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E-I E-I Woe: Mossy Cell Regulation of Granule Cell Activity in Temporal Lobe Epilepsy

An Excitatory and Epileptogenic Effect of Dentate Gyrus Mossy Cells in a Mouse Model of Epilepsy

Botterill JJ, Lu YL, LaFrancois JJ, Bernstein HL, Alcantara-Gonzalez D, Jain S, Leary P, Scharfman HE. *Cell Rep*. 2019;29(9):2875-2889.e6. doi:10.1016/j.celrep.2019.10.100.

The sparse activity of hippocampal dentate gyrus (DG) granule cells (GCs) is thought to be critical for cognition and behavior, whereas excessive DG activity may contribute to disorders such as temporal lobe epilepsy. Glutamatergic mossy cells (MCs) of the DG are potentially critical to normal and pathological functions of the DG because they can regulate GC activity through innervation of GCs or indirectly through GABAergic neurons. Here, we test the hypothesis that MC excitation of GCs is normally weak, but under pathological conditions, MC excitation of GCs is dramatically strengthened. We show that selectively inhibiting MCs during severe seizures reduced manifestations of those seizures, hippocampal injury, and chronic epilepsy. In contrast, selectively activating MCs was proconvulsant. Mechanistic in vitro studies using optogenetics further demonstrated the unanticipated ability of MC axons to excite GCs under pathological conditions. These results demonstrate an excitatory and epileptogenic effect of MCs in the DG.

Commentary

The dentate gyrus has long been a focus of research seeking to understand temporal lobe epilepsy (TLE). This is in no small part due to the dentate gating hypothesis—the idea that the dentate gyrus "gates" input to the hippocampus to prevent overexcitation.[1,2] The context provided by the dentate gating hypothesis has often led researchers to explore the role of granule cell (GC) function in TLE because GCs are excitatory neurons of the dentate gyrus that provide glutamatergic input to pyramidal neurons of the hippocampal region CA3.[3] Presynaptic to the GCs are excitatory neurons of the entorhinal cortex and other neurons of the dentate—namely mossy cells (MCs) and a subclass of inhibitory interneurons known as basket cells. The relationship between MC activity and GC activity in TLE has been a source of debate, largely due to MCs providing both direct excitatory input and indirect inhibitory input to GCs. Indirect inhibition is mediated via excitation of basket cells, which in turn inhibit GCs.[4] Botterill and colleagues elucidate the relationship between MC activity and GC activity and, in doing so, clarify the role of MC function in epileptogenesis in TLE.

In order to explore the relationship between MC function and epileptogenesis in TLE, Botterill et al first observed the impact of MC inhibition on pilocarpine-induced status epilepticus (SE) in mice. In this model, the induced episode of SE is followed by the development of spontaneous recurrent seizures. Mossy cells were inhibited via the introduction of a viral construct into the dentate gyrus of mice that allowed for targeted expression of an exogenous receptor (iDREADDs) that, in response to an exogenous drug (CNO), would cause inhibition of MCs. These mice and controls, following administration of the drug to inhibit MCs, were then treated with pilocarpine. Mice with inhibited MC activity exhibited decreased spike number and frequency during pilocarpine-induced SE. These mice also experienced decreased numbers of seizures 1 day post-SE, decreased hippocampal damage 3 days post-SE, and decreased seizure occurrence 4 weeks after pilocarpine-induced SE. These data show that MC activity modulates SE severity, hippocampal damage, and epileptogenesis in response to pilocarpine injection. It is also important to emphasize that this effect of MC dynamics is specific for SE. Changes in MC dynamics at other times have differing effects. In a study in epileptic rodents, MC excitation at the onset of milder individual spontaneously occurring seizures mitigated their generalization and the frequency of subsequent seizures, while MC inhibition had the opposite effect.[5] Both studies highlight the significance of MC activity in TLE, but Botterill et al[4] explore how MC activity during the initial SE relates to development of epilepsy, instead of how MC activity relates to individual seizures as they occur.

In order to better contextualize how MC activity mediates epileptogenesis, Botterill et al used slice electrophysiology to

explore the circuit in which these MCs function. Viral injection was used to selectively insert a fluorescently tagged light-sensitive protein (ChR2-eYGP) into MCs, such that transduced MCs would depolarize upon exposure to light of a specific frequency. Recording from GCs following light activation of MCs revealed that under standard recording conditions modest GC activation was observed, but following simulated SE, paroxysmal depolarizing shifts—correlates of epileptiform discharges—were observed. Observed depolarizing shifts following MC activation in simulated SE, however, were not limited to GCs but were also observed in downstream hippocampal structures—CA3, CA1, and the subiculum. Furthermore, paroxysmal depolarizing shifts were not observed when the N-methyl-D-aspartate (NMDA) receptor antagonist DL-APV was introduced, suggesting that generation and propagation of epileptiform activity is NMDA receptor mediated. These findings suggest a circuit mechanism for enhancing excitability resultant from increased MC activity: Increased MC activation causes an NMDA receptor-mediated increase in GC activation which, in turn, propagates through the hippocampus and affects the development and severity of seizures.

This work is significant for contributing to the debate regarding 2 hypotheses that describe the relationship MCs have with GCs in TLE. One hypothesis, the "dormant basket cell" hypothesis, suggests that MC cell loss observed in TLE limits MC excitation of inhibitory interneuron basket cells, which in turn limits inhibition of GCs by these basket cells.[6] This would ultimately result in increased excitation of GCs and, subsequently, the propagation of epileptiform activity through the hippocampus. A diametrically opposed mechanistic hypothesis that proposes the same outcome is the "irritable" MC hypothesis, which suggests that those MCs that remain are more active in TLE and thereby facilitate overexcitation of GCs.[7] Botterill and colleagues suggest that the results support the view that "both hypotheses are partly correct." However, their results do not strongly support the "dormant basket cell" hypothesis. Inhibition of MCs should result in decreased inhibition on GCs by basket cells and might exacerbate the severity of SE and resultant spontaneous seizures. However, the results show that inhibition of MCs instead diminished the severity of SE and resultant seizures. This result doesn't strongly support the role of basket cells as a functional mediator during SE, and the role of these cells requires further study.

This study is really important for understanding MC role in SE. However, there are a few caveats. For one, the use of simulated SE in the slice electrophysiology experiments is an approximation of ongoing seizure activity since it is an in vitro model and can't recapitulate all of the physiological changes from prolonged status. Furthermore, SE is unlikely to be a fixed condition and levels of *gamma*-Aminobutyric acid (GABA) and timing of GABA depletion are likely to influence the exact effect of MC activation, along with development of changes from excitotoxicity. Future experiments exploring the impact of MC activation during development of status on downstream hippocampal circuits in vivo would provide valuable data to understand exactly when the gating function of the GC fails during SE. Given the relevance of MC in TLE, we believe that future experiments exploring other cell types and features of this microcircuit would be important. For example, how MC and basket cell activity are balanced or regulated in order to change GC activity in TLE would provide critical context to the results of this study. Further defining how MC activity becomes strongly excitatory may provide therapeutic targets for the treatment of TLE. Targeting this microcircuit to restore excitatory–inhibitory homeostatic balance may help mediate the severity of TLE.

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References

1. Lothman EW, Stringer JL, Bertram EH. The dentate gyrus as a control point for seizures in the hippocampus and beyond. *Epilepsy Res Suppl*. 1992;7:301-313.
2. Heinemann U, Beck H, Dreier JP, Ficker E, Stabel J, Zhang CL. The dentate gyrus as a regulated gate for the propagation of epileptiform activity. *Epilepsy Res Suppl*. 1992;7:273-280.
3. Scharfman HE. The dentate gyrus and temporal lobe epilepsy: an "exciting" era. *Epilepsy Curr*. 2019;19(4):249-255.
4. Botterill JJ, Lu YL, La Francois JJ, et al. An excitatory and epileptogenic effect of dentate gyrus mossy cells in a mouse model of epilepsy. *Cell Rep*. 2019;29(9):2875-2889. e6. doi:10.1016/j.celrep.2019.10.100.
5. Bui AD, Nguyen TM, Limouse C, et al. Dentate gyrus mossy cells control spontaneous convulsive seizures and spatial memory. *Science*. 2018;359(6377):787-790.
6. Sloviter RS. Permanently altered hippocampal structure, excitability, and inhibition after experimental status epilepticus in the rat: the "dormant basket cell" hypothesis and its possible relevance to temporal lobe epilepsy. *Hippocampus*. 1991;1(1):41-66.
7. Ratzliff AD, Santhakumar V, Howard A, Soltesz I. Mossy cells in epilepsy: rigor mortis or vigor mortis? *Trends Neurosci*. 2002; 25(3):140-144.