

ACTIVATION OF FOS DURING SPONTANEOUS HIPPOCAMPAL SEIZURES IN A MODEL OF TEMPORAL LOBE EPILEPSY

Temporal Patterns of Fos Expression in the Dentate Gyrus after Spontaneous Seizures in a Mouse Model of Temporal Lobe Epilepsy

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Identifying the brain regions and neuronal cell types that become active at the time of spontaneous seizures remains an important challenge for epilepsy research, and the involvement of dentate granule cells in early seizure events continues to be debated. Although Fos expression is commonly used to evaluate patterns of neuronal activation, there have been few studies of Fos localization after spontaneous seizures. Thus, in a pilocarpine model of recurrent seizures in C57BL/6 mice, Fos expression was examined at multiple time points after spontaneous seizures to follow the temporal and spatial patterns of Fos activation. By 15 minutes after the beginning of a spontaneous behavioral seizure, Fos labeling was evident in dentate granule cells. This labeling was particularly striking because of its wide extent and relatively uniform

appearance in the granule cell layer. At later time points, from 30 minutes to 4 hours after a spontaneous seizure, Fos labeling was also detected in interneurons within the dentate gyrus and in widespread regions of the temporal lobe. Interestingly, the timing of Fos activation appeared to differ among different types of GABAergic interneurons in the dentate gyrus, with labeling of parvalbumin neurons along the base of the granule cell layer preceding that of GABA neurons in the molecular layer. The findings in this mouse model are consistent with previous suggestions that spontaneous seizures in temporal lobe epilepsy may result from a periodic breakdown of the normal filter functions of the dentate gyrus and a resulting increase in hypersynchronous activity of dentate granule cells.

COMMENTARY

The dentate gyrus continues to attract the attention of neuroscience researchers as being an important structure that serves as a “gate” to the hippocampus and is thought to be involved in the initiation and/or propagation of seizures in temporal lobe epilepsy. The dentate gyrus in the normal brain is an enigma, because it is considered to have a high threshold for electrophysiological activation. However, histopathological changes in the dentate that occur with temporal lobe epilepsy have led to the theory that it might become hyperexcitable and, thus, be involved in the time-dependent process of epileptogenesis.

Over 20 years ago, *in vitro* electrophysiological studies on hippocampal slices from normal animals established that the dentate gyrus has a high threshold for generation of epileptiform activity (1), which probably stems from the relatively negative resting membrane potential of dentate granule cells, their high degree of spike accommodation during maintained depolarizations, and strong GABAergic inhibition. Consistent with

these early observations, *in vivo* studies showed that the dentate gyrus is relatively resistant to electrically induced seizure activity, which is part of the basis for viewing the normal dentate as a closed gate, blocking seizures from entering the hippocampus (2). On these grounds, one would expect that the dentate gyrus would be inexcitable during acute seizures, at least when the normal brain is challenged with convulsant drugs or repetitive electrical stimulations. An important issue, however, is whether changes occur in the chronically epileptic brain. The present authors have provided evidence for expression of Fos (the protein product of the gene *c-fos*) in dentate granule cells within 15 minutes of the onset of spontaneous behavioral seizures in mice with pilocarpine-induced epilepsy, strongly suggesting that the dentate is prone to electrical activation during epileptic seizures.

Recently, Harvey and Sloviter (3,4) reported that interneurons but not dentate granule cells show Fos (referred to as “c-fos” by the authors) staining after chronic spontaneous seizures in rats with pilocarpine-induced epilepsy (5). The finding contradicts the hypothesis that the dentate gyrus is important in controlling temporal lobe epilepsy. The data of Harvey and Sloviter (3) support a hypothesis that maintains that the dentate gyrus undergoes time-dependent hyperinhibition. This hypothesis, in turn, arises from the previous proposal that mossy

fiber sprouting leads to the enhancement of recurrent inhibitory circuits rather than recurrent excitation (6). Thus, Harvey and Sloviter (3) argue that the granule cells are not activated during spontaneous seizures, because mossy fiber sprouting leads to enhanced inhibition. By contrast, numerous laboratories have provided evidence that mossy fiber sprouting leads to recurrent excitatory circuits (7). The present study by Peng and Houser, using a mouse model of pilocarpine-induced epilepsy, reports that Fos expression was high in granule cells after spontaneous behavioral seizures, was most intense at 15–30 minutes after a seizure, and decayed with time after a seizure; staining of interneurons was most robust 1–2 hours after a seizure (i.e., when Fos in granule cells was fading). Harvey and Sloviter (3) measured Fos 1 hour after the seizures in rats occurred—a time when Peng and Houser suggest Fos activity in mice would be decreasing in granule cells and increasing in interneurons. It is presently unclear whether these differences are due to the animal model, the postseizure time at which Fos was studied, or other technical factors.

The concept of sequential Fos activation, based on the observation that granule cells showed Fos activation before interneurons, is potentially quite complex. Because normal dentate granule cells are relatively inexcitable compared with most interneurons, which generally have a lower threshold for action potential generation and less accommodation than granule cells, one might expect many of the interneurons to fire more intensely and in a more prolonged manner than granule cells during the strong depolarizations typical of seizure activity. Even with mossy fiber sprouting and new recurrent excitatory circuits as well as the loss of a fraction of the GABAergic interneurons, it is quite possible that dentate granule cells do not initiate seizures. It may be that electrographic seizures are propagated from the entorhinal cortex to the dentate gyrus and that the perforant path may more quickly and effectively activate dentate granule cells than inhibitory interneurons, although this issue remains to be addressed with electrophysiological techniques. The problem is that both Fos staining and field-potential recordings lack the resolution needed to answer definitively the specific question of which type of neurons are activated at what time during a seizure; that is, Fos staining lacks temporal resolution while field-potential recordings lack cellular resolution to differentiate the time course of the relative activation of granule cells versus interneurons. Thus, the question ultimately requires single-cell recording from electrophysiologically identified neurons in the dentate gyrus during a spontaneous seizure.

The Fos-based analysis of neuronal activation by Peng and Houser suggests that parvalbumin interneurons along the base of the granule cell layer are activated before GABAergic neu-

rons in the molecular layer. According to these data, if the temporal sequence of Fos activation is related to the sequence of electrical activation, recurrent inhibitory circuits involving parvalbumin interneurons would be activated before the interneurons in the molecular layer that are thought to mediate feed-forward inhibition. As the authors point out, however, it is difficult to determine whether the sequence of the Fos activation actually reflects the sequence of electrical activation. The anticipated time course of electrical activation would be expected to be in the order of milliseconds to seconds, while the expression of Fos protein is manifest in the range of tens of minutes to an hour or two. The time course of Fos activation may represent the degree to which neurons ultimately undergo intense seizure-induced depolarization (possibly with depolarization inactivation), which could be earlier in dentate granule cells than interneurons. The degree and time course of Fos activation also could represent other mechanisms that are not directly related to electrophysiological activation, such as intracellular signaling cascades, gene transcription, and protein translation. Regardless of precisely how Fos activation is to be interpreted, these studies begin to address the important question of what occurs in the chronically epileptic brain during a spontaneous seizure. The authors show activation of Fos in dentate granule cells soon after the onset of spontaneous seizures in the pilocarpine model of temporal lobe epilepsy, which suggests that the dentate is electrically activated during epileptic seizures.

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