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Mossy Cells and Epileptogenesis: From Synaptic Strengthening to Seizures

Seizure-Induced Strengthening of a Recurrent Excitatory Circuit in the Dentate Gyrus Is Proconvulsant

Nasrallah K, Frechou MA, Yoon YJ, Persaud S, Gonçalves JT, Castillo PE. *Proc Natl Acad Sci*. 2022;119(32):e2201151119. doi:10.1073/pnas.2201151119

Epilepsy is a devastating brain disorder for which effective treatments are very limited. There is growing interest in early intervention, which requires a better mechanistic understanding of the early stages of this disorder. While diverse brain insults can lead to epileptic activity, a common cellular mechanism relies on uncontrolled recurrent excitatory activity. In the dentate gyrus, excitatory mossy cells (MCs) project extensively onto granule cells (GCs) throughout the hippocampus, thus establishing a recurrent MC-GC-MC excitatory loop. MCs are implicated in temporal lobe epilepsy, a common form of epilepsy, but their role during initial seizures (i.e., before the characteristic MC loss that occurs in late stages) is unclear. Here, we show that initial seizures acutely induced with an intraperitoneal kainic acid (KA) injection in adult mice, a well-established model that leads to experimental epilepsy, not only increased MC and GC activity in vivo but also triggered a brain-derived neurotrophic factor (BDNF)-dependent long-term potentiation (LTP) at MC-GC excitatory synapses. Moreover, in vivo induction of MC-GC LTP using MC-selective optogenetic stimulation worsened KA-induced seizures. Conversely, Bdnf genetic removal from GCs, which abolishes LTP, and selective MC silencing were both anticonvulsant. Thus, initial seizures are associated with MC-GC synaptic strengthening, which may promote later epileptic activity. Our findings reveal a potential mechanism of epileptogenesis that may help in developing therapeutic strategies for early intervention.

Commentary

A mossy cell (MC) is a type of excitatory neuron that resides in the hilus of the dentate gyrus (DG) of the hippocampus. These cells have elaborate clusters of spines called thorny excrescences on their proximal dendrites which gives them the likeness of a cell covered in moss.[1,2] Mossy cells directly innervate and excite ipsilateral and contralateral dentate granule cells (GC) and are activated by mossy fibers, the axons of the dentate GCs, thereby forming an intrinsic excitatory loop (GC→MC→GC) in the hippocampal DG. Hilar MCs also indirectly inhibit GCs, however, by activating GABAergic neurons to inhibit GC activity (GC→MC→Int→GC). The dual role of MCs in promoting and controlling GC activity makes understanding their function under physiological and pathological conditions complex (for review see studies by Scharfman & Myers[1] and Scharfman[2]). While the exact functions of MCs are not definitively known, these cells have been implicated in the control of adult neural stem cell quiescence and neurogenesis.[2,3] They also contribute to synaptic plasticity and theta rhythm oscillations of hippocampal neurons as well as to spatial memory encoding and spontaneous convulsive seizures.[1,2,4]

The population of hilar MCs is drastically reduced in human temporal lobe epilepsy (TLE). This histopathological feature is widely recapitulated in preclinical models with chronic TLE and is thought to contribute to hyperexcitability within the DG through impairment of feedback inhibition.[1,2] To interrogate their role in the pathophysiology of epilepsy, numerous studies have applied sophisticated pharmaco-, opto-, or chemo-genetic approaches to either suppress or enhance the activity of MCs and measure their impact on GC activity as well as in the extent of electrographic and behavioral seizures in mouse models of TLE.[1,2,4-6] Some findings show that selective activation of remaining MCs in chronically epileptic mice dampens the electrographic seizure activity that precedes and leads to convulsive seizures, while selective inhibition of MCs during a seizure exacerbates the event.[4] This evidence suggests that in neural networks with fully established epilepsy feedback inhibition may be dominant, with remaining MCs exciting inhibitory GABAergic neurons to control GC hyperactivity and reduce/prevent convulsive seizures. In contrast, selective inhibition of MC activity during the induction of status epilepticus (SE) with the chemoconvulsant pilocarpine in an otherwise healthy system showed anticonvulsive effects[5] suggesting that

the excitatory loop is dominant, with MC activation enhancing SE severity. This evidence suggests evolving roles for MCs during SE and in chronic epilepsy.

To further understand how MCs modulate neural activity following an acutely induced seizure, Nasrallah et al determined the extent of synaptic transmission/strength between MCs and GCs along with the impact of brain-derived neurotrophic factor (BDNF) signaling in this process during an episode of SE.[6] The authors utilized viral vectors to deliver and selectively express the G_i inhibitory Designer Receptor Exclusively Activated by a Designer Drug (iDREADD) under the control of the CaMKII promoter in hilar MCs. A series of *in vitro* electrophysiology experiments confirmed a reduction of evoked excitatory postsynaptic currents recorded from GCs following stimulation of MC axons in acute hippocampal slices in the presence of the DREADD agonist clozapine N-oxide (CNO). The study also confirmed that MC inhibition with iDREADD and CNO during SE dampened the severity and susceptibility to behavioral seizures induced with the chemoconvulsant kainic acid. *In vivo* calcium imaging revealed that the temporal progression of calcium waves in MCs preceded that of GCs during SE and *in vitro* electrophysiology supported an increase in synaptic strength between MCs and GCs when a stage of convulsive seizures was reached. This set of initial experiments validated and confirmed previous findings that the direct excitatory loop between MCs and GCs can be effectively manipulated with chemogenetics and that it is altered during SE-induced seizures. Other strengths of this study include the comprehensive assessment of the effectiveness of the tools, approaches, and control groups in the experimental design.

The novel findings of Nasrallah et al support the conclusion that SE-induced increases in MC→GC synaptic strength contribute to increased seizure susceptibility and severity during SE in a BDNF-dependent fashion, suggesting that MC activity is pro-convulsant during SE. To support this conclusion, the authors first established that the induction of presynaptic long-term potentiation (LTP) with electrical or chemical stimulation of the MC axons was blocked in acute hippocampal slices obtained from mice with stage 3 convulsive seizures induced with kainic acid. Impaired LTP can occur when synapses are already maximally potentiated, and likely reflects prior SE-induced enhancement of MC→GC synaptic transmission. The authors next interrogated whether blocking or enhancing LTP within the MC→GC excitatory loop before the administration of kainate could alter the seizure outcome. For this, conditional knockout mice were combined with viral vectors to suppress presynaptic MC→GC LTP by deleting BDNF, which is needed for LTP induction. Conversely, MC→GC LTP was enhanced through optogenetic-mediated stimulation *in vivo*. BDNF deletion in GCs attenuated the SE-induced increases in MC→GC synaptic strength, mitigated the MC→GC LTP deficit observed after SE, and reduced the severity of the kainic acid-induced behavioral seizures. In contrast, optogenetically enhancing MC→GC LTP prior to kainic acid administration aggravated the behavioral seizure phenotype. These findings suggest that that SE-induced MC→GC synaptic strengthening may be proconvulsant early after SE.

A limitation noted in this study is that kainic acid was given at either 20 or 30 mg/kg to mice of different genetic backgrounds. A confounding factor is associated with variable susceptibilities to chemoconvulsant-induced seizures in the different mouse strains, and this in turn may impact the extent of synaptic transmission and potential pathological outcomes. The effects of a dose–response test for kainate along with the use of EEG recordings could help further elucidate whether convulsive or electrographic seizures, or just an increase in neuronal excitability within the DG is sufficient to trigger the MC→GC synaptic strengthening that increases the vulnerability for behavioral seizures. Furthermore, activation of GABAergic cells by MCs may contribute to decreasing GC excitability during a sudden seizure occurring in a normal neural network. However, the contribution of inhibitory neurons was not investigated here.

The take home message of the study by Nasrallah et al is that the initial seizure-induced activation of MCs may trigger excessive synaptic transmission onto GCs that promotes the transition to convulsive seizures in an otherwise normal brain. Nasrallah et al convincingly show that MC→GC strengthening parallels increasing seizure severity in a mouse model of acquired TLE. However, the precipitating molecular events may go beyond those mediated by BDNF signaling. It is also possible that an abrupt seizure induced in an otherwise healthy system may be met by activation of homeostatic mechanisms such as, for example, the Neuron-Restrictive Silencer Factor in excitatory dentate GCs and/or MCs to dampen further excitation of the GC→MC→GC neuronal loop. Furthermore, there is extensive evidence for the activation of homeostatic mechanisms in both normal and epileptic brains.[7] Engagement of homeostatic mechanisms in excitatory cells along with the impact of GABAergic cell activation may be key differences underlying the contrasting pro- and anti-convulsant roles for MCs reported as a result of SE induced in normal brains versus neural networks with fully established epilepsy, respectively. Nevertheless, understanding how MCs contribute to healthy and epileptic networks is still a work in progress that will be advanced as we develop new tools and approaches that allow clean and specific targeting of this interesting and paradoxical cell population.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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