

AXON SPROUTING AND SYNAPTIC REORGANIZATION OF GABAERGIC INTERNEURONS: A FOCUSED LOOK AT A GENERAL QUESTION

Surviving Hilar Somatostatin Interneurons Enlarge, Sprout Axons, and Form New Synapses with Granule Cells in a Mouse Model of Temporal Lobe Epilepsy. Zhang W, Yamawaki R, Wen X, Uhl J, Diaz J, Prince DA, Buckmaster PS. *J Neurosci* 2009;29(45):14247–14256. In temporal lobe epilepsy, seizures initiate in or near the hippocampus, which frequently displays loss of neurons, including inhibitory interneurons. It is unclear whether surviving interneurons function normally, are impaired, or develop compensatory mechanisms. We evaluated GABAergic interneurons in the hilus of the dentate gyrus of epileptic pilocarpine-treated GIN mice, specifically a subpopulation of somatostatin interneurons that expresses enhanced green fluorescence protein (GFP). GFP-immunocytochemistry and stereological analyses revealed substantial loss of GFP-positive hilar neurons (GPHNs) but increased GFP-positive axon length per dentate gyrus in epileptic mice. Individual biocytin-labeled GPHNs in hippocampal slices from epileptic mice also had larger somata, more axon in the molecular layer, and longer dendrites than controls. Dual whole-cell patch recording was used to test for monosynaptic connections from hilar GPHNs to granule cells. Unitary inhibitory postsynaptic currents (uIPSCs) recorded in control and epileptic mice had similar average rise times, amplitudes, charge transfers, and decay times. However, the probability of finding monosynaptically connected pairs and evoking uIPSCs was 2.6 times higher in epileptic mice compared to controls. Together, these findings suggest that surviving hilar somatostatin interneurons enlarge, extend dendrites, sprout axon collaterals in the molecular layer, and form new synapses with granule cells. These epilepsy-related changes in cellular morphology and connectivity may be mechanisms for surviving hilar interneurons to inhibit more granule cells and compensate for the loss of vulnerable interneurons.

COMMENTARY

The role of axonal sprouting and synaptic reorganization in acquired epileptogenesis, particularly temporal lobe epilepsy, has garnered considerable attention for over a quarter century. Interest in this issue derived originally from the observation of Timm stain in the inner molecular layer of the dentate gyrus (i.e., mossy fiber sprouting) from patients with and animal models of temporal lobe epilepsy and mesial temporal sclerosis. Most research has focused on recurrent excitation (1,2), and only a few studies have used cellular techniques to test the hypothesis that synaptic reorganization also occurs within the GABAergic inhibitory system.

Several reports, based on electrophysiological field-potential recordings with the paired-pulse technique and qualitative light-microscopic observations, have proposed that synaptic reorganization occurs in the GABAergic system and that this reorganization leads to *increased* GABAergic inhibition in animal models of temporal lobe epilepsy (3). Two types of synaptic reorganization within GABAergic circuits have been proposed. One hypothesis is that principal cell axon collaterals (e.g., the mossy fibers of the granule cells) enhance their con-

nectivity to GABAergic interneurons (3). A second hypothesis, the basis for the experiments by Zhang et al. considered here, is that the GABAergic interneurons sprout axon collaterals and increase their synaptic connections back to the principal cells (4). Both of these hypothetical forms of synaptic reorganization would be expected to augment the efficacy of local GABAergic circuits during acquired epileptogenesis and would generally be seen as compensatory. The present study by Zhang and coworkers uses converging anatomic and electrophysiological techniques at the cellular level to test directly the specific hypothesis that somatostatin-immunoreactive GABAergic interneurons, in the hilus of the dentate gyrus, sprout axon collaterals and enhance their connectivity to dentate granule cells. The evidence provided to support this hypothesis is compelling; however, as the authors themselves state, the GABAergic interneuron system is complex and the implications are presently tentative.

One of the more long-standing and well-supported hypotheses concerning cellular mechanisms that likely contribute to acquired epileptogenesis is a selective loss of specific types of GABAergic interneurons (5). Numerous studies have shown that the somatostatin-immunoreactive GABAergic interneurons are particularly vulnerable, and most of them are lost in human temporal lobe epilepsy and in several animal models. The present study, using paired whole-cell recordings from identified somatostatin-immunoreactive interneurons

(i.e., interneurons tagged with gene-targeted, green fluorescent protein) and dentate granule cells, shows that the remaining somatostatin-immunoreactive interneurons formed more synaptic connections to granule cells (i.e., the number of granule cells that received connections was increased two- or three-fold) but probably not more connections per granule cell (i.e., the amplitude of the unitary evoked IPSC was not changed). These data, however, are considered in relation to results showing that the frequency of miniature IPSCs is decreased shortly after experimental status epilepticus and remains decreased for many months as the animals undergo epileptogenesis. This finding, which was essentially replicated by several laboratories, suggests immediate loss of interneurons and a failure of those interneurons to undergo axon sprouting and formation of new inhibitory circuits. Although the paired recordings provide direct evidence that axon sprouting of inhibitory interneurons occurs and that GABAergic input is augmented to the principal cells (at least for some types of interneurons), the details of the alterations of the reorganized GABAergic interneuron circuit are quite complicated, as discussed by Zhang and coworkers.

One of the reasons that synaptic reorganization, particularly axon sprouting of principal cells and the formation of new recurrent excitatory circuits, has attracted so much attention is because this mechanism would be expected to provide a slow and continuous process that could contribute to the latent periods seen in many patients and animal models of temporal lobe and other forms of acquired epilepsy. The present experiments were performed at a mean time of roughly 45 days after pilocarpine-induced status epilepticus, when the animals were shown to have had spontaneous recurrent seizures. Mossy fiber sprouting, seen in the form of Timm stain in the inner molecular layer, is usually clearly visible after 45 days; however, the full effects of synaptic reorganization are best seen many months after status epilepticus. Unfortunately, it is expensive to maintain experimental animals for many months, and it is technically difficult to perform the paired whole-cell recordings with the visualized patch technique in animals much older than 45 days. Nonetheless, an interesting question is whether changes in the GABAergic interneurons continue to occur for several more months after status epilepticus or whether they are potentially maximal shortly after the insult and then slowly decay. An answer to this question is important because many studies have shown that animal models of acquired epilepsy generally undergo progressive increases in seizure frequency and severity (6–8). Many animals seem to have periods in which seizure frequency is not increased, however, and a lack of progression has been reported in some animals (9). One possible explanation could be that during the periods when the animals are without progression, the reorganization of inhibitory GABAergic circuits is more profound and essentially compromises the enhanced recurrent excitation. One could hypothesize that spontaneous remission arises from reorganization

of GABAergic circuits, as described here. Such a mechanism, hypothetically, could even be a pathway for therapeutic disease modification or antiepileptogenesis.

Although the present paper provides some of the strongest evidence to date that inhibitory interneurons undergo synaptic reorganization, this effect may be relatively small and seen only in some interneurons, and it may provide no more than partial compensation for the epilepsy-associated decrease in the number of GABAergic interneurons. Furthermore, the observation that the mean amplitude of the unitary evoked IPSCs was unchanged has other interpretations than the possible conclusion that the number of GABAergic synaptic connections from the somatostatin-immunoreactive interneurons in the reorganized hippocampus is the same as in normal brain. Several studies have suggested that the properties of GABA_A receptors are altered during epileptogenesis, which might be expected also to modify the mean amplitude of unitary evoked IPSCs (10); thus, changes in the amplitude of IPSCs (or lack thereof) could reflect both changes in connectivity *and* receptor subunits.

The conservative interpretations of the data by the authors actually serve to highlight the complexity of the issue. For example, numerous types of interneurons are present in the hippocampus and cortex, and the question is whether all or only some of them undergo reorganization. Another less obvious question is whether these interneurons and other types of interneurons sprout axon collaterals to yet *other interneurons*—a distinct possibility since it is well known that interneurons normally have synaptic connections among themselves. One could argue that increased connectivity among interneurons, whereby interneurons inhibit other interneurons, could lead to a *decrease* of GABAergic inhibition. Additional studies combining anatomic and electrophysiological mapping at the single-cell level, with quantitative analyses of axonal distributions and postsynaptic currents, should ultimately reveal the degree to which reorganization occurs among the GABAergic inhibitory interneuron system and identify the role it may play in promoting, or compensating for, acquired epileptogenesis.

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