

THE HYPERINHIBITION HYPOTHESIS IN EPILEPTOGENESIS: AN ASSESSMENT OF THE EVIDENCE

Hippocampal Granule Cell Activity and *c-fos* Expression During Spontaneous Seizures in Awake, Chronically Epileptic, Pilocarpine-treated Rats: Implications for Hippocampal Epileptogenesis

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J Comp Neurol 2005;488(4):442–463

The process of postinjury hippocampal epileptogenesis may involve gradually developing dentate granule cell hyperexcitability caused by neuron loss and synaptic reorganization. We tested this hypothesis by repeatedly assessing granule cell excitability after pilocarpine-induced status epilepticus (SE) and monitoring granule cell behavior during 235 spontaneous seizures in awake, chronically implanted rats. During the first week post-SE, granule cells exhibited diminished paired-pulse suppression and decreased seizure discharge thresholds in response to afferent stimulation. Spontaneous seizures often began during the first week after SE, recruited granule cell discharges that followed behavioral seizure onsets, and evoked *c-fos* expression in all hippocampal neurons. Paired-pulse suppression and epileptiform discharge thresholds increased gradually after SE, eventually becoming abnormally elevated. In the chronic epileptic state, interictal granule cell hyperinhibition extended to the ictal state; granule

cells did not discharge synchronously before any of 191 chronic seizures. Instead, granule cells generated only low-frequency voltage fluctuations (presumed “field excitatory postsynaptic potentials”) during 89% of chronic seizures. Granule cell epileptiform discharges were recruited during 11% of spontaneous seizures, but these occurred only at the end of each behavioral seizure. Hippocampal *c-fos* after chronic seizures was expressed primarily by inhibitory interneurons. Thus, granule cells became progressively less excitable, rather than hyperexcitable, as mossy fiber sprouting progressed and did not initiate the spontaneous behavioral seizures. These findings raise doubts about dentate granule cells as a source of spontaneous seizures in rats subjected to prolonged SE and suggest that dentate gyrus neuron loss and mossy fiber sprouting are not primary epileptogenic mechanisms in this animal model.

COMMENTARY

The dentate gyrus has traditionally been regarded as a gateway to the hippocampus and has attracted considerable attention in epilepsy research. The dentate undergoes relatively consistent and substantive pathological alterations in temporal lobe epilepsy, such as neuronal loss in the hilus and synaptic reorganization accompanying mossy fiber sprouting, which occur in both human tissue and animal models. Sloviter has proposed that hilar neuron loss in the dentate gyrus is a critical component of temporal lobe epilepsy, because it is consistently associated with mesial temporal sclerosis and leads to a loss of excitatory input to basket cell interneurons (1).

Many *in vitro* studies from several laboratories support the hypothesis that mossy fiber sprouting in the dentate gyrus forms

a positive-feedback network among granule cells, although this process occurs in the presence of synaptic inhibition (2). In contrast, after a period of hyperexcitability associated with neuronal loss from kainate-induced status epilepticus, Sloviter previously reported that mossy fiber sprouting leads to progressive hyperinhibition of the dentate gyrus (3). Harvey and Sloviter replicate these data with pilocarpine-treated rats, and also report that (i) spontaneous recurrent electrographic and behavioral seizures have few if any population spikes (i.e., synchronous action potentials) in the dentate gyrus and (ii) the seizures are associated with a lack of *c-fos* staining of dentate granule cells, while GABAergic interneurons show intense *c-fos* staining. Expression of *c-fos* has generally been interpreted to represent increased electrical activity, although the details of the underlying mechanism remain unclear. Replicating the results from repetitive paired-pulse stimulation experiments from this laboratory, the authors conclude that the effect of mossy fiber sprouting is to enhance synaptic GABAergic inhibition of granule cells. Thus, the authors argued that interneurons and not granule cells become active during hippocampal seizures, which they believe

accounts for the failure of population spikes to occur during seizures and the presence of c-fos staining of interneurons and not of granule cells after a seizure.

Hyperexcitability and hyperinhibition were assessed by repeated paired stimulation of the perforant path. An increase in the second response in relation to the first was taken to indicate hyperexcitability, whereas the reverse indicated hyperinhibition. The observed hyperinhibition is attributed to enhanced GABAergic inhibition resulting from sprouting of excitatory connections to GABAergic interneurons. Although the authors state that “responses to paired-pulse stimulation do not provide direct measures of synaptic GABA-mediated inhibition,” the interpretations are based on the view that repetitive paired-pulse suppression is equivalent to GABA-mediated inhibition. Increased paired-pulse suppression, however, does not necessarily indicate enhanced GABA-mediated inhibition since other alterations in dentate gyrus circuits and granule cell electrophysiological properties could cause the same effect. Furthermore, the results from this approach likely depend on several parameters, such as stimulus intensity. More direct approaches are necessary for confirmation.

Harvey and Sloviter report that during chronic behavioral seizures, the dentate gyrus did not consistently generate population spikes. Although the presence of large population spikes indicates synchronous firing of action potentials, the failure to record population spikes does not show that action potentials have not occurred within the granule cell network. In a focal kainate model, chronic multichannel recordings of field potentials revealed that fast oscillations, which are small population spikes, are one of the earliest events in the generation of electrographic seizures, although these potentials may only occur in limited locations (4). Furthermore, neurons may fire action potentials that are not obviously synchronized, but nonetheless may contribute to seizure generation. For example, recent experiments by Bower and Buckmaster using single-unit recording of electrophysiologically identified granule cells and interneurons in the dorsal hippocampus of freely behaving animals with pilocarpine-induced epilepsy have shown that granule cell action potentials start to occur before the actual seizures and before the presence of population spikes (5). Thus, although the presence of population spikes may be a useful indicator of synchronized action potentials in hippocampal structures, the failure to detect population spikes is not evidence for a lack of action potential activity in a hippocampal network. In addition, Harvey and Sloviter did not evaluate the ventral hippocampus—an area more likely to be the site of seizure generation than the dorsal hippocampus.

The authors report that interneurons, but not dentate granule cells, show c-fos staining after dentate seizures, which appears consistent with the hypothesis that seizures do not ac-

tivate the granule cells because of hyperinhibition. By contrast, a recent study in a mouse model of pilocarpine-induced epilepsy reported that Fos expression was high in granule cells, was strongest 15–30 minutes after a seizure, and decayed with time after a seizure (6). These investigators also demonstrated that staining of interneurons was most robust 1–2 hours after a seizure when Fos in granule cells was fading. Harvey and Sloviter measured c-fos 1 hour after the seizures. It presently is unclear whether these differences are due to the animal model, the postseizure time at which c-fos was studied, or other technical factors.

In summary, the data of Harvey and Sloviter are provocative; however, it should be emphasized that the dentate gyrus provides a *model* for epileptogenesis, but it is not the *only* place that epileptogenesis may occur. On the basis of several independent lines of evidence, Bertram and coworkers have proposed that a more broadly based limbic network may be responsible for the generation and synchronization of seizures in temporal lobe epilepsy (7,8). This view suggests that mechanistic alterations within and possibly among many limbic structures (of which the dentate gyrus is only one) should be considered (9). The concept that hilar neuron loss and other alterations in the dentate gyrus are critical in temporal lobe epilepsy (1) has been challenged from many perspectives. Although the properties of spontaneous seizures are best studied in vivo with freely behaving animals, other experimental techniques (e.g., whole-cell and single-channel recordings with in vitro experiments) allow investigations that more directly test specific hypotheses concerning synaptic inhibition and excitation at the circuit and receptor levels. These experiments will need to be performed not only in the dentate gyrus but also elsewhere in the limbic system.

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References

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