

SYNAPTIC REORGANIZATION IN AN EXPERIMENTAL MODEL OF POSTTRAUMATIC EPILEPSY

Synaptic Activity in Chronically Injured, Epileptogenic Sensorimotor Neocortex

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We recorded spontaneous and evoked synaptic currents in pyramidal neurons of layer V in chronically injured, epileptogenic neocortex to assess changes in the efficacy of excitatory and inhibitory neurotransmission that might promote cortical hyperexcitability. Partial sensory–motor neocortical isolations with intact blood supply (“undercuts”) were made in 20 rats on postnatal day 21–25 and examined 2–6 weeks later in standard brain slice preparations by using whole-cell patch-clamp techniques. Age-matched, uninjured naive rats ($n = 20$) were used as controls. Spontaneous and miniature excitatory and inhibitory postsynaptic currents (s- and mEPSCs; s- and mIPSCs) were recorded by using patch-clamp techniques. The average frequency of s- and mEPSCs was significantly higher, whereas that of s- and mIPSCs was significantly lower in neurons of undercuts versus controls. The increased frequency of excitatory events was due to an increase in both s- and mEPSC frequency, suggesting an increased number of excitatory contacts and/or increased release probability at excitatory terminals. No significant difference was observed in 10–90% rise time of these events. The input–output slopes of fast, short-latency, α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid/kainate (AMPA/KA) receptor-mediated components of evoked EPSCs were steeper in undercuts than in controls. The peak amplitude of the AMPA/KA component of EPSCs evoked by suprathreshold stimuli was significantly greater in the partially isolated neocortex. In contrast, the *N*-methyl-D-aspartate receptor-mediated component of evoked EPSCs was not significantly different in neurons of injured versus control cortex, suggesting that the increased AMPA/KA component was due to postsynaptic alter-

ations. Results support the conclusion that layer V pyramidal neurons receive increased AMPA/KA receptor-mediated excitatory synaptic drive and decreased γ -aminobutyric acid type A (GABA_A) receptor-mediated inhibition in this chronically injured, epileptogenic cortex. This shift in the balance of excitatory and inhibitory synaptic activation of layer V pyramidal cells toward excitation might be maladaptive and play a critical role in epileptogenesis.

COMMENTARY

Traumatic brain injury is probably the most important cause of acquired epilepsy, yet we know remarkably little about its cellular mechanisms. In this article, Li and Prince provide a detailed analysis of changes in spontaneous and evoked excitatory and inhibitory postsynaptic events in pyramidal cells of “undercut” neocortex in the rat, which was used as an experimental model of a penetrating head injury. Comparatively few *in vitro* experiments have been done on animal models of posttraumatic epilepsy, and even fewer of them have been done with quantitative analyses of alterations in both excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs). This animal model involves deep cuts into the cortical white matter with minimal disruption of the blood supply. This research is an extension of previous work from this group using this model and is focused on synaptic reorganization of both excitatory and inhibitory circuits. The experiments analyze changes in the frequency and amplitude of spontaneous and miniature EPSCs and IPSCs by using whole-cell patch-clamp recordings from visualized layer V pyramidal cells. Other experiments incorporated focal extracellular stimulation to evoke EPSCs and IPSCs; therefore this line of research strongly suggests a fundamental role for synaptic reorganization in the progressive changes that underlie the mechanisms of epileptogenesis after head injury.

One of the key results is an increase in the frequency of spontaneous and miniature EPSCs (sEPSCs and mEPSCs); the enhanced mEPSC frequency, in particular, suggests an in-

crease in the number of synaptic terminals and/or an enhanced probability of transmitter release after the injury. This group previously found an increase in the number of axon collaterals and swellings, which together with the data described earlier, suggests that axon sprouting has led to the formation of excitatory synaptic circuits near the recorded neuron. As the authors point out, several hypothetical alterations in the synaptic circuitry can account for the data, but one interesting possibility is that the layer V pyramidal cells have formed new recurrent excitatory circuits. Evidence in support of this hypothesis is that local extracellular stimulation evoked all-or-none polysynaptic EPSCs with a variable latency after the initial response in some slices of undercut neocortex. These responses to extracellular stimulation—when local synaptic inhibition is depressed (see later)—are the hallmark of recurrent excitatory circuits, because they represent the expected properties of a network of neurons interconnected with excitatory synapses (Traub and Wong, 1982). Posttraumatic epilepsy usually occurs after a latent period after the injury, and these experiments were performed weeks after the undercut (i.e., after the injury). This hypothesis provides a possible explanation of the latent period and progressive increase in seizure frequency and severity that usually occurs after injury-induced epilepsy. Future research that is focused on the temporal characteristics of the alterations in synaptic circuitry may provide data relevant to this hypothesis and the possible role of synaptic reorganization in the time-dependent aspects of epileptogenesis.

These experiments also addressed changes in inhibitory synaptic transmission and found a decrease in the frequency of sIPSCs and mIPSCs, but no change in the amplitude of these events was detected. The most parsimonious interpretation of these electrophysiologic data is a loss of interneurons and/or inhibitory synapses. The authors discuss their results in regard to the difficulties in interpretation of apparently conflicting data from immunocytochemical studies. Nonetheless, these results point to a shift in the balance of excitation relative to inhibition and lend support to the hypothesis that the progressive formation of new recurrent excitatory circuits, in combination with compromised inhibition, creates a network that is prone to synchronous bursting. This new hypothetical synaptic circuit would be more likely to generate all-or-none net-

work bursts and afterdischarges, either spontaneously or in response to excitatory synaptic input.

In addition to these alterations in synaptic circuits, experiments with pharmacologic antagonists provided evidence that the injured neocortex has increased α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid/kainate (AMPA)/kainate receptor-mediated EPSCs, rather than alterations in *N*-methyl-D-aspartate (NMDA) receptors. An NMDA antagonist did, however, depress the enhanced polysynaptic bursts. As the authors point out, increased AMPA/kainate-mediated transmission would secondarily augment the NMDA component of the responses. Many of the experiments on animal models of injury-induced epilepsy implicating enhanced NMDA-receptor mechanisms have not fully controlled for possible augmentation of AMPA/kainate receptors or depression of γ -aminobutyric acid (GABA)-mediated inhibition. Either of these alternative mechanisms could increase the *apparent* role of NMDA receptors in excitatory postsynaptic responses and epileptiform bursts, and incorrectly be viewed as support for the hypothesis that increased NMDA-receptor function contributes to epileptogenesis. The study by Li and Prince addressed these concerns and tested hypotheses concerning synaptic reorganization and NMDA receptors in relative isolation, and thus tends to minimize or eliminate these potential problems of interpretation.

Finally, this study highlights the potential that injury-induced epilepsy is likely a multifactorial disorder. Increased local excitatory circuits alone, for example, may not lead to epileptiform activity unless inhibitory circuits are simultaneously depressed or other changes are present that allow excitatory neuron-to-neuron interactions. Furthermore, the tendency to discuss epileptogenicity only in terms of “hyperexcitability” leads to electrophysiologic vagueness, whereas this research points to multiple ways that alterations in specific synaptic mechanisms may increase seizure susceptibility.

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Reference

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