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Not All Paths are Equal: Stimulating Specific Cerebellar Outputs Inhibits Hippocampal Seizures

Gordon F. Buchanan, MD, PhD[1]

Distinct Fastigial Output Channels and Their Impact on Temporal Lobe Seizures

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Despite being canonically considered a motor control structure, the cerebellum is increasingly recognized for important roles in processes beyond this traditional framework, including seizure suppression. Excitatory fastigial neurons project to a large number of downstream targets, and it is unclear whether this broad targeting underlies seizure suppression, or whether a specific output may be sufficient. To address this question, we used the intrahippocampal kainic acid mouse model of temporal lobe epilepsy, male and female animals, and a dual-virus approach to selectively label and manipulate fastigial outputs. We examined fastigial neurons projecting to the superior colliculus, medullary reticular formation, and central lateral nucleus of the thalamus, and found that these comprise largely nonoverlapping populations of neurons that send collaterals to unique sets of additional, somewhat overlapping, thalamic and brainstem regions. We found that neither optogenetic stimulation of superior colliculus nor reticular formation output channels attenuated hippocampal seizures. In contrast, on-demand stimulation of fastigial neurons targeting the central lateral nucleus robustly inhibited seizures. Our results indicate that fastigial control of hippocampal seizures does not require simultaneous modulation of many fastigial output channels. Rather, selective modulation of the fastigial output channel to the central lateral thalamus, specifically, is sufficient for seizure control. More broadly, our data highlight the concept of specific cerebellar output channels, whereby discrete cerebellar nucleus neurons project to specific aggregates of downstream targets, with important consequences for therapeutic interventions.

Commentary

Temporal lobe epilepsy (TLE) is among the most common forms of epilepsy. Seizures in TLE are often refractory to medications.[1] Refractory epilepsies are associated with enormous morbidity and carry a high risk of mortality in the form of sudden unexpected death in epilepsy (SUDEP).[2] Thus, there is a critical need to identify improved treatment options to prevent seizures in TLE to reduce morbidity, mortality, and societal cost.

Refractory epilepsies may be amenable to surgical intervention including resection, if a specific seizure focus can be identified, or various forms of neuromodulation, if the patient is not a candidate for resection.[3] Currently available FDA-approved forms of neuromodulation include vagal nerve stimulation (VNS), responsive neural stimulation (RNS), and deep brain stimulation (DBS), primarily of the anterior nucleus of the thalamus.[3] The regions stimulated with these devices are rather broad. Improved precision in circuits and/or networks stimulated could be desirable to interrupt the seizure generating circuit or network and minimize side effects from stimulating other off-target circuits. In TLE, seizures most often arise from the hippocampus (HPC). The HPC is modulated by a wide range of brain loci, including the cerebellum.[4] The cerebellum has been long thought to be a motor control modulator; however, emerging work demonstrates a broad influence of the cerebellum on many functions and diseases, including epilepsy.[5] Interestingly, though there are no known direct synaptic connections from cerebellum to the HPC,[6] it was previously shown that stimulation of one of the deep cerebellar nuclei, the fastigial nucleus (FN), could stop seizures in a rodent model of TLE.[4]

[1] Neurology, University of Iowa Roy J and Lucille A Carver College of Medicine, Iowa City, IA, USA, USA

Streng, et al set out to determine how FN stimulation could stop TLE seizures despite there being no direct connections between FN and HPC.[7] They hypothesized that anti-seizure effects of FN stimulation are mediated by connections from FN to another intermediary site with direct connections to HPC. They chose three candidate sites to test including superior colliculus (SC), ventral mediodorsal reticular nucleus (MdV), and the central lateral nucleus of the thalamus (CL), each of which impinges upon networks relevant to epilepsy. They were interested in determining whether these sites are targeted by distinct groups of fastigial neurons, whether individual fastigial neurons target multiple sites, and whether targeting any one of these connections is sufficient to stop seizures. They employed AAV-mediated labeling and on-demand optogenetic stimulation in the intrahippocampal kainic acid model of TLE in mice.

For the labeling studies, C57BL/6 mice of both sexes were injected with an adeno-associated virus (AAV) expressing green fluorescent protein (GFP) in neurons and terminals into FN. They identified robust connections between FN and all three sites of interest. Terminals were also labeled in other sites including other thalamic nuclei, other parts of the reticular formation, and periaqueductal gray, among others.

To assess specific circuit connections between FN and the three sites of interest, they injected an AAV allowing retrograde expression of CRE-recombinase into each of the three sites in separate groups of C57BL/6 mice of both sexes. They then injected an AAV allowing CRE-dependent expression of channel rhodopsin (ChR2) conjugated to yellow fluorescent protein (YFP) into FN. This selectively expressed ChR2 in neurons that were connected to each of the three sites. At least two weeks following AAV injections, mice received an intrahippocampal injection of kainic acid to induce epileptogenesis. At least one week after kainic acid injections, mice were implanted with optical fibers into the FN and a bipolar recording electrode into the HPC. At least five days after implants, to allow surgical recovery, and at least six weeks from AAV injection, to allow full viral transfection, mice began HPC local field potential (LFP) recordings to verify that seizures were occurring. With the aid of a closed-loop system that they developed, blue light stimulation was triggered by seizures detected in the HPC. 50% of seizures were targeted in a random fashion, allowing for half unstimulated control seizures. Through this rather elegant paradigm, they found that stimulation of the FN neurons targeting SC or MdV was insufficient to stop seizures; however, stimulation of the FN neurons targeting CL was sufficient to stop seizures. This demonstrates a specific circuit to target for seizure cessation, and also indentifies distinct fastigial output channels.

To assess the sufficiency of FN terminal stimulation in CL to halt seizures, they used two AAV strategies to express ChR2 in terminals. In the first, the FN of C57BL/6 mice was injected with an AAV allowing for expression of ChR2 in all FN neurons. In the other, the FN of VGlut2-Cre (to selectively target excitatory glutamatergic neurons) mice was injected with AAV allowing Cre-dependent expression of ChR2. In both cases, optical fibers were implanted into CL, and light pulses delivered to half of the detected seizures in the same on-demand closed-loop fashion as above. Terminal stimulation within the CL in both cases was sufficient to terminate seizures, suggesting that though there are several collaterals of the FN to CL circuit, the main output to CL plays a significant role in seizure maintenance. Through their detailed analysis the authors also determined that each of these three channels from FN to CL, FN to SC, and FN to MdV constitute separate nonoverlapping populations of neurons within the FN.

This is an intriguing study that demonstrates a distinct fastigial output channel sufficient to halt seizures originating from the HPC. That they were able to do this in a cerebellar nucleus suggests a relatively accessible site for stimulation. While at present it would be easier to stimulate this region more generically, as was done in the authors' earlier study, the added precision will be of great value as improved techniques emerge to better target specific neuron types and output tracks. As they demonstrated, targeting this specific output channel reduced motor side effects of general FN stimulation.[7] Stimulating the projection site in the CL could also be a possibility. CL stimulation is under intense investigation to minimize arousal impairment associated with seizures;[8] and thalamus is the target of stimulation in DBS for epilepsy, albeit in the anterior nucleus.[3] In the future, more could be done to identify specific neuron types within the FN that project to these different locations. The authors targeted excitatory glutamatergic neurons originating in FN that reach CL in at least a portion of this study, but whether other FN neuron types could be involved and which specific neurons within the CL are targeted is yet to be determined. Of course, there is always the question of translatability of optogenetic strategies from rodents to humans, including optimizing methods for gene delivery to express opsins, minimizing possible immune reactions, and ensuring adequate light penetration in human brain, among others.[9] Nevertheless, this group has developed a powerful approach to continue to probe the promising potential of targeting the cerebellum for seizure cessation.

ORCID iD

Gordon F. Buchanan https://orcid.org/0000-0003-2371-4455

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