



Waking Up the Dormant Dentate Gyrus

Hilar Mossy Cell Degeneration Causes Transient Dentate Granule Cell Hyperexcitability and Impaired Pattern Separation.

Jinde S, Zsiros V, Jiang Z, Nakao K, Pickel J, Kohno K, Belforte JE, Nakazawa K. *Neuron* 2012;76:1189–1200.

Although excitatory mossy cells of the hippocampal hilar region are known to project both to dentate granule cells and to interneurons, it is as yet unclear whether mossy cell activity's net effect on granule cells is excitatory or inhibitory. To explore their influence on dentate excitability and hippocampal function, we generated a conditional transgenic mouse line, using the Cre/loxP system, in which diphtheria toxin receptor was selectively expressed in mossy cells. One week after injecting toxin into this line, mossy cells throughout the longitudinal axis were degenerated extensively, theta wave power of dentate local field potentials increased during exploration, and deficits occurred in contextual discrimination. By contrast, we detected no epileptiform activity, spontaneous behavioral seizures, or mossy-fiber sprouting 5–6 weeks after mossy cell degeneration. These results indicate that the net effect of mossy cell excitation is to inhibit granule cell activity and enable dentate pattern separation.

Commentary

Identification and characterization of the vast variety of hippocampal principal neurons and interneurons are necessary to understand the role that each cell type plays in the complex network alterations leading to epilepsy (1). Over several decades, research has focused on which cells are necessary, sufficient, or extraneous for hippocampal dysfunction often observed in animal models of epilepsy. In particular, temporal lobe epilepsy (TLE) is characterized by the death of many cells of the dentate hilus, and investigators have demonstrated that dentate granule cells (DGC) could be hyperexcitable (2) or hyperinhibited (3). In human TLE, it is also uncertain whether DGC are hyperexcitable (4, 5). A complete understanding of whether—and how—specific cell loss is related to epilepsy remains elusive; in particular, how the dentate gyrus (DG) contributes to epilepsy is uncertain since it is not clear that seizures begin there.

A greater understanding of the role of the DG in epilepsy has been hampered by the inability to selectively delete or ablate specific cell types for selective analysis of the remaining circuitry. In particular, the role of mossy cells has been controversial (6). Mossy cells are glutamatergic hilar neurons that can either excite or inhibit DGC. An excitatory effect occurs via direct activation of DGC by mossy cells (feedback excitation); an inhibitory effect of mossy cells on DGC occurs when mossy cells excite inhibitory hilar interneurons, which in turn inhibit DGC by a “feedforward” mechanism. Owing to their location and physiological functions, hilar mossy cells have been predicted to play a crucial role in the regulation of hippocampal

excitability after excitotoxic insults, such as status epilepticus and traumatic brain injury. Therefore, the consequences of mossy cell loss are key to understanding the pathophysiology of TLE. Possible explanations include the “dormant basket cell hypothesis” (7), which holds that mossy cell death eliminates the feedforward inhibition by disinhibiting granule cells and allows them to fire repetitively (which they do not ordinarily do); as a consequence, there is increased circuit excitability and seizures. Conversely, the “irritable mossy cell hypothesis” (6) posits that mossy cells surviving an insult (such as status epilepticus) provide excessive excitatory innervation to granule cells, leading to their hyperexcitability. Finally, the role of mossy fiber sprouting in epileptogenesis remains uncertain; it is possible that in addition to autoinnervation of granule cells, mossy fibers might also sprout onto mossy cells and interneurons, altering the excitability of the network.

The present study by Jinde and colleagues addresses these hypotheses by a relatively selective ablation of mossy cells (some CA3c cells were killed as well). The investigators used a Cre/loxP system to create a transgenic mouse line in which mossy cells selectively express diphtheria toxin (DT) receptors. Subsequent treatment of these mice with DT caused degeneration of a large percentage of mossy cells (75–90% by their estimation, increasing over time), allowing for investigation of anatomic, physiological, and behavioral correlates in mice with major deficits of this cell type.

First, the authors determined that mossy cells are specifically affected by DT treatment: They assessed cell morphology, degeneration by Fluro-Jade B staining, and mossy cell markers (GluA2/3 and calretinin). Mutant mice were shown to lack mossy cells (but not other cell types) in the dentate hilus by all three markers. The investigators focused on the dorsal hippocampus; details as to mossy cells of the ventral

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hippocampus are less clear, but this information is important because of the interactions of ventral and dorsal regions of the hippocampus and the fact that the dorsal hippocampus is the site of many *in vivo* recordings, including those reported here. Next, electrophysiology was employed to assess the effect of decreased mossy cell input onto granule cells. Effects were divided into acute (5–7 days after DT treatment) and chronic phases (4–6 weeks after DT treatment). In the acute phase, spontaneous inhibitory postsynaptic currents (sIPSCs) were decreased in DGCs, a consequence of mossy cell loss-induced disinhibition. However, there was also a decrease in spontaneous excitatory postsynaptic currents (sEPSCs), which might be predicted to decrease excitability. The net effect of these physiological alterations is uncertain. Further, in the acute phase, perforant path stimulation resulted in enhanced firing of DGCs. These results were interpreted as supporting the notion that the dentate gyrus becomes hyperexcitable and could mediate long-term seizure activity. That said, when tested in the chronic phase, the decreases in sEPSCs and sIPSCs were no longer apparent, suggesting recovery from the transient hyperexcitability. Furthermore, the investigators found no spontaneous recurrent seizures either by visual inspection or with video-EEG monitoring.

Mossy fiber sprouting was not detected in the dentate gyrus, suggesting that mossy fiber sprouting is not an inevitable consequence of mossy cell degeneration and is not required for increased DGC excitability (8). Therefore, loss of hilar mossy cells in this preparation caused transient hyperexcitability of dentate granule neurons but no longstanding epilepsy or sustained physiological evidence of hyperexcitability. Intriguingly, the authors noted the gradual appearance of GAD67-positive fibers from GABAergic interneurons to the inner molecular layer of the dentate. As previously suggested (9), this sprouting of inhibitory fibers might represent a form of compensatory synaptic reorganization that reduces transient hyperexcitability, but seizure generation likely requires additional cellular or circuit alterations.

Lastly, the authors queried whether mossy cell loss caused any behavioral or functional deficits in mutant mice, since the DG has been implicated in a variety of cognitive and behavioral tasks, including pattern separation, anxiety, learning, and memory. They evaluated several hippocampal-dependent behaviors and found impaired context discrimination and increased anxiety during the acute phase only (when DGC excitability was greatest), with resolution of these deficits when the animals were tested in the chronic phase. The role of other hippocampal subfields, such as CA1 and CA3, need to be examined as well. Interestingly, controls and mutants did not differ on contextual fear learning, suggesting that mossy cell loss has specific effects only on certain aspects of cognition. Mutant mice exhibited a transient increase in theta oscillation power during exploratory tasks, which the authors interpret as consistent with the transient increase in DGC excitability—although it is recognized that hippocampal theta rhythms are generated in multiple hippocampal and extrahippocampal structures (10).

Taken together, the authors interpret their results as supporting the dormant basket cell hypothesis, to explain, at least in part, the transient DGC hyperexcitability. Nonetheless, they cannot fully exclude the possibility that surviving mossy cells or other factors could be contributing to dentate excitability in this model. Jinde and colleagues' use of transgenic models, selectively depleting specific cell types, opens new avenues to dissect complex neural circuit activity such as that underlying temporal lobe epilepsy. These findings should awaken investigators to the potential of such novel techniques to examine complex neuronal circuits in epilepsy, allowing correlation from the molecular to the behavioral levels.

by Carl E. Stafstrom, MD, PhD

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