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Loss of GABAergic Interneurons in Seizure-Induced Epileptogenesis—Two Decades Later and in a More Complex World

Commentary on: Dudek FE, Shao LR. Loss of GABAergic Interneurons in Seizure-Induced Epileptogenesis. *Epilepsy Currents*. 2003;3:159-161. doi:10.1046/j.1535-7597.2003.03503.x

Commentary

Our commentary[1] from 2003 discussed 3 publications[2-4] that showed an epilepsy-associated loss of GABAergic interneurons in different regions of the temporal lobe using 2 models of temporal lobe epilepsy (TLE)—pilocarpine-induced status epilepticus and kindling. The interneuron loss caused a reduction in GABA-mediated inhibition, which in turn likely contributed to hyperexcitability and spontaneous recurrent seizures (SRSs). These studies supported, but certainly did not prove, a simple hypothesis—loss of interneurons and their inhibitory function causes (or at least *contributes to*) epilepsy. As one might expect, however, 20 years of research have shown that this concept is probably too simple.

Interneuron Loss, Decreased $GABA_A$ Inhibition, Hyperexcitability, and a Role in TLE?

Most of the neuronal loss in TLE is from glutamatergic pyramidal cells, compared to GABAergic interneurons. GABAergic interneurons are only a small fraction (5%-15%) of the neurons in the temporal lobe, and so one line of research concerning the general phenomenon of neuronal loss in TLE has related more to glutamatergic pyramidal cells. For example, the substantive degeneration of glutamatergic pyramidal cells has been proposed as a trigger for subsequent axonal sprouting and/or neurogenesis. Classic studies showed that blocking the effects of GABA on $GABA_A$ receptors can rapidly lead to paroxysmal depolarizing shifts, seizure-like activity, and convulsions in animals, thus leading to the long-standing concept that GABA is normally a powerful *inhibitory* transmitter system. Thus, many researchers had proposed that loss of GABAergic interneurons could cause—or at least contribute to—many forms of epilepsy.

Models of TLE

Although animal models are generally considered more approachable for experimental studies (eg, availability of controls) than human patients, a consensus on the best models of TLE for studying the effects of interneuron loss has been lacking. Several decades ago, researchers began to study the mechanisms of epileptogenesis in rodent models of TLE based on kindling or induction of status epilepticus (eg, kainate and pilocarpine), both of which are controversial and imperfect. Although models of TLE based on status epilepticus have consistently shown some loss of GABAergic interneurons, this issue has been more controversial in the kindling model. Much interest 30 to 40 years ago concerning epileptogenesis focused on synaptic reorganization of excitatory circuits (increased positive feedback) and loss of GABAergic inhibition (decreased negative feedback), the latter hypothetically by loss of interneurons or alterations of GABAergic circuits and/or receptors. Although a recent quantitative study comparing 2 models of acquired epilepsy (traumatic brain injury with status epilepticus) did not find any relationship between the amount of GABAergic interneuron loss and the likelihood of epilepsy with SRSs,[5] this may have been because only the rats with status epilepticus had SRSs. A more recent study with the pilocarpine model found that ictal onset sites were variable within and between rats, but seizures most frequently occurred in the ventral hippocampus, where interneuron loss was most apparent.[6] Thus, at least so far, it has been difficult to find a definitive role of interneuron loss in these models of acquired epileptogenesis.

Interneuron Loss When and Where?

Temporal lobe epilepsy is generally considered to be a progressive acquired epilepsy, where seizure frequency increases over

time and may sequentially involve different brain areas. This begs the question: When and where is loss of GABAergic interneurons most important during epileptogenesis? Does the interneuron loss occur during the initial brain injury, between the precipitating injury and the onset of SRSs (ie, latent period), and/or later—during the fully developed TLE? Even with the most "focal" examples of TLE, numerous brain structures are almost certainly involved, and a partial loss of interneurons could occur in any or all of them. Although some consider the dentate gyrus to be the cornerstone of TLE, others would argue that the amygdala, piriform cortex, subiculum, thalamus, or other parts of the hippocampus could be as important as the dentate gyrus. These points were raised in the commentary publications, and many recent studies have considered the *timing* and *location* of interneuron loss.

Which Interneurons Are Lost, and Are Their Interconnections Altered in TLE?

The Commentary publications addressed specific types of interneurons and the target site of their axonal projections. Are some types of interneurons—and their loss—more important for TLE than others? In the pilocarpine model, more recent studies with several immunohistochemical and electrophysiological techniques reported that in the CA1 area cholecystokinin-positive basket cells were preferentially lost compared to parvalbumin-positive basket cells.[7] In the original Commentary, we briefly mentioned that interneurons normally connect to other interneurons, possibly creating a complex GABAergic circuitry. Electrophysiological studies using optogenetic techniques combined with several structural methods in the pilocarpine model showed that somatostatin-containing neurons undergo axonal reorganization and form aberrant connections outside their normal synaptic territory.[8] Thus, the issues of which interneurons are lost, and how does synaptic reorganization affect the circuitry of the remaining interneurons have been studied with combinations of optogenetics, electrophysiology, and ultrastructure; these more recent data add considerable complexity to the original concept that GABAergic interneuron loss may suppress inhibition and thus promote the generation of seizures.

GABA Can Be Excitatory

Another critical issue is that early in development, and in some types of epileptogenic tissue, GABA—acting on $GABA_A$ receptors—can be excitatory.[9] The general mechanism is a shift in the chloride equilibrium potential (E_{Cl}) from negative of resting membrane potential to a depolarized level that is positive of threshold for action potential generation. Recent electrophysiological and optogenetic studies in brain slices from normal rats have shown that acute *repetitive seizure-like activity* can cause GABA to be transiently excitatory.[10] Although the Commentary publications reported that loss of GABAergic neurons decreased $GABA_A$-mediated inhibition, GABA could be excitatory at certain sites and times during epileptogenesis or even in normal brain immediately after high levels of seizure activity.

Are Interneurons Active at Seizure Onset?

Several studies, using analyses of the frequency of *identified* single-neuron action potentials at the beginning of a seizure, have reported that interneurons are among the first cell types to become active at the beginning of a SRS. For example, in either brain slices from normal mice using convulsive drugs[11] or in recordings from human patients with epilepsy,[12] the *activity of GABAergic interneurons* appeared to increase at the beginning of the seizures. If GABA becomes excitatory in epileptogenic tissue and if GABAergic interneurons fire early during the onset of a seizure, maybe GABA neurons actually trigger some seizures? Their loss would then have the opposite effects proposed previously concerning loss of GABAergic interneurons, and their presence would seem to be pro-epileptogenic. Another possible explanation, however, is that GABAergic interneurons fire action potentials at seizure onset *only because they have a lower threshold and a higher baseline firing rate*; thus, they might be activated *before* glutamatergic pyramidal cells *not because they contribute to seizure onset, but only because they are activated first*. In this scenario, activation of GABAergic interneurons at seizure onset and subsequent $GABA_A$-receptor activation might still be inhibitory (rather than excitatory). A silent period (ie, a decrease in electrical activity) is commonly seen to precede some seizure onsets, which may be due to interneuron activation of a surrounding inhibitory restraint mechanism[13] before the moving wave-front of the seizure, as shown to occur with single-neuron recordings from humans. Therefore, these studies suggest that a loss of GABAergic interneurons has several possible interpretations, and they emphasize the complexity associated with interneuron loss during epileptogenesis.

Selective Interneuron Silencing and Ablation Cause Seizures and Epilepsy?

Obviously, the best way to test the hypothesis about loss of GABAergic interneurons for TLE would be to *selectively* ablate them and then determine if this manipulation *alone* leads to epileptogenesis. Focal ablation of GABAergic interneurons causes hyperexcitability[14] and repetitive seizures, at least transiently.[15,16] Recent studies have proposed that focal interneuron ablation could cause hippocampal sclerosis and epilepsy over time.[17] A critical question is how selective ablation of specific interneuron subtypes before and at different phases of epileptogenesis—and in different locations—induces or alters seizures/epilepsy.

In the 20 years since we wrote our Commentary, the complexity of the field has increased dramatically. The present commentary has only touched on this complexity and has only considered TLE; however, many other types of acquired epilepsy exist—all of which could involve interneuron

loss . . . plus alterations in interneuron development, their axonal projections, and their electrical activity.

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