

## Current Literature

In Basic Science



# Hot Spots Light Up the Recurrent Excitation Hypothesis of Temporal Lobe Epilepsy

## Increased Excitatory Synaptic Input to Granule Cells From Hilar and CA3 Regions in a Rat Model of Temporal Lobe Epilepsy.

Zhang W, Huguenard JR, Buckmaster PS. *J Neurosci* 2012;32:1183–1196.

One potential mechanism of temporal lobe epilepsy is recurrent excitation of dentate granule cells through aberrant sprouting of their axons (mossy fibers), which is found in many patients and animal models. However, correlations between the extent of mossy fiber sprouting and seizure frequency are weak. Additional potential sources of granule cell recurrent excitation that would not have been detected by markers of mossy fiber sprouting in previous studies include surviving mossy cells and proximal CA3 pyramidal cells. To test those possibilities in hippocampal slices from epileptic pilocarpine-treated rats, laser-scanning glutamate uncaging was used to randomly and focally activate neurons in the granule cell layer, hilus, and proximal CA3 pyramidal cell layer while measuring evoked EPSCs in normotopic granule cells. Consistent with mossy fiber sprouting, a higher proportion of glutamate-uncaging spots in the granule cell layer evoked EPSCs in epileptic rats compared with controls. In addition, stimulation spots in the hilus and proximal CA3 pyramidal cell layer were more likely to evoke EPSCs in epileptic rats, despite significant neuron loss in those regions. Furthermore, synaptic strength of recurrent excitatory inputs to granule cells from CA3 pyramidal cells and other granule cells was increased in epileptic rats. These findings reveal substantial levels of excessive, recurrent, excitatory synaptic input to granule cells from neurons in the hilus and proximal CA3 field. The aberrant development of these additional positive-feedback circuits might contribute to epileptogenesis in temporal lobe epilepsy.

### Commentary

Development of acquired temporal lobe epilepsy (TLE) is accompanied by selective cell loss and reorganization of excitatory synaptic circuits in key brain regions. Of particular research focus, the axons of granule cells in the dentate gyrus (i.e., mossy fibers) have long been known to sprout collaterals and form new excitatory synapses with other granule cells after an epileptogenic insult. Thus, so-called “mossy fiber sprouting” (MFS) is associated with the formation of new, recurrent, excitatory circuits amongst granule cells, capable of supporting seizure-like activity in the dentate gyrus. The longstanding hypothesis that MFS and synaptic reorganization in the dentate gyrus serve as a potential substrate for seizure generation or propagation is intuitive and attractive to many research scientists. However, efforts to correlate the degree of MFS with seizure frequency have met with mixed results and more often than not have been inconclusive. Moreover, the loss of large numbers of hilar neurons and CA3 pyramidal cells—the typical targets of mossy fibers—during TLE acquisition complicate understanding of the functional significance of MFS as it relates to hippocampal excitability. Among the hypotheses to explain

the lack of a strong relationship between inner molecular layer MFS and seizure susceptibility include the concepts that 1) the relationship is nonlinear (i.e., some quantitatively or spatially relevant “threshold” for new connections might be necessary to support seizure-like activity); 2) newly sprouted axons form synapses with inhibitory neurons, activation of which serves to shunt some excitatory activity expressed after epilepsy development; 3) mossy fiber reorganization occurs in the hilus (e.g. onto ectopic granule cells or granule cell hilar basal dendrites); and 4) axon sprouting occurs in neurons other than granule cells, contributing cumulatively to the positive feedback circuit that may underlie seizures. These hypotheses are not mutually exclusive, and credible results consistent with many of these hypotheses have been published. The study by Zhang et al. extends previous work showing that granule cells make new connections with other granule cells in the pilocarpine-treated rat model of TLE by identifying and mapping new recurrent excitatory connections onto granule cells arising from CA3 and hilar regions.

Normally, granule cells are sparsely interconnected, and occasional projections from CA3 pyramidal cells to the inner molecular layer of the dentate gyrus have been shown (1). Excitatory hilar mossy cells project to granule cells, but their axons tend to project to septotemporally distant hippocampal levels and are thus not prominent in terms of local connections (2). Axon sprouting in any of these cell types after epi-

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leptogenic lesions could contribute to circuit reorganization during epileptogenesis (3–5). Whereas granule cells have been shown to make new excitatory synaptic connections in several models of TLE, the contribution of putatively sprouted CA3 or hilar neurons to granule cell excitability has not been as carefully documented. Using patch-clamp recordings in hippocampal slices, changes in synaptic input to granule cells associated with TLE development can be examined with high resolution. Electrical stimulation methods can be used to examine these changes, but this technique activates intact neurons as well as axons of passage, leaving uncertainty regarding the spatial nature of the activated afferent. Glutamate application depolarizes the somatodendritic domain of intact cells in the slice and can be used to assess more directly the hypothesis that neurons within the slice form new functional connections after TLE development. Focal, photolytic uncaging of glutamate in the granule cell layer has demonstrated the presence of robust recurrent excitatory connections between granule cells associated with MFS and epilepsy development in several models (6–8). Yet, these studies and others have only hinted at the possibility of recurrent excitation to the dentate gyrus arising from other cell groups.

Using high-resolution laser-scanning glutamate photolysis technology to activate surviving CA3 pyramidal and hilar neurons, Zhang et al. were able to activate neurons at multiple, discrete locations within the slice and identify synaptic connections with recorded granule cells with great spatial and temporal resolution. Of immense value to the interpretation of their results is the care with which control experiments were conducted to demonstrate that responses resulted from direct synaptic activation of granule cells following glutamate stimulation of other regions. Stimulation site “hot spots” were located within the slice, where glutamate photolysis activated CA3 and hilar neurons, resulting in direct, excitatory synaptic input to individual granule cells in slices from epileptic rats. Granule cell-to-granule cell connections were also robust. Similar connections were rarely observed in controls. Ectopic granule cells in the hilus, which appear in both animal models of TLE and in patients, received inputs arising from granule cells and CA3 pyramids, implying that they too may participate in the recurrent circuit reorganization. In addition to increased recurrent connection ratios, the synaptic strength of identified inputs to granule cells was greater in the epileptic group. This occurred despite a dramatic reduction in excitatory pyramidal cell and hilar neuron density during epileptogenesis, suggesting a critical role as contributors to the novel circuit for the surviving neurons. These findings provide important support for the hypothesis that the functional recurrent excitatory circuitry that forms in the dentate gyrus during epileptogenesis also includes new and robust inputs from glutamatergic neurons located in both the CA3 pyramidal layer and hilus.

Epilepsy-related hilar neuron and CA3 pyramidal cell axon sprouting and synaptic reorganization have been suggested previously (3, 5, 9), consistent with the hypothesis that recurrent excitation can arise from hippocampal regions outside the dentate gyrus. The findings by Zhang et al.,

which demonstrate new and robust synaptic connections to granule cells from CA3 and hilar neurons, are consistent with the hypothesis that axon sprouting and new recurrent circuit formation associated with TLE are not limited to mossy fibers. Glutamatergic hippocampal neurons, recruited into the synaptically reorganized dentate gyrus circuit during epileptogenesis, may help form the functional, positive-feedback excitatory circuitry hypothesized to participate in epileptiform activity in the dentate gyrus, irrespective of MFS. Axons of principal neurons in other hippocampal regions also sprout and form new excitatory connections outside the dentate gyrus and thus may also participate in the excessive, recurrent excitatory circuit that develops regionally in association with epileptogenesis. Axon sprouting and synaptic reorganization in principal neurons throughout the hippocampus, and likely other cortical areas, may help explain the apparent “mismatch” between seizure frequency and MFS density. Functional roles for these new circuits in modulating other neuron types, as well as the degree of reorganization required to support spontaneous seizure development in epilepsy, are yet to be determined. The findings of Zhang et al. provide important support for the hypothesis that, in addition to MFS, widespread formation of recurrent excitatory circuits may contribute to TLE and other chronic epilepsies.

by Bret N. Smith, PhD

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