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Septal Signaling Suppresses Seizures Through Stimulating Somatostatin Cells

Direct Septum-Hippocampus Cholinergic Circuit Attenuates Seizure Through Driving Somatostatin Inhibition

Wang Y, Wang Y, Xu C, Wang S, Tan N, Chen C, Chen L, Wu X, Fei F, Cheng H, Lin W, Qi Y, Chen B, Liang J, Zhao J, Xu Z, Guo Y, Zhang S, Li X, Zhou Y, Duan S, Chen Z. *Biol Psychiatry*. 2020;87(9):843-856. doi:10.1016/j.biopsych.2019.11.014.

Background: Previous studies indicated the involvement of cholinergic neurons in seizure; however, the specific role of the medial septum (MS)–hippocampus cholinergic circuit in temporal lobe epilepsy (TLE) has not yet been completely elucidated. Methods: In the current study, we used magnetic resonance imaging and diffusion tensor imaging to characterize the pathological change of the MS-hippocampus circuit in 42 patients with TLE compared with 22 healthy volunteers. Using optogenetics and chemogenetics, combined with in vivo or in vitro electrophysiology and retrograde rabies virus tracing, we revealed a direct MS-hippocampus cholinergic circuit that potently attenuates seizure through driving somatostatin inhibition in animal TLE models. Results: We found that patients with TLE with hippocampal sclerosis showed a decrease of neuronal fiber connectivity of the MS-hippocampus compared with healthy people. In the mouse TLE model, MS cholinergic neurons ceased firing during hippocampal seizures. Optogenetic and chemogenetic activation of MS cholinergic neurons (but not glutamatergic or GABAergic [γ-aminobutyric acidergic] neurons) significantly attenuated hippocampal seizures, while specific inhibition promoted hippocampal seizures. Electrophysiology combined with modified rabies virus tracing studies showed that direct (but not indirect) MS-hippocampal cholinergic projections mediated the anti-seizure effect by preferentially targeting hippocampal GABAergic neurons. Furthermore, chemogenetic inhibition of hippocampal somatostatin-positive (rather than parvalbumin-positive) subtype of GABAergic neurons reversed the anti-seizure effect of the MS-hippocampus cholinergic circuit, which was mimicked by activating somatostatin-positive neurons. Conclusions: These findings underscore the notable anti-seizure role of the direct cholinergic MS-hippocampus circuit in TLE through driving the downstream somatostatin effector. This may provide a better understanding of the changes of the seizure circuit and the precise spatiotemporal control of epilepsy."

Commentary

In preclinical epileptology, when one thinks of acetylcholine, one likely thinks of pilocarpine. Half of PubMed abstracts that include the keywords acetylcholine (or cholinergic) and epilepsy also contain pilocarpine. This is unsurprising, given the widespread adoption of this muscarinic acetylcholine receptor (mAChR) agonist as a method to evoke status epilepticus and model temporal lobe epilepsy (TLE). Alternatively, one might instead think of soman or other nerve gases which inhibit acetylcholinesterase, or even high dose nicotine, which can produces seizures. On this evidence, one might place cholinergic neurotransmission squarely in the "pro-convulsant" camp.

However, a smaller literature examining the medial septum (MS), the primary source of cholinergic input to the hippocampus, has raised the possibility that cholinergic neurons in this region may *suppress* seizure activity. Immunotoxin-mediated depletion of septal cholinergic neurons facilitates hippocampal kindling,[1] whereas intrahippocampal cholinergic neuron grafts suppress hippocampal kindling.[2] While several studies have demonstrated anti-seizure effects of septal stimulation,[3] the precise role of cholinergic neurons in these effects has remained unclear. For example, both glutamatergic neurons[4] and GABAergic neurons[5] in the MS can drive distal rhythmic activity. Recently, Wang et al[6] have turned to MS cholinergic neurons as a target for the control of seizures. Through selective optogenetic and chemogenetic mapping and modulation of the septo-hippocampal projections, they describe a pathway by which activation of cholinergic, but not glutamatergic or GABAergic septo-hippocampal projections suppressed seizures. They report that MS cholinergic neurons target somatostatin-positive interneurons (SST-INs) in the hippocampus and find that activation of SST-INs is both sufficient to

suppress seizures and necessary for the anti-seizure effects of MS activation.

In the hippocampal kindling model of TLE, the authors examined the profile of cellular activation of MS cholinergic neurons using a combination of fiber photometry and optogenetic identification of single units. Surprisingly, during kindled seizures, the authors reported a suppression of putative cholinergic neuron activity, with little to no change in the firing of other neurons. This pattern differs substantially from the increased septal activity reported by functional magnetic resonance imaging, cerebral blood flow, multiunit activity during hippocampal-evoked seizures in rats,[7] and the striking elevation of hippocampal acetylcholine levels by chemoconvulsants.[8]

The suppression of cholinergic activity during seizures led the authors to hypothesize that activation of these cells might be an effective strategy to suppress seizures. They found that optogenetic activation of cholinergic neurons in the septum increased the number of stimulations needed to produce a fully kindled state and decreased the duration of after discharges during kindling. These effects were mirrored in the intrahippocampal kainite model using designer receptors exclusively activated by designer drugs (DREADDs) to activate cholinergic neurons. Using a within-subject design, they found a decrease in seizure burden during a week of designer drug treatment (clozapine-n-oxide [CNO]) as compared to the baseline (pretreatment) period. Interestingly, this reduction in seizure activity outlasted the duration of CNO treatment, raising the possibility of a disease modifying effect of prolonged cholinergic neuron activation. While the authors show that CNO was ineffective against acute intrahippocampal kainaic acid-evoked seizures in DREADD-negative animals, they did not include similar controls for chronic CNO administration. In this regard, it is noteworthy that CNO can be metabolized to clozapine which in turn is a high affinity antagonist of mAChRs. This is of particular concern with chronic administration of CNO, during which time appreciable concentrations of clozapine may be produced. However, their findings are consistent with the data they obtained using optogenetics. Moreover, antimuscarinic effects of clozapine would likely result in an *underestimation* of chemogenetic efficacy, as they report that focal muscarinic or nicotinic receptor blockade in hippocampus abolished their anti-kindling effect of optogenetic stimulation. In contrast to optogenetic/chemogenetic activation of MS cholinergic neurons, the authors found that either optogenetic inhibition or genetic ablation of these cells sped the kindling process, suggesting that not only can they be activated to suppress seizures, but also consistent with prior studies using immunotoxic lesions,[1] that endogenous activity of these cells plays an anticonvulsant role.

While Wang and colleagues reported reduced EEG power during hippocampal seizures in the presence of optogenetic and chemogenetic activation, they did not report effects on baseline EEG power. This is perhaps a missed opportunity, given that septal cholinergic neurons have been proposed to play a key role in hippocampal θ oscillations.[9] Moreover, activation of septal cholinergic neurons has been reported to disrupt hippocampal ripple oscillations,[10] which are thought to play a central role in memory consolidation. Whether their manipulations altered these "healthy" rhythms, and whether long-term chemogenetic treatment would thus impair memory consolidation and retrieval remain an important consideration.

The MS projects widely throughout the hippocampus—and signaling through muscarinic receptors—can have both excitatory and inhibitory effects. To determine which cell types were the target of MS cholinergic input, Wang et al used transsynaptic viral tracing and found that a higher proportion of GABAergic neurons as compared to glutamatergic neurons received cholinergic input from MS, that activation of MS results in increased firing in a subset of hippocampal INs, and that selective activation of MS neurons that project to hippocampal GABAergic neurons suppresses kindling. There is a diversity of IN populations in HPC, which can be been categorized based on morphology, function, and genetic profiles[11] and have different impacts on pyramidal cell firing. While the authors found that similar proportions of parvalbumin INs (PV-INs) and SST-INs received MS cholinergic input, they found through chemogenetic silencing that SST but not PV INs were necessary for the anti-seizure effects of septal cholinergic neuron activation. This effect is presumably due to SST-mediated inhibition of pyramidal cells, as others have shown that optogenetic activation of septal cholinergic neurons triggers IPSCs on CA1 pyramidal cells.[12] Somewhat paradoxically, it has previously been suggested that inhibition secondary to activation of MS cholinergic neurons is predominantly perisomatic, a form of inhibition that is thought to be mediated by PV, as compared to SST Ins.[11] These data also differ from direct activation of PV or SST cells—optogenetic activation of both cell types has been shown to suppress hippocampal seizure activity in other studies.[13,14] Moreover, while the authors target SST and PV neurons genetically and found clear differences between activating these populations, it is important to note that there can be overlap based on these promoters alone. Thus, the hippocampal microcircuit engaged by MS cholinergic neurons remains understudied and is the next logical gap to be addressed.

These recent findings raise a new potential target for TLE, help clarify the role of the septum in regulating seizure activity, and raise several areas for future direction. First, will activation of septo-hippocampal projections suppress seizures originating in other temporal lobe regions? Second, will activation of cholinergic projections to other ictogenic regions of the temporal lobe suppress seizures? Regional differences in either the local role of acetylcholine could begin to explain the discrepancy between the present findings and the proconvulsant effects reported in other models. Third, SST neurons in the hippocampus consist of multiple populations with different effects on the hippocampal microcircuit. Defining the precise SST neurons responsible for suppressing seizures and, moreover, the particular receptor subtypes mediating the cholinergic effects may reveal new anticonvulsant targets.

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References

1. Ferencz I, Leanzabc G, Nanobashvil A, et al. Septal cholinergic neurons suppress seizure development in hippocampal kindling in rats: comparison with noradrenergic neurons. *Neuroscience*. 2001;102(4):819-832.
2. Ferencz I, Kokaia M, Elmér E, Keep M, Kokaia Z, Lindvall O. Suppression of kindling epileptogenesis in rats by intrahippocampal cholinergic grafts. *Eur J Neurosci*. 1998;10(1):213-220.
3. Miller JW, Turner GM, Gray BC. Anticonvulsant effects of the experimental induction of hippocampal theta activity. *Epilepsy Res*. 1994;18(3):195-204.
4. Robinson J, Frédéric M, Guillaume D, et al. Optogenetic activation of septal glutamatergic neurons drive hippocampal theta rhythms. *J Neurosci Off J Soc Neurosci*. 2016;36(10):3016-3023.
5. Boyce R, Glasgow SD, Williams S, Adamantidis A. Causal evidence for the role of REM sleep theta rhythm in contextual memory consolidation. *Science*. 2016;352(6287):812-816.
6. Wang Y, Yi W, Cenglin X, et al. Direct septum-hippocampus cholinergic circuit attenuates seizure through driving somatostatin inhibition. *Biol Psychiatry*. 2020;87(9):843-856.
7. Englot DJ, Peter KM, Peter H, Fahmeed H, Hal B. Remote effects of focal hippocampal seizures on the rat neocortex. *J. Neurosci*. 2008;28(36):9066-9081.
8. Hillert MH, Imran I, Martina Z, et al. Dynamics of hippocampal acetylcholine release during lithium-pilocarpine-induced status epilepticus in rats. *J Neuro Chem*. 2014;131(1):42-52.
9. Vandecasteele M, Viktor V, Antal B, et al. Optogenetic activation of septal cholinergic neurons suppresses sharp wave ripples and enhances theta oscillations in the hippocampus. *Proc Natl Acad Sci U S A*. 2014;111(37):13535-13540.
10. Ma X, Yiyao Z, Lina W, et al. The firing of theta state-related septal cholinergic neurons disrupt hippocampal ripple oscillations via muscarinic receptors. *J Neurosci*. 2020;40(18):3591-3603.
11. Pelkey KA, Ramesh C, Michael TC, et al. Hippocampal GABAergic inhibitory interneurons. *Physiol Rev*. 2017;97(4):1619-1747.
12. Nagode DA, Tang AH, Karson MA, Klugmann M, Alger BE. Optogenetic release of ACh induces rhythmic bursts of perisomatic IPSCs in hippocampus. *PLoS One*. 2011;6(11):e27691.
13. Ledri M, Madsen MG, Nikitidou L, Kirik D, Kokaia M. Global optogenetic activation of inhibitory interneurons during epileptiform activity. *J Neurosci*. 2014;34(9):3364-3377.
14. Krook-Magnuson E, Armstrong C, Oijala M, Soltesz I. On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy. *Nat Commun*. 2013;4:1376.