

MOSSY FIBER SPROUTING AND RECURRENT EXCITATION: DIRECT ELECTROPHYSIOLOGIC EVIDENCE AND POTENTIAL IMPLICATIONS

Electrophysiological Evidence of Monosynaptic Excitatory Transmission between Granule Cells after Seizure-induced Mossy Fiber Sprouting

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Mossy fiber sprouting is a form of synaptic reorganization in the dentate gyrus that occurs in human temporal lobe epilepsy and animal models of epilepsy. The axons of dentate gyrus granule cells, called mossy fibers, develop collaterals that grow into an abnormal location, the inner third of the dentate gyrus molecular layer. Electron microscopy has shown that sprouted fibers form synapses on both spines and dendritic shafts in the inner molecular layer, which are likely to represent the dendrites of granule cells and inhibitory neurons. One of the controversies about this phenomenon is whether mossy fiber sprouting contributes to seizures by forming novel recurrent excitatory circuits among granule cells. To date, a great deal of indirect evidence suggests that this is the case, but counterarguments exist. The purpose of this study was to determine whether functional monosynaptic connections exist between granule cells after mossy fiber sprouting. By using simultaneous recordings from granule cells, we obtained direct evidence that granule cells in epileptic rats have monosynaptic excitatory connections with other granule cells. Such connections were not obtained when age-matched, saline control rats were examined. The results suggest that indeed mossy fiber sprouting provides a substrate for monosynaptic recurrent excitation among granule cells in the dentate gyrus. Interestingly, the characteristics of the excitatory connections that were found indicate that the pathway is only weakly excitatory. These characteristics may contribute to the empirical observation that the sprouted dentate gyrus does not normally generate epileptiform discharges.

COMMENTARY

Mossy fiber sprouting has been the subject of extensive research and controversy since it was first described two decades ago. The hypotheses concerning this phenomenon are based largely on evidence derived from Timm staining of the inner molecular layer of the dentate gyrus in humans and animal models that have undergone injury-induced epileptogenesis. The primary hypothesis has been that neuronal injury leads to the formation of new axon collaterals of dentate granule cells (i.e., mossy fiber sprouting), which form recurrent excitatory synaptic connections with other granule cells whose proximal dendrites are in the inner molecular layer (1,2). Other proposed hypotheses are that new excitatory synapses are formed with dormant basket cells, although only the pyramidal-shaped basket cells have dendrites in the inner molecular layer (3), and that mossy fibers in the inner molecular layer arise from newly born granule cells (i.e., neurogenesis), rather than sprouting of existing granule cells (4,5). The present article by Scharfman et al. provides the most direct evidence, to date, to support the hypothesis that mossy fiber sprouting, after status epilepticus and during the subsequent epileptogenesis, leads to formation of recurrent excitatory circuits among dentate granule cells.

Probably the most valuable aspect of the studies by Scharfman et al. is their use of dual intracellular recording, which permits a *direct* analysis of the synaptic connections. Initial electrophysiologic experiments on mossy fiber sprouting used electrical stimulation of the hilus (1,6). This approach is considered *indirect* because, although it would provide antidromic activation of the mossy fibers, such stimulation could also activate afferent fibers from other areas. Recent studies using focal photoactivation of caged glutamate in the granule cell layer have provided additional evidence for connections between granule cells (7,8). Unlike electrical stimulation, this technique would not be expected to evoke action potentials in fibers of passage. By stimulating one intracellularly recorded granule cell, while simultaneously recording from another granule cell, Scharfman et al. now have provided the strongest direct evidence to date of formation of recurrent excitatory circuits associated with mossy fiber sprouting.

Another issue addressed by these experiments is the strength of the synaptic connections. Scharfman et al. reported

that the amplitude of the monosynaptic excitatory postsynaptic potentials (EPSPs) appeared small, and the percentage of demonstrated connections seemed low, which, at the surface, implies that the mossy fiber–mediated recurrent excitatory system is weak. The electrophysiologic data of Scharfman et al. from the dentate gyrus of pilocarpine-treated rats, however, are quite similar to the experimental results of Miles and Wong (9,10) from the normal CA3 area that showed a monosynaptic connection in 7 (1.75%) of 400 and 8 (1.6%) of 500 pairs of CA3 pyramidal cells, with an EPSP amplitude of about 1 to 2 mV and a transmission failure rate of up to 23%. Thus the recurrent excitatory circuits demonstrated by Scharfman et al. are roughly comparable in synaptic strength and density of connections to the CA3 area, where recurrent excitation is thought to play an important role in all-or-none epileptiform bursting when GABA_A receptor–mediated inhibition is blocked pharmacologically.

Most recent studies on the electrophysiologic consequences of mossy fiber sprouting have used pharmacologic (6,11) or ionic treatments (12–14) to unmask hypothetical abnormalities. The rationale is that GABA_A receptor–mediated inhibition prevents synchronous bursting by interrupting the propagation of activity from one granule cell to another within an interconnected neuronal network. Traub and Wong (15) provided the theoretical basis for how recurrent excitation mediates network bursting in the CA3 area when GABA_A receptor–mediated inhibition is depressed. Miles and Wong (9,10) then used dual intracellular recordings to show how divergence and multisynaptic interactions, via recurrent excitation, could lead to epileptiform activity. In fact, Miles and Wong directly showed that polysynaptic interactions occur when inhibition is depressed and that GABA_A receptor–mediated inhibition blocks or shunts the positive feedback effects of recurrent excitation. Furthermore, for positive feedback from recurrent excitation to recruit the granule cell population, the recurrent excitatory circuits must actually cause the postsynaptic neurons to fire action potentials. Granule cells are known to have a very negative resting potential and a high threshold, and thus single EPSPs do not generally reach the threshold for action-potential initiation. Both the initial evidence from studies with hilar stimulation by Tauck and Nadler (1) and recent studies using photoactivation of caged glutamate (7,8) have provided evidence for recurrent excitatory connections in normal medium, without the requirement of unmasking, although the dual intracellular experiments of Scharfman et al. are the most direct.

Another hypothesis that has been proposed is that dentate granule cells become GABAergic after repeated seizures (16,17), as would occur after the pilocarpine-induced status epilepticus used in the studies of Scharfman et al. Evidence for inhibitory synaptic connections was found in the recordings of Scharfman et al.; however, based on the latency between the presynap-

tic action potentials and the inhibitory postsynaptic potentials (IPSPs), these inhibitory connections were disynaptic—apparently mediated by an intercalated interneuron. The paired recordings thus demonstrate monosynaptic excitatory connections under conditions in which inhibitory connections clearly would have been detectable; therefore these dual recordings also provide evidence *against* the hypothesis that dentate granule cells become GABAergic and release GABA at their synapses. It remains possible, however, that the dentate granule cells release GABA nonsynaptically or at their synapses under conditions other than the ones studied by Scharfman et al.

The studies of Scharfman et al. have focused on the hypothesis of new, recurrent *excitatory* circuits, and these particular experiments do not directly address the opposing hypothesis of new, recurrent *inhibitory* circuits (18). Three independent ultrastructural studies, one on human tissue resected for the treatment of intractable temporal lobe epilepsy (19) and two others on pilocarpine-treated rats with spontaneous seizures (20,21), argue that most if not all new synapses from sprouted mossy fibers in the inner molecular layer connect directly to granule cells rather than to interneurons. The recent quantitative electron microscopic studies of Buckmaster et al. (21,22), in particular, reported that 93% to 97% of the new excitatory synaptic connections from mossy fibers in the inner molecular layer were onto non-GABAergic targets (i.e., dentate granule cells). Because ultrastructural data suggest that some mossy fibers in normal animals make connections to the dendrites of GABAergic interneurons in the inner molecular layer (23), it is possible that none of the sprouted mossy fibers project to interneurons. Nearly all of the previous studies (18) supporting the recurrent inhibitory circuits hypothesis are based on light microscopic data, which does not have the structural resolution to detect actual synaptic connections, or on field-potential recordings during repetitive paired-pulse stimulation in intact animals, which lacks the electrophysiologic resolution to identify a cellular mechanism—unlike the experiments of Scharfman et al. that provide direct evidence for the mechanism of new, recurrent excitatory circuits. Future studies, using quantitative electron microscopy and intracellular or whole-cell recordings from basket cells and other interneurons, will be necessary to test more directly the hypothesis that sprouted mossy fibers form new connections to GABAergic neurons.

Is the dentate gyrus unique in regard to seizure-induced axonal sprouting, and is this structure critical to epileptogenesis? Several lines of indirect evidence for axonal sprouting and new, recurrent excitation are available in the CA1 area (e.g., 24–28), suggesting that synaptic reorganization could occur in many other temporal lobe areas. Several experiments with undercut neocortex, a potential model of posttraumatic brain injury, suggest that chronic injury leads to axonal sprouting of pyramidal cells and new excitatory circuits (29,30). Although

evidence points to the importance of the hippocampus, particularly the hilus, the classic histopathologic work of Margerison and Corsellis (31) clearly indicated that many temporal lobe structures are damaged in a variable manner in temporal lobe epilepsy. The late Pierre Gloor (32) eloquently and effectively emphasized that other areas, such as the amygdala, may be more important than the hippocampus. Future experiments should determine whether other areas show axonal sprouting and new, recurrent excitatory circuits after injury.

Are the new, recurrent excitatory circuits epileptogenic? If epileptogenicity is simply defined as hyperexcitability in normal medium, then maybe they are not. However, this mechanism of axonal sprouting and new, recurrent excitatory circuits potentially contributes to epileptogenesis without directly leading to hyperexcitability under resting conditions in normal medium. The fundamental nature of positive feedback systems is that they become unstable and explosive—even when individual interactions are weak. Many mechanistic components may have to be in register for increased recurrent excitation to contribute to seizure generation or epileptogenesis. Other issues contribute to the overall process of epileptogenesis (33), including depression of GABA-mediated inhibition as well as various other mechanisms. The early modeling work of Traub and Wong (15) suggested that intrinsic burst-generating mechanisms are important for propagation of seizure-like events through the CA3 pyramidal cell network, and recent studies with the pilocarpine model have suggested that upregulation of T-type calcium-channel genes and calcium-dependent intrinsic bursting in the CA1 area contributes to long-lasting alterations in repetitive firing during epileptogenesis (34–36), although this has yet to be demonstrated in the dentate granule cells.

An understanding of why and how seizures occur in the epileptic brain and why people with epilepsy are usually *not* having seizures is needed. The concept that epileptogenesis simply causes hyperexcitability and that hyperexcitability is equivalent to epileptogenesis does not adequately address these questions. New hypotheses are most certainly needed.

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