonus, and vibrissa twitching. In contrast, in wt littermates, SWO induction did not cause epileptic SWDs and motor behaviors. To our knowledge, this is the first mechanism to explain why epileptic SWDs preferentially happen during non-rapid eye-movement sleep and quiet-wakefulness in human GGE patients.

Commentary

The influence of slow-wave sleep on seizure incidence in epilepsy has long been recognized.[1] In 400 BC the Greek philosopher Aristotle first noted "In many cases, epilepsy sets in during sleep." Investigations to determine the underlying mechanisms that drive this relationship with the goal to help improve treatment outcomes in patients with epilepsy are needed. Sleep is a critical component of all homeostatic plasticity at neuronal synapses. It underlies circadian rhythms that determine the efficiency of long-term learning and memory. Synaptic homeostatic scaling also contributes to the behavioral state-dependent modulation of electroencephalogram (EEG) changes associated with slow-wave sleep versus wake states. Slow-wave sleep refers to phase 3 (N3) sleep, which is the deepest phase of non-rapid eye movement (NREM) sleep, characterized by delta waves (0.5-4 Hz on EEG) and critical for memory consolidation. Studying impairments of slow-wave oscillations (SWO, 0.5 Hz) in generalized genetic epilepsies (GGE) has emerged as an important tool to gain insights that will help guide investigation of underlying mechanisms and identify new EEG biomarkers[2] associated with increased incidence of spikes and seizure activity during NREM sleep. The EEG phenotype of GGEs are bilateral, synchronous, symmetric, and generalized spike-wave discharges (SWDs). Sleep, sleep deprivation, eye closure, and fixation-off are often used as activation techniques to increase the diagnostic yield of EEG recordings[3], indicating that in addition to sleep states, suppression of visual cues also plays a role in seizure initiation. Several anti-seizure medications approved for seizure suppression act by either subduing runaway excitation or enhancing inhibition to help curb occurrence and frequency of global seizures, which are common in GGEs. Some GGEs are notoriously refractory and commonly treated with polytherapy cocktails introduced in an empirical manner due to lack of evidence-based guidelines. The known potential for interictal discharges to disrupt sleep-related memory consolidation provides a perspective for understanding the association of childhood epilepsy with a high rate of intellectual disability. For patients with tuberous sclerosis-related epilepsy where seizures cluster when the child is falling asleep or soon after waking, it has been reported that when the epilepsy begins before the age of 2 years, the frequency and severity of

intellectual disability is much higher.[4] Added to the poor sleep-efficiency, day-time sleepiness is commonly reported in pediatric patients with severe GGEs, which further aggravates poor learning and cognition.

The impairment of cognitive functions via sleep is present especially in epileptic networks involving the thalamocortical system and the hippocampo-cortical memory encoding system. Although the thalamus may contribute to shaping the rhythm, SWOs are a cortical phenomenon.[5] During NREM sleep, cortical neurons oscillate approximately once every second between a depolarized upstate, when cortical neurons are actively firing, and a hyperpolarized downstate, when cortical neurons are virtually silent. The bistable behavior of the thalamocortical circuit during NREM sleep that allows for rapid and synchronous neuronal depolarization is inevitably followed by a massive hyperpolarization.[6] At the EEG level, this leads to an "enhanced" slow wave that displays larger amplitude, steeper slope, and involves broader cortical regions. Investigation of SWOs using high-density EEGs, which combine both temporal precision and the opportunity to record from the entire cortex, indicate that the negative peak of the scalp-recorded SWO likely reflects the beginning of the transition from downstate to upstate and the resumption of cortical neural firing. These waves of SWO could therefore be driving waves of sEPSPs and sIPSPs in groups of cortical neurons during depolarized versus hyperpolarized states.

γ-aminobutyric acid type A ($GABA_A$) receptors are the primary mediators of fast inhibitory synaptic transmission in the central nervous system and reduction of $GABA_A$ receptor-mediated inhibition has been shown to produce seizures. The $GABA_A$ receptor γ2(Q390X) subunit is associated with epileptic encephalopathy, Dravet syndrome, and the epilepsy syndrome genetic epilepsy with febrile seizures plus.[7] The mutation generates a premature stop codon that results in translation of a truncated and misfolded γ2 subunit that accumulates in neurons and disrupts incorporation of γ2 subunits into $GABA_A$ receptors. The authors of the current study have previously suggested that the aggregated protein likely causes neuronal stress and apoptosis, resulting in the severe neurological phenotype.[8] Het Gabrg2+/Q390X knock-in (KI) mice have been shown to have reduced cortical inhibition, SWD on EEG, a lower seizure threshold to the convulsant drug pentylenetetrazol, and spontaneous generalized tonic–clonic seizures. To investigate the phenomenon of slow-wave sleep potentiation of generalized seizures, this study investigated het Gabrg2+/Q390X KI mice, transgenic wild-type (wt), and heterozygous (het) Gabrg2+/Q390X KI mice expressing halorhodopsin in cortical neurons for *ex vivo* and *in vivo* optogenetic SWO induction protocols.[9] They showed that induction of SWO in het KI mice triggered SWDs accompanied by behaviors typical of generalized absence seizures which did not occur in wt mice. In *vitro* experiments showed that SWOs (0.5 Hz) potentiated sEPSCs and sIPSCs in cortical pyramidal neurons (layer V) from wt mice. In contrast, only sEPSCs, but not sIPSCs, were enhanced in cortical neurons from het Gabrg2+/Q390X KI mice. The impaired sIPSC potentiation during SWOs prompted the neurons to more readily generate action potentials in the het mice than in wt. The data presented indicate that in *ex vivo* brain slices from Gabrg2+/Q390X KI mice during induced up and down states in cortical neurons, the significant deficit in sIPSC potentiation may be a cause of the emergence of SWDs during SWO. The same experiment done *in vivo* in the Gabrg2+/Q390X KI mice, which expressed halorhodopsin to laser-induce cortical neuronal up and down states and SWO, showed post-SWO EEGs with a significant increase in SWDs compared to pre-SWO EEG baselines in the same KI mice.

Spike-wave discharges during SWO are commonly reported in neurodevelopmental disorders (NDDs) associated with early-life seizures and epilepsy. However, sleep disorders associated with frequent nighttime awakenings and difficulty falling asleep are also commonly reported for NDDs.[10] The SWD potentiation during NREM could be one of the underlying causes of the sleep disorders. Interestingly, SWDs in NDDs are also potentiated by eye closure, fixation off, and reflex seizures when awake[11] highlighting the likely role of loss of GABAergic potentiation in diverse circuits activated during behavioral and sensory-motor transition-states that trigger the awake events in addition to SWOs during NREM.

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