

SPROUTED MOSSY FIBERS FORM PRIMARILY EXCITATORY CONNECTIONS

Axon Sprouting in a Model of Temporal Lobe Epilepsy Creates a Predominantly Excitatory Feedback Circuit

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The most common type of epilepsy in adults is temporal lobe epilepsy. After epileptogenic injuries, dentate granule cell axons (mossy fibers) sprout and form new synaptic connections. Whether this synaptic reorganization strengthens recurrent inhibitory circuits or forms a novel recurrent excitatory circuit is unresolved. We labeled individual granule cells *in vivo*, reconstructed sprouted mossy fibers at the electron-microscopic (EM) level, and identified postsynaptic targets with γ -aminobutyric acid (GABA) immunocytochemistry in the pilocarpine model of temporal lobe epilepsy. Granule cells projected an average of 1.0 and 1.1 mm of axon into the granule cell and molecular layers, respectively. Axons formed an average of one synapse every 7 μ m in the granule cell layer and every 3 μ m in the molecular layer. Most synapses were with spines (76 and 98% in the granule cell and molecular layers, respectively). Almost all of the synapses were with GABA-negative structures (93 and 96% in the granule cell and molecular layers, respectively). By integrating light microscopic and EM data, we estimate that sprouted mossy fibers form an average of >500 new synapses per granule cell, but <25 of the new synapses are with GABAergic interneurons. These findings suggest that almost all of the synapses formed by mossy fibers in the granule cell and molecular layers are with other granule cells; therefore after epileptogenic treatments that kill hilar mossy cells, mossy fiber sprouting does not simply replace one recurrent excitatory circuit with another. Rather, it replaces a distally distributed and disynaptic excitatory feedback circuit with one that is local and monosynaptic.

COMMENTARY

The experimental finding that axons of hippocampal dentate granule cells, termed mossy fibers, sprout and innervate aberrant targets in the hippocampus of animals and humans with temporal lobe epilepsy has been known for more than a decade. A Medline search of the terms “sprouting and epilepsy” results in >290 hits dating back to 1983. Despite (or perhaps because of) this extensive body of literature, there is considerable controversy concerning the consequences of this synaptic reorganization within the dentate gyrus. This controversy breaks down into primarily two camps, centered on two basic questions. Does mossy fiber axon reorganization create a recurrent excitatory pathway and contribute to seizure initiation and/or propagation? Alternatively, do these axons innervate primarily inhibitory interneurons, enhance inhibition in the dentate gyrus, and potentially resist seizure initiation and propagation?

Two primary experimental methods are capable of addressing this question: functional approaches (patch-clamp and intracellular recording studies), and anatomic approaches (electron microscopy; EM). The present study adopted the latter approach, conducting a quantitative EM study examining the innervation targets of nine hippocampal dentate granule cells labeled by using intracellular electrodes *in vivo* in six epileptic rats.

The authors present several lines of evidence supporting the hypothesis that sprouted mossy fibers create predominantly a recurrent excitatory pathway. First, sprouted mossy fibers primarily synapse with dendritic spines in the molecular layer of the dentate gyrus (93% of synapses), consistent with a recurrent excitatory pathway. Excitatory neurons (i.e., granule cells) tend to receive excitatory input on dendritic spines, whereas most, but not all, inhibitory neurons lack spines and tend to receive excitatory input on dendritic shafts. To exclude extensive innervation of spiny interneurons as a potential caveat in their data, the authors examined tissue immunostained for γ -aminobutyric acid (GABA). From this set of studies, the authors conclude that most (93–97%) of synapses formed by sprouted mossy fibers in the molecular and cell body layer of the dentate gyrus were with GABA-negative postsynaptic targets. The authors further calculated that each sprouted axon formed >500 new synapses with aberrant targets in the gran-

ule cell and molecular layer, and that >95% of these synapses were with GABA-negative targets.

Clearly, these data argue that aberrant recurrent connectivity is primarily excitatory in the dentate gyrus of epileptic animals. Given the role of the dentate gyrus as a gateway for inputs into the hippocampus, and also that its normal function is to filter this information, these changes could certainly contribute to enhanced excitability in the hippocampus of animals and humans with epilepsy. Given this strong anatomic evidence, several questions remain. Why is it so difficult to see these connections functionally? Recordings from dentate gyrus *in vitro* have clearly demonstrated examples in which synchro-

nous bursting occurs in normal medium. However, these are somewhat rare, and finding synchronous bursting usually requires manipulating conditions to highlight collateral function, such as blocking inhibition, or increasing extracellular potassium concentrations. Are other compensatory mechanisms in place restricting the potential of these recurrent collaterals to synchronize dentate granule cells? These questions will require further functional studies, focused on the synaptic and circuit function of the dentate gyrus in animals and humans with temporal lobe epilepsy.

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