

LASER SCANNING PHOTOSTIMULATION: NEW EVIDENCE FOR ENHANCED RECURRENT EXCITATION IN A MODEL OF POSTTRAUMATIC EPILEPSY

Enhanced Excitatory Synaptic Connectivity in Layer V Pyramidal Neurons of Chronically Injured Epileptogenic Neocortex in Rats

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Formation of new recurrent excitatory circuits after brain injuries has been hypothesized as a major factor contributing to epileptogenesis. Increases in total axonal length and the density of synaptic boutons are present in layer V pyramidal neurons of chronic partial isolations of rat neocortex, a model of posttraumatic epileptogenesis. To explore the functional consequences of these changes, we used laser-scanning photostimulation combined with whole-cell patch-clamp recording from neurons in layer V of somatosensory cortex to map changes in excitatory synaptic connectivity after injury. Coronal slices were submerged in artificial CSF (23°C) containing 100 μ M caged glutamate, APV (2-amino-5-phosphonovaleric acid), and high divalent cation concentration to block polysynaptic responses. Focal uncaging of glutamate, accomplished by

switching a pulsed UV laser to give a 200–400 μ s light stimulus, evoked single- or multiple-component composite EPSCs. In neurons of the partially isolated cortex, there were significant increases in the fraction of uncaging sites from which EPSCs could be evoked (“hot spots”) and a decrease in the mean amplitude of individual elements in the composite EPSC. When plotted along the cortical depth, the changes in EPSCs took place mainly between 150 and 200 μ m above and below the somata, suggesting a specific enhancement of recurrent excitatory connectivity among layer V pyramidal neurons of the undercut neocortex. These changes may shift the balance within cortical circuits toward increased synaptic excitation and contribute to epileptogenesis.

COMMENTARY

Increased local excitation and decreased local inhibition are among the dominant hypotheses for the mechanisms of epileptogenesis after injury of cortical neurons. A critical technical problem is how to analyze these hypothetical changes in local synaptic circuits, independent of projection pathways. Extracellular electrical stimulation of afferent pathways and extracellular field-potential recording are frequently used approaches to study the alterations within local circuitry. The most direct but technically challenging approach involves dual intracellular or whole cell recordings; however, the small percentage of neuron pairs that are actually connected often makes this technique quite difficult, if not impractical, particularly when measuring quantitative differences between epileptogenic and normal conditions. The study of Jin, Prince, and Huguenard used photic activation of caged glutamate to stimulate neurons (i.e., pyramidal cells) at specific locations in neocortical slices, without simultaneous activation of fibers of passage. Although other investigators have used variations of this approach in hippocampal

slices from animal models of temporal lobe epilepsy, the present study differed by using a laser-scanning technique to provide more defined stimulation sites and an animal model of post-traumatic rather than temporal lobe epilepsy.

Although many studies in experimental epilepsy research, including some on humans, have used electrical stimulation protocols to analyze local synaptic circuits, the problems associated with simultaneous electrical activation of fibers of passage can result in seriously confounded analyses of local synaptic circuitry. In particular, extracellular stimulation often has been used with paired-pulse techniques and field-potential recordings; however, these methods are extremely hard to interpret and quantify, particularly across epileptic and control preparations. Glutamate microapplication previously had been shown to activate the soma-dendritic regions of hippocampal granule and pyramidal cells without causing action potentials when applied directly to axons (1). Jin and colleagues used a previously developed technique (2) to activate small groups of neurons. The technique involves recording of excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) during direct stimulation of local neurons but without activating fibers of passage from projection neurons, which allows more rigorous and direct analyses of alterations in local synaptic circuits than does extracellular electrical stimulation.

In this study, small photostimuli were moved in a raster pattern to determine which areas (i.e., "hot spots") triggered EPSCs in layer V pyramidal cells. More hot spots were found surrounding layer V pyramidal cells in undercut cortex than in control cortex, and photostimulations were more likely to evoke EPSCs in layer V than other layers, suggesting that compared to controls, layer V pyramidal cells show increased recurrent excitatory connectivity in the undercut cortex. The greater connectivity probably results from enhanced monosynaptic connections from nearby layer V pyramidal cells to the recorded pyramidal cells. The experiments were conducted with high extracellular concentrations of divalent cations (i.e., elevated magnesium and calcium), which would be expected to leave the mechanisms of chemical synaptic transmission intact but raise the threshold for action potential firing. Previous studies showed that axon sprouting occurs in the undercut cortex (3) and that the frequency of spontaneous EPSCs was higher in this model (4). Although similar data on spontaneous EPSCs were obtained in the present study, the increases were not statistically significant. The authors suggest that the elevated extracellular concentration of divalent cations used to elevate firing threshold also may have prevented multisynaptic interactions, which normally would be enhanced in the presence of more recurrent excitation and could have been responsible for the previously observed increase in the frequency of spontaneous EPSCs. Thus, increased recurrent excitation associated with axon sprouting, which is thought to occur in at least two areas of the hippocampus in models of temporal lobe epilepsy (i.e., after kainate- or pilocarpine-induced status epilepticus and in the kindling model), also occurs in the neocortex in a model of posttraumatic epilepsy.

The photostimulation experiments were conducted 2–3 weeks after the neocortical injury (i.e., after the undercut). Although previous studies have shown that epileptiform activity is present at this time period, as is axon sprouting, experiments on dentate granule cells in hippocampal slices from rats with kainate-induced epilepsy have suggested that axonal sprouting and increased recurrent excitation continues to occur over a period of several months after the kainate-induced status epilepticus (5). If a similar phenomenon occurs in the neocortex, then the relative number of hot spots would be expected to increase with time after the injury; a time-dependent increase in the number of local synaptic circuits hypothetically could explain the progressive worsening that is seen in some cases of temporal lobe and posttraumatic epilepsy.

Glutamate receptors on the soma and dendrites presumably mediated the responses to photostimulation in this study. The primary question of the study related to enhancement of recurrent excitation, and accordingly the elevated number of hotspots (defined here as photostimulation-evoked EPSCs occurring temporally after the direct response of the recorded neu-

rons to uncaged glutamate) was interpreted to reflect recurrent excitatory input from other pyramidal cells. Another question, however, is whether the direct responses to glutamate uncaging at points distant from the soma also were increased, possibly reflecting enhanced dendritic arborization following trauma. Although this issue apparently was not studied, if such an increase does occur, it could reflect a dendritic counterpart to the sprouting of recurrent axons back into their network of origin. Such sprouted axons might cause formation of dendritic targets beyond those made available from injury-induced loss of axon terminals. Future studies could conceivably examine whether an increase in the spatial extent of dendritic glutamate responses reflects dendritic sprouting, in addition to axonal sprouting and elevated recurrent excitation.

As the authors point out, enhanced recurrent excitation alone is not likely to be responsible for epileptogenesis or the generation of seizures. A reduction in GABA-mediated inhibition possibly is operating in concert with elevated recurrent excitation. Even slight reductions in GABA-mediated inhibition may shift the balance in favor of epileptogenesis and seizure generation (6). The concept that depressed inhibition leads to epileptiform activity in the presence of recurrent excitation derives from early modeling studies by Traub and Wong (7). Further increases in recurrent excitation in cortical circuits may promote seizure generation and epilepsy. Thus, a series of studies involving both the hippocampus and neocortex now point to the concept that progressive increases in axon sprouting and enhanced recurrent excitation, in the presence of potentially small reductions in inhibition, may underlie at least some types of epileptogenesis.

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