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One Seizure Please, Hold the Sprouts: The Role of Hippocampal Mossy Fiber Sprouting in Epilepsy

Early Detonation by Sprouted Mossy Fibers Enables Aberrant Dentate Network Activity

Hendricks WD, Westbrook GL, Schnell E. *Proc Natl Acad Sci U S A*. 2019;116(22):10994-10999. doi:10.1073/pnas.1821227116. Epub 2019 May 13. PMID: 31085654.

In temporal lobe epilepsy, sprouting of hippocampal mossy fiber axons onto dentate granule cell dendrites creates a recurrent excitatory network. However, unlike mossy fibers projecting to CA3, sprouted mossy fiber synapses depress upon repetitive activation. Thus, despite their proximal location, relatively large presynaptic terminals, and ability to excite target neurons, the impact of sprouted mossy fiber synapses on hippocampal hyperexcitability is unclear. We find that despite their short-term depression, single episodes of sprouted mossy fiber activation in hippocampal slices initiated bursts of recurrent polysynaptic excitation. Consistent with a contribution to network hyperexcitability, optogenetic activation of sprouted mossy fibers reliably triggered action potential firing in postsynaptic dentate granule cells after single light pulses. This pattern resulted in a shift in network recruitment dynamics to an "early detonation" mode and an increased probability of release compared with mossy fiber synapses in CA3. A lack of tonic adenosine-mediated inhibition contributed to the higher probability of glutamate release, thus facilitating reverberant circuit activity.

Commentary

When scientists first began looking at neurons under the microscope, they did what humans are good at doing; namely, they began sorting what they saw into categories. When classifying neurons in the hippocampus, Ramon y Cajal noted that certain cells and nearby axons had messy outgrowths resembling moss.[1] As hippocampal mossy cells have been reviewed previously,[2] the remainder of this commentary will focus on the separate entity of *mossy fibers*. Mossy fibers are the axons originating from dentate granule cells and are morphologically distinct from other neuronal axons in that they possess a variety of synaptic terminals.[3] Mossy fibers form synapses onto neurons of the CA3 region of the hippocampus, a connection mediated by glutamate. The mossy fiber-CA3 synapse is a "detonator synapse," which means it robustly and reliably activates postsynaptic CA3 neurons; however, this detonation is conditional insofar a brief burst of presynaptic activity is required for expression.[4] In contrast, the more prevalent CNS connection is the nondetonator synapse, which has a low success rate of triggering spikes in postsynaptic targets, and often requires teamwork (from neighboring synapses) to evoke a robust postsynaptic response.[5]

In response to injury, new connections grow out from mossy fibers, a process called *mossy fiber sprouting* (Figure 1A). The extent to which these new synaptic connections contribute to the epileptogenic process remains hotly debated,[6] namely, the question remains whether the newly sprouted excitatory synapses form recurrent, hyperexcitable networks among dentate granule cells capable of driving seizures. Indeed, Heng et al discovered, in 2013, that pharmacologically blocking mossy fiber sprouting with rapamycin did not alter seizure frequency in the pilocarpine-induced mouse model of temporal lobe epilepsy.[7]

Regardless of their involvement in epilepsy, isolating and interrogating sprouted synapses have been difficult. This challenge was overcome in 2017 by using tamoxifen to temporally express channelrhodopsin and a reporter gene specifically in dentate granule cells and then provoking mossy fiber sprouting by inducing seizures with pilocarpine.[8] This approach yielded a population of sprouted dentate granule cells labeled with a fluorescent protein that could be optogenetically activated. Repeated, optogenetic stimulation of putative sprouted mossy fibers led to progressively smaller postsynaptic responses in dentate granule cells, a phenomenon called *synaptic depression*.

But what exactly does it mean to have a depressing, mossy fiber synapse? The authors of the current study now address this question. One important parameter governing synaptic transmission is the probability that the presynaptic neuron releases its pool of neurotransmitter-containing vesicles

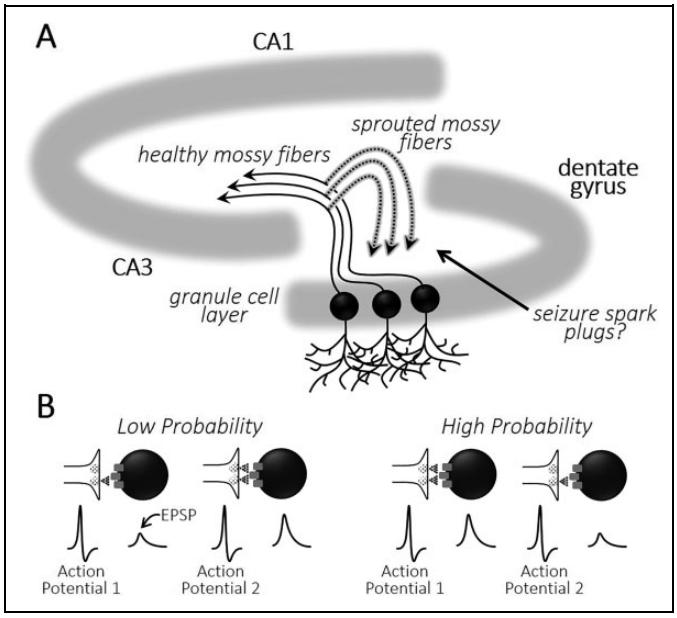

Figure 1. Sprouted mossy fibers release glutamate with a high probability. A, Schematic of hippocampal structures including the dentate gyrus, CA3, and CA1. Mossy fibers in the healthy brain correspond to the axons originating from glutamatergic dentate granule cells located within the granule cell layer of the dentate gyrus. Healthy mossy fibers innervate CA3 pyramidal cells. Many humans with temporal lobe epilepsy—and the animals used to model the disorder—exhibit sprouted mossy fibers. Sprouted mossy fibers correspond to aberrant, recurrent axons originating from dentate granule cells that return to the dentate gyrus. B, Low versus high neurotransmitter release probability. Calcium-dependent release of neurotransmitter is probabilistic. *Left.* Relatively little neurotransmitter is released at neuronal connections characterized by a low probability of release, and therefore, postsynaptic responses (eg, excitatory postsynaptic potentials, EPSPs) are relatively small. However, if a second presynaptic action potential occurs with a sufficiently brief latency after the first action potential, then the postsynaptic response is often larger (ie, *facilitated*). The larger, second response is often attributed to (1) ample neurotransmitter remaining in the presynaptic neuron and (2) an accumulation of presynaptic calcium. *Right.* Plenty of neurotransmitter is initially released at connections characterized by a high release probability, and therefore, postsynaptic responses are relatively large. As neurotransmitter pools are depleted, subsequent responses become smaller (ie, *depressed*).

(Figure 1B). A presynaptic neuron with a high release probability will discharge much of its neurotransmitter upon action potential-mediated depolarization of the terminal. As a consequence, less transmitter is available for subsequent terminal activations, and postsynaptic responses are smaller.[9] In contrast, the postsynaptic responses at a low-release probability synapse generally become larger during repeated stimulation (ie, *synaptic facilitation*). The normal mossy fiber-CA3 synapse is characterized by a low probability of release, and only after brief repetitive stimulation can it robustly excite the postsynaptic cell.[4]

The authors of the study highlighted herein evaluated the release probability associated with the aberrant, mossy fiber-dentate granule cell synapses and discovered that sprouted mossy fibers release glutamate with a high probability. Thus, the authors suggest that sprouted mossy fibers serve as "spark plugs" that reliably activate (ie, detonate) recurrent excitatory networks within the dentate gyrus. Using an approach similar to their aforementioned 2017 study, Hendricks et al (2019) selectively delivered the light-activated channelrhodopsin to granule cells possessing sprouted mossy fibers in mice subjected to pilocarpine-induced status epilepticus (SE). Because channelrhodopsin delivery was not fully effective, not every granule cell expressed the light-activated channel or its associated fluorescent protein reporter (ie, yellow fluorescent protein); thus, while some of the granule cells in the dentate gyrus were activatable by light, others were not, thereby permitting the recording of postsynaptic responses uncontaminated by action potentials.

The authors found that the optogenetic stimulation of granule cells from brain slices obtained from SE-mice reliably produce responses in neighboring granule cells—both single spikes and epileptiform bursts—whereas the same procedure in control mice failed to trigger any activity in dentate granule cells. The authors next applied brief trains of light pulses to their slice preparations to evaluate the release probability of sprouted synapses. Consistent with previous work, mossy fibers synapsing onto CA3 exhibited robust facilitation to repeated stimuli (with a low-release probability), while sprouted mossy fibers synapsing onto granule cells exhibited rapid depression due to a high-release probability. In fact, the release probability was sufficiently high in the sprouted mossy fibers to reliably trigger action potentials at the start of the stimulus train. Moreover, the single, evoked action potential in the sprouted mossy fiber preparation was often sufficient to generate bursts of activity in SE-derived hippocampal slices. Thus, the authors coined the term *early detonation* to distinguish such rapid and robust activation from synapse detonation requiring an initial burst of presynaptic activity, as occurs at the healthy mossy fiber-CA3 synapse (see above).

The authors next speculated that the high-release probability occurring at mossy fiber synapses could be accounted for by changes in adenosine modulation of the synapse. Under normal circumstances, extracellular adenosine, a byproduct of neuronal ATP-driven vesicle release, establishes an inhibitory tone on synaptic terminals expressing the A1 adenosine receptor and provides negative feedback to active synapses.[10] Interestingly, when the authors delivered an adenosine receptor antagonist to sprouted mossy fiber slices from the brains of epileptic mice, they saw no effect on the evoked postsynaptic current. Thus, they concluded that the sprouted mossy fiber synapse lacks the normal inhibitory tone mediated by adenosine, thereby enabling a higher release probability.

Although these data support the hypothesis that sprouted mossy fibers produce synapses with a high-release probability, thereby placing the aberrant outgrowths in a solid position to reliably drive recurrent seizure activity, the authors recognize the limitations of translating observations found in acute brain slices to seizures occurring in the intact brain. One compelling future test of their model includes determining whether

selectively activating sprouted mossy fibers with light can trigger a seizure in the whole animal. Other, perhaps larger, questions also remain. As highlighted above, pharmacologically blocking mossy fiber sprouting does not alter seizure frequency in mouse models.[7] Perhaps sprouting alters other features of seizures besides the frequency. Alternatively, perhaps seizures in the pilocarpine model rely less heavily on sprouting. Regardless, it is noteworthy that temporal lobe resection effectively treats 65% of humans with temporal lobe epilepsy, yet over half of those treated patients still require at least 1 anticonvulsant to achieve seizure freedom.[11] Thus, if an exuberantly sprouted dentate gyrus was the sole cause of temporal lobe epilepsy, then resection should be more curative, not simply provide less pharmacoresistance. Until more discoveries are made, we must wait to learn if indeed it is all about the sprout.

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