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

Commentary on: Huguenard J. Thalamocortical Circuits and Excitability. *Epilepsy Curr.* 2001 Sep;1(1):13. doi: 10.1046/j.1535-7597.2001.00008.x

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 commentary[1] 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 optogenetics[7] 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 probes[10] 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

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 $GABA_AR$ α1 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 stargazer 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.

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References

1. Huguenard J. Thalamocortical circuits and excitability. *Epilepsy Curr*. 2001;1(1):13.
2. Bal T, Debay D, Destexhe A. Cortical feedback controls the frequency and synchrony of oscillations in the visual thalamus. *J Neurosci*. 2000;20(19):7478-7488.
3. Blumenfeld H, McCormick DA. Corticothalamic inputs control the pattern of activity generated in thalamocortical networks. *J Neurosci*. 2000;20(13):5153-5162.
4. von Krosigk M, Bal T, McCormick DA. Cellular mechanisms of a synchronized oscillation in the thalamus. *Science*. 1993; 261(5119):361-364.
5. Huguenard JR, Prince DA. Intrathalamic rhythmicity studied in vitro: nominal T current modulation causes robust anti-oscillatory effects. *J Neurosci*. 1994;14(9):5485-502.
6. Warren RA, Agmon A, Jones EG. Oscillatory synaptic interactions between ventroposterior and reticular neurons in mouse thalamus in vitro. *J Neurophysiol*. 1994;72(4):1993-2003.
7. Paz JT, Huguenard JR. Optogenetics and epilepsy: past, present and future. *Epilepsy Curr*. 2015;15(1):34-38.
8. Krook-Magnuson E, Soltesz I. Beyond the hammer and the scalpel: selective circuit control for the epilepsies. *Nat Neurosci*. 2015;18(3):331-338.
9. Akerboom J, Tsai-Wen C, Trevor JW, et al. Optimization of a GCaMP calcium indicator for neural activity imaging. *J Neurosci*. 2012;32(40):13819-13840.

10. Jun JJ, Nicholas AS, Joshua HS, et al. Fully integrated silicon probes for high-density recording of neural activity. *Nature*. 2017; 551(7679):232-236.
11. Avanzini G, Vergnes M, Spreafico R, Marescaux C. Calcium-dependent regulation of genetically determined spike and waves by the reticular thalamic nucleus of rats. *Epilepsia*. 1993;34(1): 1-7.
12. Slaght SJ, Leresche N, Deniau JM, Crunelli V, Charpier S. Activity of thalamic reticular neurons during spontaneous genetically determined spike and wave discharges. *J Neurosci*. 2002;22(6): 2323-2334.
13. Huguenard JR, McCormick DA. Thalamic synchrony and dynamic regulation of global forebrain oscillations. *Trends Neurosci*. 2007;30(7):350-356.
14. Zaman T, Lee K, Park C, et al. Cav2.3 channels are critical for oscillatory burst discharges in the reticular thalamus and absence epilepsy. *Neuron*. 2011;70(1):95-108.
15. Cain SM, Tyson JR, Choi HB, et al. CaV 3.2 drives sustained burst-firing, which is critical for absence seizure propagation in reticular thalamic neurons. *Epilepsia*. 2018; 59(4):778-791.
16. Paz JT, Bryant AS, Peng K, et al. A new mode of corticothalamic transmission revealed in the Gria4(-/-) model of absence epilepsy. *Nat Neurosci*. 2011;14(9):1167-1173.
17. Zhou C, Ding L, Deel ME, Ferrick EA, Emeson RB, Gallagher MJ. Altered intra thalamic GABAA neurotransmission in a mouse model of a human genetic absence epilepsy syndrome. *Neurobiol Dis*. 2015;73(1):407-417.
18. Bomben VC, Aiba I, Qian J, Mark MD, Herlitze S, Noebels JL. Isolated P/Q Calcium channel deletion in Layer VI corticothalamic neurons generates absence epilepsy. *J Neurosci*. 2016;36(2):405-418.
19. Hammad M, Schmidt SL, Zhang X, Bray R, Frohlich F, Ghashghaei HT. Transplantation of GABAergic interneurons into the neonatal primary visual cortex reduces absence seizures in stargazer mice. *Cereb Cortex*. 1991;25(3):2970-2979.
20. Makinson CD, Tanaka BS, Sorokin JM, et al. Regulation of thalamic and cortical network synchrony by Scn8a. *Neuron*. 2017; 93(5):1165-1179.e6.
21. Papale LA, Beyer B, Jones JM, et al. Heterozygous mutations of the voltage-gated sodium channel SCN8A are associated with spike-wave discharges and absence epilepsy in mice. *Hum Mol Genet*. 2009;18(9):1633-1641.
22. Makinson CD, Tanaka BS, Lamar T, Goldin AL, Escayg A. Role of the hippocampus in Nav1.6 (Scn8a) mediated seizure resistance. *Neurobiol Dis*. 2014;68:16-25.
23. Wong JC, Makinson CD, Lamar T, et al. Selective targeting of Scn8a prevents seizure development in a mouse model of mesial temporal lobe epilepsy. *Sci Rep*. 2018;8(1):126.
24. Huntsman MM, Porcello DM, Homanics GE, De Lorey TM, Huguenard JR. Reciprocal inhibitory connections and network synchrony in the mammalian thalamus. *Science*. 1999; 283(5401):541-543.
25. Cheong E, Zheng Y, Lee K, et al. Deletion of phospholipase C beta4 in thalamocortical relay nucleus leads to absence seizures. *Proc Natl Acad Sci U S A*. 2009;106(51):21912-21917.
26. Hammelmann V, Stieglitz MS, Hülle H, et al. Abolishing cAMP sensitivity in HCN2 pacemaker channels induces generalized. *JCI Insight*. 2019;4(9):e126418. https://doi.org/10.1172/jci.insight.126418.