

EVIDENCE AGAINST A PATHOGENIC ROLE FOR MOSSY FIBER SPROUTING

Is mossy fiber sprouting present at the time of the first spontaneous seizures in rat experimental temporal lobe epilepsy?

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PURPOSE: The contribution of mossy fiber sprouting to the generation of spontaneous seizures in the epileptic brain is under dispute. This study addressed this question by examining whether sprouting of mossy fibers is present at the time of appearance of the first spontaneous seizures in rats, and whether all animals with increased sprouting have spontaneous seizures.

METHODS: Epileptogenesis was induced in 16 rats by electrically stimulating the lateral nucleus of the amygdala for 20–30 min until the rats developed self-sustained status epilepticus (SSSE). During and after SSSE, rats were monitored in the long term with continuous video-EEG until they developed a second spontaneous seizure (8–54 days). Thereafter, monitoring was continued for 11 days to follow up seizure frequency. The density of mossy fiber sprouting was analyzed from Timm-stained preparations. The density of hilar neurons was assessed from thionine-stained sections.

RESULTS: Of 16 rats, epilepsy developed in 14. In epileptic rats, the density of mossy fiber sprouting did not correlate with the severity or duration (115–620 min) of SSSE, delay from SSSE to occurrence of first (8–51 days) or second (8–54 days) spontaneous seizure, or time from SSSE to perfusion (20–63 days). In the temporal end of the hippocampus, the sprouting correlated with the severity of neuronal damage (ipsilateral: $r = -0.852$, $p < 0.01$; contralateral: $r = -0.748$, $p < 0.01$). The two animals without spontaneous seizures also had sprouting. Increased density of sprouting in animals without seizures and its association with the severity of neuronal loss was confirmed in another series of 30 stimulated rats that were followed up with video-EEG monitoring for 60 days.

CONCLUSIONS: Our data indicate that although mossy fiber sprouting is present in all animals with spontaneous seizures, its presence is not necessarily associated with the occurrence of spontaneous seizures.

COMMENTARY

Mossy fibers are the axons that arise from hippocampal dentate gyrus granule cells that normally innervate hilar neurons and the apical dendrites of CA3 pyramidal cells. It has been demonstrated in clinical cases and in vivo experimental models of temporal lobe epilepsy that mossy fibers sprout and show aberrant innervation of granule cell dendrites and CA3 pyramidal neuron basal dendrites. Anatomic and physiologic studies have shown that these sprouted abnormal afferent pathways form functional synapses with their targets. Several lines of evidence suggest that seizure frequency may have an association with the density of sprouting, and other studies have shown that pretreatment maneuvers that reduce sprouting likewise can prevent or delay epileptogenesis. Whether such interventions interfered with the sprouting process directly, as opposed to the antecedent steps necessary for sprouting to occur, is unclear, and accordingly, a cause-and-effect relation cannot be construed. Consequently, the contribution of mossy fiber sprouting to the development of spontaneous seizures remains controversial. Other work shows that the sprouting and epileptogenesis can be distinguished, such that protein-synthesis inhibitors that prevent sprouting have no effect on epileptogenesis. Sprouting onto inhibitory interneurons, which likewise occurs, may even protect against developing seizures. Suffice it to say that ample studies exist supporting both sides of the argument over the relation between sprouting and epileptogenesis.

In this setting, the authors proposed to study whether spontaneous seizures occur only in animals with mossy fiber sprouting, and whether the degree of sprouting correlates with seizure frequency. Epileptogenesis was induced in rats by electrical stimulation of the lateral amygdala nucleus for 20–30 min until the rats developed self-sustained status epilepticus (SSSE). During and after this procedure, rats were monitored

by using continuous video-EEG until they had a second spontaneous seizure. Monitoring continued for 11 days, and seizure frequency was ascertained. Sprouting density was then assessed in Timm-stained sections (to reveal mossy fiber distribution) for correlation with seizure frequency. Cell counts of hilar neurons assessed the extent of neuronal damage. No correlations were seen between the density of mossy fiber sprouting and the severity and duration of the SSSE, or delay from SSSE to occurrence of first or second spontaneous seizure. Sprouting was correlated with neuronal loss at the temporal end of hippocampus, as well as with seizure frequency. However, robust sprouting was still seen in the animals that did not develop seizures. These results were confirmed in another series of animals followed up for 60 days.

In sum, whereas sprouting was seen in all animals developing epilepsy, not all animals demonstrating sprouting developed chronic seizures. The density of sprouting was found not to correlate with sprouting when this was analyzed in the long-term animals. Although correlation was seen at the earlier time, the authors argued this was a kind of “sampling error,” in that the seizure frequency may not have stabilized. Thus the role of sprouting in epileptogenesis is as yet not completely resolved. This article clearly demonstrates that sprouting per se does not ensure that seizures will develop. Conversely, there are certainly

scenarios in which sprouting, with overgrowth of excitatory synapses, could certainly bias the system toward hyperexcitability, contributing to seizure generation. Whether sprouting is the obligate component seems unlikely, given the results of this study.

Sprouting has only relatively recently been considered in the epilepsy field. However, sprouting in mammalian limbic structures after damage to various intrinsic and extrinsic pathways has been known for decades (from the work of Raisman, Cotman and Lynch, and others). Although these studies demonstrated robust reinnervation of denervated territories, seizures were not obviously detected in the experimental animals; therefore not all sprouting is associated with epileptogenesis. Sprouting may be triggered separate from the epileptogenic process and may be secondary to it, rather than causal. Sprouting, synonymous with reactive synaptogenesis, is a process postulated to be responsible for recovery of function after brain damage (and there is ample evidence in support of this). Therefore a more fruitful approach to preventing epileptogenesis would seem to be to focus on defining the true mechanisms of epileptogenesis, as opposed to targeting the sprouting process, which indeed could be involved in recovery of function after neuronal damage.

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