

FUNCTIONAL CONSEQUENCES OF HILAR MOSSY CELL LOSS IN TEMPORAL LOBE EPILEPSY: PROEPILEPTIC OR ANTIEPILEPTIC?

Translamellar Disinhibition in the Rat Hippocampal Dentate Gyrus after Seizure-induced Degeneration of Vulnerable Hilar Neurons

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Longitudinally restricted axonal projections of hippocampal granule cells suggest that transverse segments of the granule cell layer may operate independently (the “lamellar” hypothesis). Longitudinal projections of excitatory hilar mossy cells could be viewed as antithetical to lamellar function, but only if longitudinal impulse flow effectively excites distant granule cells. We therefore determined the effect of focal granule cell discharges on granule cells located more than 2 mm along the longitudinal axis. During perforant pathway stimulation in urethane-anesthetized rats, passive diffusion of the γ -aminobutyric acid (GABA)_A-receptor antagonist bicuculline methiodide, from the tip of a glass recording electrode, evoked granule cell discharges and c-Fos expression in granule cells, mossy cells, and inhibitory interneurons, within an approximate 400- μ m radius. This focally evoked activity powerfully suppressed distant granule cell-evoked responses recorded simultaneously 2.5–4.5 mm longitudinally. Three days after kainic acid-induced status epilepticus or prolonged perforant pathway stimulation, translamellar inhibition was intact in rats with less than 40% hilar neuron loss but was consistently abolished after extensive (>85%) hilar cell loss. Retrograde transport of Fluoro-Gold (FG) from the rostral dentate gyrus revealed that few inhibitory interneurons were among the many retrogradely labeled hilar neurons approximately 2.5 to 4.5 mm longitudinally. Although many somatostatin-positive hilar interneurons effectively transported FG from the distant septum, few of these neurons transported detectable FG from much closer hippocampal injection sites. Inhibitory basket and chandelier cells also exhibited minimal longitudinal FG transport. These findings suggest that translamellar disinhibition may result from the loss of vulnerable, longitudinally projecting mossy cells and may represent

a network-level mechanism underlying postinjury hippocampal dysfunction and epileptic network hyperexcitability.

Rapid Deletion of Mossy Cells Does Not Result in a Hyperexcitable Dentate Gyrus: Implications for Epileptogenesis

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Loss of cells from the hilus of the dentate gyrus is a major histologic hallmark of human temporal lobe epilepsy. Hilar mossy cells, in particular, are thought to show dramatic numeric reductions in pathologic conditions, and one prominent theory of epileptogenesis is based on the assumption that mossy cell loss directly results in granule cell hyperexcitability. However, whether it is the disappearance of hilar mossy cells from the dentate gyrus circuitry after various insults or the subsequent synaptic–cellular alterations (e.g., reactive axonal sprouting) that lead to dentate hyperexcitability has not been rigorously tested, because of the lack of available techniques to remove specific classes of nonprincipal cells rapidly from neuronal networks.

We developed a fast, cell-specific ablation technique that allowed the targeted lesioning of either mossy cells or γ -aminobutyric acid (GABA)ergic interneurons in horizontal as well as axial (longitudinal) slices of the hippocampus. The results demonstrate that mossy cell deletion consistently decreased the excitability of granule cells to perforant path stimulation both within and outside of the lamella where the mossy cell ablation took place. In contrast, ablation of interneurons caused the expected increase in excitability, and control aspirations of the hilar neuropil or of interneurons in the presence of GABA-receptor blockers caused no alteration in granule cell excitability.

These data do not support the hypothesis that loss of mossy cells from the dentate hilus after seizures or traumatic brain injury directly results in hyperexcitability.

COMMENTARY

Understanding how pathologic changes in the hippocampus of epileptic animals contribute to alterations in limbic circuit excitability requires a detailed knowledge of both the anatomy and function of the fundamental components of the neuronal circuit. The two studies discussed in the present commentary address the same question: what is the consequence of loss of hilar mossy cells in the epileptic brain on circuit excitability in the dentate gyrus? Hilar mossy cells are glutamatergic (excitatory) and are among the most injury-prone hippocampal neurons, although this fragility has been questioned in recent studies (1). Because of their propensity for being damaged, mossy cells frequently are lost during injurious stimuli, such as those triggering the development of epilepsy. The functional impact of mossy cell loss has been the subject of much recent controversy. At the center of the controversy are two main, interrelated questions regarding function: (a) does mossy cell innervation of granule cells or interneurons predominate, and (b) does their loss promote more or less excitability in the dentate gyrus?

Loss of hilar neurons is one of the pathologic hallmarks of temporal lobe epilepsy. Many of the missing neurons are hilar mossy cells, which constitute about half of the neurons in the normal hilus and are the main population of excitatory neurons. The remaining 50% of hilar neurons are various populations of γ -aminobutyric acid (GABA)ergic interneurons, subsets of which also may be susceptible to loss in epilepsy. The fact that large numbers of excitatory hilar neurons are lost in the hippocampus of animals with epilepsy would, at first blush, appear to be hard to reconcile with a hyperexcitable state, because loss of excitatory neuron populations would be hypothesized to decrease excitability. A possible solution to this apparent contradiction was proposed by Dr. Robert Sloviter more than a decade ago when he speculated that the primary role of mossy cells is to excite interneurons (2). Therefore mossy cell loss would lead to hypoinnervation of inhibitory cells. Because these cells would no longer be activated appropriately, the decreased input, in turn, would lead to disinhibition. This hypothesis was termed the “dormant basket cell” hypothesis, and the two studies discussed in the present commentary both address aspects of this experimental concept.

The anatomy of mossy cells provides insight into the possible relation between their loss and dentate gyrus excitability. Mossy cells receive excitatory inputs from dentate granule cells and from the entorhinal cortical afferents to the dentate gyrus, the perforant path. They also receive inhibitory inputs from local GABAergic interneurons. Mossy cells send axons for several millimeters along the long (septotemporal) axis of the hippocampus, interconnecting neurons in several of the 600- μ m transverse lamellae that constitute a functional unit important

in hippocampal processing. Serial electron microscopic reconstructions of mossy cell axons demonstrate that these fibers predominantly target the inner molecular layer of adjacent lamellae, with 90% of the synaptic contacts on spines of dentate granule cells (3). These anatomic features support the concept that mossy cells participate principally in a recurrent excitatory loop.

However, anatomic evidence of a synaptic connection does not provide sufficient information to determine the functional impact of that synapse. Although mossy cells primarily innervate other excitatory neurons, they also send a subset of contacts onto inhibitory interneurons. Excitatory inputs onto GABAergic interneurons can be quite powerful, exceeding the efficacy of similar synapses on excitatory neurons. To assess the relative contribution of mossy cells to dentate gyrus circuit excitability requires functional studies, such as those articles discussed here.

The Zappone and Sloviter study used *in vivo* approaches to examine interactions between lamellae (i.e., translamellar interactions) along the septotemporal axis of the hippocampus. The investigators recorded perforant path stimulus-evoked responses at two dentate granule cell layer sites along the long axis of the hippocampus, while using a weeper electrode to infuse the GABA_A antagonist bicuculline locally at the site proximal to the stimulating electrode. This protocol enabled strong, stimulus-evoked local activation of one area of the dentate gyrus, while recording the response to the activation at a second site 2.5 to 4.0 mm away from the proximal site. By using this technique, Zappone and Sloviter demonstrated that strong activation at the proximal site appears to trigger translamellar inhibition at the distant site. For three main reasons, they attributed this inhibition to activation of mossy cells. First, *c-Fos* staining at the proximal, bicuculline-treated, activated site demonstrates a strong signal (corresponding to neuronal activation) in dentate granule cells, hilar interneurons, and mossy cells. Second, the investigators incorporated both retrograde transport studies, using fluorogold (FG) infused at the inhibited site and then examining which cells extend processes to the bicuculline-activated site, as well as double-labeling studies, using FG and GluR2 (labeling mossy cells) or somatostatin (labeling interneurons). The retrograde and double-labeling studies demonstrated that the hippocampal hilar neuronal subtypes with projections 2.5 to 4.0 mm along the long axis (capable of generating the recorded translamellar inhibition) were limited to mossy cells and somatostatin-positive interneurons. A significant majority of the FG-positive neurons were mossy cells, colabeled with GluR2 antibody. Third, the translamellar inhibition was compromised in kainic acid-treated or perforant path-stimulated animals. Both of these manipulations kill hilar neurons, and the degree of loss of inhibition was found to correlate with the extent of hilar cell loss.

How does the Zappone and Sloviter study differ from previous and, apparently, contradictory publications, describing intact translamellar inhibition in kainate-treated epileptic animals with extensive hilar neuron loss (4,5)? A translamellar inhibition study by Buckmaster and Dudek (5) was very similar in design to the Zappone and Sloviter study in that it used two recording electrodes in different lamellae of the dentate gyrus (separated by 1.0 mm) and a perforant path–stimulating electrode, with the proximal recording electrode weeping bicuculline. Several substantial differences are evident, however. First, Zappone and Sloviter examined animals much earlier after kainic acid–induced status epilepticus (3 days), compared with the Buckmaster and Dudek studies (several months). Second, the Zappone and Sloviter study examined translamellar inhibition at much greater distances (2.5–4 mm), compared with the 1.0-mm distance examined in the study by Buckmaster and Dudek. Because the Buckmaster and Dudek study was conducted in animals with significant mossy fiber sprouting, which can extend into the 1.0-mm area and, potentially, restore translamellar inhibition by innervating interneurons, this aspect of the study design introduced a potential confounding variable, which was identified by Zappone and Sloviter. Third, the Zappone and Sloviter study described a threshold effect in which at least 40% of hilar neuronal loss was necessary to see significant translamellar inhibition deficits. It is unclear whether this level of hilar neuronal loss was achieved in all of the animals recorded in the Buckmaster and Dudek or the Buckmaster and Jongen-Relo studies.

Despite these important caveats, the fact remains that the Zappone and Sloviter study arrived at opposite conclusions from the two studies by Buckmaster and colleagues. The Zappone and Sloviter study concluded that mossy cells were mediating translamellar inhibition in the dentate gyrus and that loss of these neurons resulted in reductions in translamellar inhibition. In contrast, Buckmaster and colleagues (4,5) reported intact translamellar inhibition in the dentate gyrus of epileptic animals and attributed this inhibition to surviving GABAergic interneurons in the hilus of epileptic animals.

Although studies such as those described are critical to understanding neuronal inhibition in the dentate gyrus, a significant factor confounding all of the experiments conducted in the epileptic brain is that, in addition to hilar neuronal loss, multiple processes exist at many levels within the circuit that could complicate assessment of the role of mossy cell loss on circuit excitability. In addition to mossy fiber sprouting, changes occur in expression of neurotransmitter receptors in dentate granule cells (including GABA receptors), sprouting of other neurotransmitter fiber systems (including inhibitory fibers), gliosis, birth of new dentate granule cells with potentially aberrant integration into the dentate gyrus circuit, entorhinal cortical damage, and other variables that may contribute to mossy cell loss. Any or all

of these changes could confound studies designed to assess the role of mossy cell loss on circuit excitability, because they could contribute to circuit alterations and covary with the degree of mossy cell loss—both issues being dependent on the severity of the injury generating the epileptic condition.

The Ratzliff et al. (2004) study addressed the question of the role of mossy cell loss in hippocampal function by using innovative methods in hippocampal slices from nonepileptic animals. In this experimental approach, mossy cells were first selectively labeled with a fluorescent dye injected into the contralateral hippocampus *in vivo*. After labeling, animals were killed, hippocampal slices were prepared, and the identified mossy cells were then ablated by using an aspiration technique and patch electrodes. The authors reported that the net effect of the mossy cell deletion was to decrease excitability. Perforant path–evoked fields within a given hippocampal lamella are significantly smaller after mossy cell ablation. This finding argues against a disinhibitory role produced by the loss of mossy cells. The data support the idea that mossy cells provide a recurrent excitatory projection, and their deletion decreases granule cell excitability.

This concept is supported in additional studies by Ratzliff et al., which demonstrate that reduced dentate granule cell excitability, after mossy cell ablation, persists in the presence of GABA antagonists, ruling out the possibility that the effect is generated by inhibitory connectivity. The authors go on to assess the effects of mossy cell loss on translamellar inhibition, by using a novel axial hippocampal slice that preserves several interconnected hippocampal lamellae in the same *in vitro* preparation. The investigators ablated mossy cells in one lamella and report that the perforant stimulation–evoked dentate granule cell responses in adjacent lamellae were reduced, demonstrating a net decrease in excitability. Again, this is a finding consistent with the notion that mossy cells provide a recurrent excitatory projection, both within and between hippocampal lamellae.

Clearly, the critical aspect of the Ratzliff et al. study is that mossy cell ablation effects on dentate granule cell excitability are assessed in the absence of the whole constellation of other potentially confounding changes associated with brain injury and the subsequent development of epilepsy. Potentially weak aspects are that (a) the deletion of mossy cells requires extensive manipulation of slices and transfer between chambers, which could seriously compromise slice health and confound analysis; and (b) only a small subset (roughly 5% to 20%, depending on various assumptions) of mossy cells are acutely ablated in these studies.

However, Ratzliff et al. clearly were aware of viability issues and ran extensive sets of controls to assess slice health and slice transfer effects. Sham handling or aspiration of hilar neuropil did not alter granule cell excitability, and aspiration of

equivalent numbers of visually identified interneurons resulted in significant increases in dentate granule cell excitability. Deterioration of slices because of manipulations should not be trimodal, with decreases, no effect, and increases in excitability evident in response to mossy cell, neuropil, and interneuron ablation, respectively. Ratzliff et al. adequately controlled for the possibility that deterioration of the slices could compromise the specificity of their results. However, the Zappone and Sloviter study reported a significant threshold effect for translamellar inhibition compromise. They saw little effect of hilar neuron cell loss on translamellar inhibition until the magnitude of depletion exceeded 40%. So the 5% to 20% loss generated in the Ratzliff et al. study may have been insufficient to see some of the effects described by Zappone and Sloviter.

Given the contradictory findings, questions remain as to the role of mossy cells in dentate gyrus circuit function in the normal brain and the degree and consequences of loss of these neurons on dentate granule cell excitability in many forms of pathology. How far do these neurons project? What is the functional consequence of activity of mossy cells on dentate circuit excitability? What is their role in translamellar processing in the hippocampus? The dentate gyrus is a critical regulator of limbic

excitability. Better understanding of the mechanisms controlling its excitability provide a more complete understanding of epileptogenic processes and could lead to improved therapeutic strategies for temporal lobe epilepsy.

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References

1. Ratzliff AH, Santhakumar V, Howard A, Soltesz I. Mossy cells in epilepsy: rigor mortis or vigor mortis? *Trends Neurosci* 2002;25:140–144.
2. Sloviter RS. The functional organization of the hippocampal dentate gyrus and its relevance to the pathogenesis of temporal lobe epilepsy. *Ann Neurol* 1994;35:640–654.
3. Buckmaster PS, Wenzel HJ, Kunkel DD, Schwartzkroin PA. Axon arbors and synaptic connections of hippocampal mossy cells in the rat in vivo. *J Comp Neurol* 1996;366:271–292.
4. Buckmaster PS, Jongen-Relo AL. Highly specific neuron loss preserves lateral inhibitory circuits in the dentate gyrus of kainate-induced epileptic rats. *J Neurosci* 1999;19:9519–9529.
5. Buckmaster PS, Dudek FE. Network properties of the dentate gyrus in epileptic rats with hilar neuron loss and granule cell axon reorganization. *J Neurophysiol* 1997;77:2685–2696.