



REVing up the Brain: A Mechanism Driving Seizure Timing

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Dysregulation of REV-ERB α impairs GABAergic function and promotes epileptic seizures in preclinical models

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To design potentially more effective therapies, we need to further understand the mechanisms underlying epilepsy. Here, we uncover the role of Rev-erb α in circadian regulation of epileptic seizures. We first show up-regulation of REV-ERB α /Rev-erb α in brain tissues from patients with epilepsy and a mouse model. Ablation or pharmacological modulation of Rev-erb α in mice decreases the susceptibility to acute and chronic seizures, and abolishes diurnal rhythmicity in seizure severity, whereas activation of Rev-erb α increases the animal susceptibility. Rev-erb α ablation or antagonism also leads to prolonged spontaneous inhibitory postsynaptic currents and elevated frequency in the mouse hippocampus, indicating enhanced GABAergic signaling. We also identify the transporters Slc6a1 and Slc6a11 as regulators of Rev-erb α -mediated clearance of GABA. Mechanistically, Rev-erb α promotes the expressions of Slc6a1 and Slc6a11 through transcriptional repression of E4bp4. Our findings propose Rev-erb α as a regulator of synaptic function at the crosstalk between pathways regulating the circadian clock and epilepsy.

Commentary

At any given time, complex and poorly understood factors can coalesce to shift the brain into a seizure state. While variables including age, diet, intercurrent or comorbid illness, and sleep deprivation are well-accepted as contributors to seizure susceptibility, a growing body of literature supports the conclusion that circadian rhythms are a distinct modulator of seizure risk. Here, we discuss an exciting recent report from Zhang et al. demonstrating how a specific molecular driver of circadian rhythms, REV-ERB α , modulates seizure risk.¹

A seminal study published in 1938 compiled data on nearly 40,000 seizures over 10 years from 114 children living at the Lingfield Epileptic Colony (Surrey, United Kingdom), a residential colony for individuals with epilepsy. This dataset revealed that seizures tend to cluster at certain times of the day.² Fast-forward nearly a century, and modern technologies are providing more granular evidence of the same. Two major studies published in 2018 corroborated previous findings by demonstrating that (1) most people have daily 24-hour cycles in seizure likelihood³ and (2) interictal epileptiform activity oscillates over 24-hour cycles.⁴ These daily cycles in seizure risk have now been appreciated for both generalized and focal epilepsies and may affect more than 80% of individuals.

Despite the recognition of time-of-day differences in seizure risk, the mechanisms by which this occurs remain unclear. One logical hypothesis is that seizures are regulated by circadian rhythms, endogenous rhythms occurring over a period of 24 hours and controlled by the “molecular clock” comprises of transcription–translation feedback loops. In the core mammalian

loop, transcriptional activators CLOCK and BMAL1 promote the expression of *PER1/PER2* (*Period*) and *CRY1/CRY2* (*Cryptochrome*) genes. The protein products of the *Period* and *Cryptochrome* genes then feed back and repress CLOCK and BMAL1 over a period of approximately 24 hours. In addition to this core loop, numerous auxiliary loops, including one formed by REV-ERB α , fine-tune the molecular clock. CLOCK and BMAL1 drive *REV-ERB α* transcription, the protein product of which represses CLOCK and BMAL1 transcription. Previous studies have demonstrated that expression of components of the molecular clock is dysregulated in resected human epileptogenic tissue.⁵ Disruption of the molecular clock was further found to be epileptogenic, as loss of *Clock* or *Bmal1* promotes seizures in mouse models of epilepsy^{5,6} through unclear mechanisms. Zhang et al. pick up where these previous studies left off and provides a compelling mechanism as to *how* the molecular clock might lead to daily fluctuations in seizures.

As previously demonstrated, the authors find that the molecular clock is disrupted in tissue resected from patients with epilepsy. After comparing resected tissue from individuals with epilepsy to glioma specimens (from individuals without seizures), REV-ERB α was found to be selectively up-regulated in tissue from patients with epilepsy. However, the use of human glioma as “control” tissue has limitations, so the authors confirmed up-regulation of Rev-erb α in a mouse model of epilepsy. Mice were injected with kainic acid at 6 timepoints throughout the day and seizures were scored for severity. Injection of kainic acid in the middle of the daytime (when mice, being nocturnal animals, typically sleep) led to more severe seizures. In contrast, injection during the middle of the night



(when mice are awake) produced less severe seizures. Timepoints with worsened seizures positively correlated with increased expression of Rev-erb α . These findings suggest that endogenous circadian rhythms may control seizure severity through the day. However, seizure rhythmicity was not quantified in mice with pilocarpine-induced chronic acquired TLE. The authors did not measure concentrations in the brain across timepoints through the day, and there could be circadian variations in blood-brain barrier permeability that influence the brain concentration of kainate. This would be important to establish, as kainic acid is a known substrate of the ABCB5 efflux transporter, and activity of efflux transporters can vary through the day.^{7,8} The possibility that rhythmic permeability of kainic acid into the brain underlies the observed rhythms in seizure severity must be excluded.

Zhang et al. then embark on a series of detailed experiments to understand how increased Rev-erb α expression exacerbates seizures. Using both genetic and pharmacological approaches, loss of Rev-erb α function was found to lead to decreased seizure susceptibility. Electrophysiological recordings from dentate gyrus granule cells from Rev-erb α knockout animals revealed enhanced GABAergic signaling without changes in glutamatergic signaling. Providing a potential mechanism for these electrophysiological changes, transcriptomics revealed that Rev-erb α knockout animals had decreased expression of Slc6a1 and Slc6a11, GABA reuptake transporters that clear extracellular GABA. Finally, a series of epistatic experiments revealed that Rev-erb α promotes Slc6a1 and Slc6a11 expression by repressing *E4bp4*, a known transcriptional repressor. These experiments support a model whereby seizures promote Rev-erb α expression, leading to repression of *E4bp4*, thereby promoting expression of Slc6a1 and Slc6a11, which act to enhance GABA clearance from the extracellular space. Reduced GABAergic signaling in turn exacerbates seizures.

While this study focuses on the “circadian” regulation of seizures, at least two other variables that are simultaneously oscillating with possible effects on seizures were not evaluated: the sleep/wake cycle and the light/dark cycle. Circadian rhythms and the sleep/wake cycle are not synonymous. Sleep is a behavior that is predominantly controlled by (1) circadian rhythms for timing and (2) a homeostatic sleep drive for quantity. Zhang et al. find that mice have the most severe seizures in the middle of the day, when mice are typically sleeping; knockout of Rev-erb α led to decreased seizure severity at this time. Future studies should clarify if this could be a sleep-related effect, by keeping animals awake during typical times of rest and then assessing seizure severity. This possibility is important to consider since changes in sleep phase are known to occur after Rev-erb α knockout.⁹ Additionally, this shifted phase raises the possibility that seizure rhythmicity is preserved in the absence of Rev-erb α but altered in phase; this could have been missed given that the authors focus attention on only two timepoints in Rev-erb α knockout mice injected with kainic acid. In support of this alternative interpretation, when Rev-erb α was inhibited in mice with pilocarpine-induced chronic acquired TLE, there appear to

be qualitative seizure rhythms, though rhythmicity was not quantified.

Zhang et al. provide a compelling mechanism demonstrating how components of the molecular clock may affect seizure severity, but further studies are needed to disentangle overlapping cyclic variables including the sleep/wake and light/dark cycles. The cell types, circuits, and brain regions that drive seizure rhythms, and how this might change through development, are yet to be resolved. Future studies will also be needed to understand how applicable these findings are to other types of epilepsies, including generalized epilepsy, genetic forms of epilepsy, and epileptic encephalopathies. The hope is that unraveling these intertwined mechanisms may reveal novel pathways regulating seizure susceptibility and pave the way to identification of the next generation of therapeutic targets.

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