

HOW INDEPENDENT ARE INITIATION, PROPAGATION, AND TERMINATION OF EPILEPTIFORM ACTIVITY?

Initiation, Propagation, and Termination of Epileptiform Activity in Rodent Neocortex In Vitro Involve Distinct Mechanisms

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Waves of epileptiform activity in neocortex have three phenomenological stages: initiation, propagation, and termination. We use a well studied model of epileptiform activity *in vitro* to investigate directly the hypothesis that each stage is governed by an independent mechanism within the underlying cortical circuit. Using the partially disinhibited neocortical slice preparation, activity is induced and modulated using neurotransmitter receptor antagonists and is measured using both intracellular recordings and a linear array of extracellular electrodes. We find that initiation depends on both synaptic excitation and inhibition and entails a slow process of recruitment at discrete

spatial locations within cortical layer 5 but not layer 2 or 3. Propagation depends on synaptic excitation but not inhibition and is a fast process that involves neurons across the spatial extent of the slice and in all cortical layers. Termination is modulated by synaptic excitation and inhibition. In space, termination occurs reliably at discrete locations. In time, termination is characterized by a strong depolarizing shift (block) and recovery of neurons in all cortical layers. These results suggest that the phenomenological stages of epileptiform events correspond to distinct mechanistic stages.

COMMENTARY

Some of the most fundamental questions in basic epilepsy research are: How do epileptic seizures begin? How do they spread between different cortical neurons and structures? And, what causes an epileptic seizure to stop? It is clear that improvements in the understanding of these three aspects of every type of epileptic seizure would have the potential to lead to better treatment of epilepsy patients. The present paper by Pinto and coworkers uses a long-standing, acute model of epileptiform activity (i.e., pharmacologically induced disinhibition) to analyze how electrically evoked bursts of synchronous action potentials start, spread, and stop in neocortical slices from normal rats.

The central hypothesis of the paper by Pinto and coworkers is that epileptiform activity, as observed in a disinhibited slice, involves three stages, each with independent mechanisms: initiation, propagation, and termination. The concept of independence means that there are different physiological actions and separate neurons or spatial locations in the neocortex for each of the three mechanisms. Electrophysiological analyses of cellular models of interictal spikes and epileptic seizures have long invoked these three particular stages in studies of epileptiform activity; thus, they have an important and long history

in epilepsy research. The experimental research in the paper by Pinto et al. builds on theoretical mathematical studies using single perturbation analysis, which investigates a dynamic process by separately analyzing its constituent components. As the authors state, an implicit assumption of single perturbation analysis is that the mechanisms responsible for each of the three stages are independent of the others. Thus, their experiments were designed to determine whether this assumption applies to epileptiform activity in the disinhibited slice model, using a linear array of extracellular electrodes with intracellular recording, and to study the three components of epileptiform activity (i.e., initiation, propagation, and termination) in picrotoxin-treated slices.

The typical disinhibited slice preparation involves reducing or blocking GABA_A receptors or the associated chloride conductance with a pharmacological treatment. The primary mechanisms assessed in these studies were synaptic excitation (i.e., glutamatergic non-*N*-methyl-D-aspartate [non-NMDA] receptors), synaptic inhibition (i.e., GABA_A receptors), and intrinsic neuronal properties (specifically, depolarization block). The non-NMDA and GABA_A-receptor mechanisms were studied in experiments in which the concentrations of 6,7-dinitroquinoxaline-2,3-dione (DNQX), an α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA)/kainate-receptor antagonist, and picrotoxin were varied systematically, as picrotoxin-induced epileptiform activity

was recorded with the linear array of electrodes. As the authors point out, these studies build on the research of Miles, Traub, and Wong using hippocampal slices, in which similar electrophysiological experiments were combined with computer modeling to develop the concept that reduction of GABA_A inhibition leads to the initiation and propagation of epileptiform bursts. The general rationale in this earlier work was that GABA_A inhibition masks local excitatory circuits and that depression of GABA_A inhibition leads to augmented synaptic transmission through recurrent excitatory circuits. This mechanism, in principle, could underlie initiation and propagation of epileptiform activity. For example, depression of GABA_A inhibition in hippocampal slices leads to initiation of network bursts in the CA3 area and to propagation of these bursts to the CA1 area. The present study of Pinto and coworkers reports parametric differences between measures of burst initiation and propagation, including differences in the effects of various doses of the pharmacological antagonists; however, their results show that the initiation and propagation of synchronized bursts derive from the disinhibited transmission of glutamatergic synapses. The slow process underlying initiation appears to be sequential transmission through recurrent excitatory circuits in layer 5, while fast propagation over longer distances is presumably the result of conventional axonal conduction among pyramidal cells in all layers; thus, the mechanisms of initiation and propagation have both similarities and differences.

Pinto et al. found that although synaptic excitation and inhibition appeared to modulate termination, strong depolarizations (i.e., depolarization block) characterized termination. The authors' interpretation of the finding is that intense and synchronized depolarization led to termination, but these experiments did not directly address the specific mechanism(s) of termination. Regardless of the actual role of excitatory or inhibitory synaptic mechanisms versus intrinsic mechanisms (e.g., voltage-dependent depolarizing shifts), intuitively it would seem that termination would depend on different mechanisms than those responsible for initiation or propagation. Although termination would be expected to be different from initiation and propagation, the experiments do not necessarily indicate that it is independent because the manner in which epileptiform activity is initiated and propagated may influence how it is terminated.

The authors raise the issue of whether the findings are limited only to the disinhibited slice preparation or whether they are applicable to other models of epileptiform activity. Over the last 25 years, *in vitro* experiments with brain slices have shown clearly that epileptiform activity can be induced by reducing or enhancing any of several different physiological mechanisms. Examples include, but are not limited to, bath application of low-magnesium solutions to remove the magnesium block and voltage dependence of NMDA receptors; K-channel

blockers, such as 4-aminopyridine (4AP), which prolong action potentials and enhance synaptic potentials; and, low-calcium solutions, which increase membrane excitability while blocking chemical synaptic transmission. According to the mechanism of action of each of these pharmacological/ionic treatments, different mechanisms of initiation, propagation, and termination are likely to be involved in the three different stages of epileptiform activity. For example, the role of GABA_A inhibition, arguably one of the most important and powerful synaptic mechanisms in cortical structures, would be expected to be less important in termination of epileptiform activity when the slices were treated with picrotoxin or low-calcium solution than with low-magnesium or 4AP. Thus, the role of depolarization block versus GABA_A receptor mechanisms could be highly model dependent. As the authors state, additional studies are necessary to answer this question.

"Epileptiform" is a widely used term, with a long history in basic epilepsy research. The standard approach to investigations concerning epileptiform activity has been to use a pharmacological and ionic treatment protocol in an *in vitro* or *in vivo* preparation to induce "hyperexcitability" or "hyperactivity," usually defined as increased electrical activity above what is considered normal. In most studies of this nature, the hyperexcitable or hyperactive events represent bursts of action potentials and then typically are called epileptiform events. Most of these events have durations in the order of tens of milliseconds, but some can persist for tens of seconds. Thus, these short-lasting epileptiform events during an EEG recording would be considered experimental models of the interictal spike rather than actual epileptic seizures, which are characteristically longer than several seconds and are often 2 or more minutes. Thus, another issue is whether brief bursts of epileptiform activity in a disinhibited slice are actually relevant to the generation of spontaneous recurrent epileptic seizures in a patient or an animal model of chronic epilepsy. The events in the study of Pinto and coworkers were usually in the order of tens of milliseconds, although some lasted up to about 500 milliseconds; therefore, the epileptiform activity under investigation could be considered to be more analogous to an interictal than an ictal event. Thus, one would interpret these studies in the context of how spikes rather than epileptic seizures occur in a cortical EEG.

Over the last two or more decades, an increasing effort in basic epilepsy research has been directed toward using animal models (and tissue from them) to understand how chronic epilepsy leads to seizures; that is, how genetic- and injury-induced alterations in cortical neurons and their circuits lead to a greatly increased likelihood that epileptic seizures will be generated. A key finding from many animal-based models of chronic epilepsy and from models using tissue from patients with epilepsy is that while most measures of inhibition show depression, other measures are either unchanged or even



enhanced. Therefore, the role of GABA_A inhibition in the initiation, propagation, and termination of chronic epileptic seizures (or interictal spikes) may not be the same as for an epileptiform burst in a disinhibited slice. The strategy, however, of using pharmacological and electrophysiological techniques to investigate the initiation, propagation, and termination of epileptic seizures will continue to be useful. Unfortunately, a common and difficult problem in contemporary epilepsy research on

animal models is that spontaneous recurrent epileptic seizures occur in awake, intact preparations (i.e., freely behaving animals), while analyses of cellular mechanisms responsible for these seizures can be studied most rigorously in isolated preparations (i.e., in vitro brain slices), which typically do not generate spontaneous epileptic seizures.

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