



Perspective: Is Cortical Hyperexcitability the Only Path to Generalized Absence Epilepsy?

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Commentary on: Huguenard J.

Thalamocortical Circuits and Excitability. *Epilepsy Curr.* 2001 Sep;1(1):13.

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How does one discover the fundamental neurobiological mechanisms leading to seizures? How can we use this information, obtained mainly in animal models, to better predict and treat seizures? In the inaugural issue of *Epilepsy Currents* I wrote a commentary¹ on 2 papers^{2,3} in which the authors each developed a novel way to generate a hybrid, semiartificial thalamocortical network in a dish. The results of the 2 papers (summarized below) suggested that in generalized absence epilepsy cortical hyperexcitability may be a key step that helps engage the thalamus thus leading to rapid generalization within the thalamocortical network and seizure genesis. Here, I review recent literature that either supports or opposes this view and provide a synthesis on the state of the field.

First a note about the powerful experimental approach of the original papers. While not fully recapitulating intact brain networks, reduced preparations can provide outstanding access for recording, stimulation, and modulating activity. Most notably, in vitro methods promote long-term stable intracellular recordings allowing direct access to voltage-clamp or current-clamp cellular responses that are necessary to support complex network activities. These responses include synaptic events, subthreshold membrane potential oscillations, and spiking, all driven by specific combinations of voltage- and ligand-gated ion channels. Previous findings had shown that brain slices retaining key connections of the *ferret* visual thalamus—those between the dorsal lateral geniculate and the perigeniculate nuclei—would generate physiological rhythms related to sleep and epilepsy.⁴ Around the same time analogous in vitro *rat*⁵ and *mouse*⁶ slice preparations of somatosensory thalamus were developed all allowing rapid advances in our understanding of thalamic network synchronizing mechanisms. In the 2 highlighted papers of 2000, each group used a simple brain slice approach to record from the visual portion of the thalamic network, and then bidirectionally couple the recorded slice responses to a virtual cortical network. When a simple form of discharge from a “normal” cortical network was fed back to the thalamus, a modest enhancement of a sleep spindle-like

thalamic oscillation was obtained. By contrast, when the virtual cortical network was made hyperexcitable such that it would respond to a thalamic barrage with a high-frequency burst discharge fed back to thalamus, this surprisingly resulted in a large increase in synchronous discharge in the thalamus, a slowing of the network activity from 6 to 10 Hz (spindle-like) to 2 to 4 Hz (seizure-like). These striking observations suggested that cortical hyperexcitability may help engage the thalamus thus leading to rapid generalization throughout the thalamocortical network at the onset of each generalized absence seizure.

What have we learned in the 20 years since these original observations? At the time of their publication at the turn of the century, no one was yet predicting what might be learned from powerful circuit modulation approaches whose development has exploded in the last 10 years. Techniques, such as optogenetics⁷ and chemogenetics,⁸ allow targeted expression of neural activity modulators in genetically tractable species, especially mouse. Similarly, genetic activity reporters,⁹ along with high-density intracranial recording methods, such as silicon Neuropixels probes¹⁰ are revolutionizing our ability to “crack” neural circuits through simultaneous recordings of hundreds to thousands of neurons.

Have these new methods confirmed the hypothesis that enhanced cortical output is the “trigger” for seizures studies? Further, as I had predicted in the original commentary, might reduction in cortical output, specifically that directed towards the thalamic reticular nucleus, suppress seizures, since the reticular nucleus appears to serve a major pacemaking role in absence seizures?¹¹⁻¹⁵ In contrast, what emerges from recent studies is the view that in fact many roads lead to excessive thalamocortical synchrony and absence seizures, and there is not one simple pathway that in every model becomes serially engaged (eg, cortex → reticular thalamus → cortex) or (basal ganglia → thalamus → cortex) to produce a seizure. For example, in mice with a spontaneously occurring loss of function of an excitatory glutamate synaptic receptor *Gria4*, we found that *in contrast* to our prediction, cortical input



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specifically to the reticular nucleus was actually *reduced*. This resulted in loss of what we then proposed was a control mechanism in the thalamus—a feedforward inhibition of thalamic relay neurons—and with the loss of this critical control the thalamic relay neurons, which retained normal inputs from cortex, then became hyperexcitable, and they in turn hyperactivated the reticular nucleus.¹⁶ In this case, even though direct cortical activation of reticular thalamus was reduced, the downstream effect was still excessive activation. A similar loss of cortically driven feedforward inhibition of relay neurons in mice with GABA_AR $\alpha 1$ subunit haploinsufficiency contributes to absence seizures in these animals.¹⁷ Complicating things further, recent studies have further supported the original view that specific cortical dysfunction *can* trigger absence seizures. For example, specific loss of P/Q calcium channels in deep layer neocortical projection cells is sufficient to cause absence seizures in mice,¹⁸ while transplantation of interneuron precursors specifically in neocortex reduce absence episodes in star-gazer mice.¹⁹

Some of the reports detailed above furthered the idea that cortical hyperexcitability is *sufficient* to induce absence seizures in mouse models. Are there examples that demonstrate that cortical dysfunction is not itself *necessary* for absences, that is, might there be subcortical mechanisms be seizure initiating on their own? A recent finding in heterozygous *med* mice, which have a spontaneous mutation in *Scn8a* encoding the voltage-gated sodium channel Na_v1.6, indicates that dysfunction limited to thalamic circuits can also be *sufficient* to trigger absence seizures in the thalamocortical system.²⁰ Of note, partial loss of *Scn8a* results in spontaneous absence seizures,²¹ yet is protective to a variety of other epileptogenic insults.^{20,22,23} How might this discrepancy between seizure-genic and seizure protective *Scn8a* mechanisms be resolved? Genetic crosses that resulted in specific deletion of *Scn8a* in different brain regions provided clues.²⁰ For example, *Scn8a* deletion driven by promoters *Emx1*, *Camk2a*, or *FoxG1* thus targeting mainly excitatory forebrain neurons all resulted in protection from flurothyl-induced seizures, while targeting forebrain interneurons (except thalamus) via the *Ppp1r2* promoter worsened flurothyl seizures. By contrast, more broadly targeting interneurons, *including thalamus*, with *Dlx5/6* resulted in robust spontaneous absence seizures. This suggested that hypofunction of Na_v channels in thalamic inhibitory neurons could trigger absences and that seizures could arise from specific thalamic dysfunction. Consistent with this were the findings that isolated in vitro thalamic slices were strongly hyperexcitable, and that recurrent collateral synapses between inhibitory thalamic reticular neurons, which are posited to regulate thalamic epileptic synchrony,²⁴ were weakened. To test whether specific disruption in thalamic reticular neuron Na_v1.6 function would be *sufficient* to generate absence seizures, a short hairpin RNA (shRNA) *Scn8a* viral construct designed to knockdown expression was injected into lateral thalamus. Notably, absence seizures were indeed induced by specific thalamic knockdown, but only when the infected region included the thalamic reticular nucleus.²⁰ Further supporting


the hypothesis that thalamic-specific mechanisms can be ictogenic, local delivery of *Plcb4* shRNA to the thalamus has been shown to result in spontaneous absence seizures,²⁵ as has local thalamic deletion of *Hcn2*.²⁶

Although this has not been an exhaustive review, from the studies described here it should be clear that within the thalamocortical loop in which absence seizures are embedded, either thalamic or cortical dysfunction can be *sufficient* to induce absence epilepsy. And by extension, neither cortical nor thalamic dysfunction on their own is *required* for seizure induction. This leaves us with the overall conclusion that coordinated thalamocortical rhythmic activity that creates the electroencephalogram spike-wave discharge (SWD) of generalized absence epilepsy is in fact an embedded feature in the network. The lack of SWD and associated absences under normal circumstances then must result from internal control mechanisms that ordinarily act as powerful suppressors of the embedded activity. Further understanding of such control mechanisms is likely to lead to better therapeutic strategies.

By John R. Huguenard

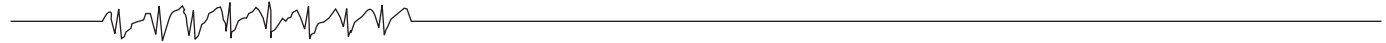
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