Current Literature
In Basic Science

Do Seizures in the Pilocarpine Model Start in the Hippocampal Formation?

Early Activation of Ventral Hippocampus and Subiculum During Spontaneous Seizures in a Rat Model of Temporal Lobe Epilepsy.

Temporal lobe epilepsy is the most common form of epilepsy in adults. The pilocarpine-treated rat model is used frequently to investigate temporal lobe epilepsy. The validity of the pilocarpine model has been challenged based largely on concerns that seizures might initiate in different brain regions in rats than in patients. The present study used 32 recording electrodes per rat to evaluate spontaneous seizures in various brain regions including the septum, dorsomedial thalamus, amygdala, olfactory cortex, dorsal and ventral hippocampus, substantia nigra, entorhinal cortex, and ventral subiculum. Compared with published results from patients, seizures in rats tended to be shorter, spread faster and more extensively, generate behavioral manifestations more quickly, and produce generalized convulsions more frequently. Similarities to patients included electrographic waveform patterns at seizure onset, variability in sites of earliest seizure activity within individuals, and variability in patterns of seizure spread. Like patients, the earliest seizure activity in rats was recorded most frequently within the hippocampal formation. The ventral hippocampus and ventral subiculum displayed the earliest seizure activity. Amygdala, olfactory cortex, and septum occasionally displayed early seizure latencies, but not above chance levels. Substantia nigra and dorsomedial thalamus demonstrated consistently late seizure onsets, suggesting their unlikely involvement in seizure initiation. The results of the present study reveal similarities in onset sites of spontaneous seizures in patients with temporal lobe epilepsy and pilocarpine-treated rats that support the model’s validity.

Commentary
Temporal lobe epilepsy is common and often is difficult to treat, making this focal epilepsy a major challenge for experimental investigation. Two of the most widely used chronic models of temporal lobe epilepsy depend on induction of status epilepticus by systemic applications of either pilocarpine or kainic acid (1). The prolonged seizures caused by these drugs result in neuronal losses that may resemble clinical hippocampal sclerosis, and in the development of spontaneous recurring epileptic seizures after a “latent period” during which the process of epileptogenesis transforms normal brain into epileptic. The fact that these drugs are administered systemically means we cannot take for granted where the resulting epileptic focus will be located, nor whether it will be a single focus or a more complex network, nor whether the seizure onset zone(s) correspond to those found in clinical temporal lobe epilepsy.

Toyoda and colleagues addressed some of these questions directly in the rat pilocarpine model, using 32 chronically implanted bipolar recording electrodes positioned in structures implicated by the known neuropathology and pathophysiology of this model. They also included recordings from electrodes that were off-target, which seems entirely sensible; judging from the table of recording sites presented for all 10 rats, they did not miss too often.

The key questions Toyoda et al. addressed were 1) which recording site produced the first electrographic seizure activity during seizures lasting >10s, 2) whether the onset site was consistent between seizures within each animal, and 3) whether the onset site(s) differed among animals.

The seizures were sampled systematically: 10 seizures in each of the 10 rats, including equal numbers of convulsive and nonconvulsive seizures. The authors used appropriate methods to minimize mortality in the pilocarpine model, although they do not quote its incidence. This group previously reported their methods for identifying seizure onsets. Here they used two methods in the time domain and two in the frequency domain: eyeballing, peak detection in low-pass filtered data, power in the 20–200 Hz (“gamma”/”ripple”) band, and power in the 200–600 Hz (“fast ripple”) band. The time of onset for each site during each seizure was taken from the earliest value among the four methods. High frequency activity may be a better marker for neuronal hyperactivity than low frequency waveforms (see papers cited in [2]), but the earliest pathophysiological activity presumably will be found...
Where Do Pilocarpine Seizures Start?

at the sites closest to the genuine seizure initiation site. As the authors discuss, the volume recorded by the 32 electrodes in each rat represents a small fraction of the total brain (they estimate ~0.1%), so a pragmatic approach of detecting the earliest seizure activity regardless of its waveform seems reasonable.

The main result is that onset sites varied both within and between animals. One question that is not answered here is whether variations between animals are related to differences in the extent and locations of lesions induced by pilocarpine. However there were clear differences between brain regions. The most common onset sites, with appropriate corrections for sampling, proved to be the ventral hippocampus and ventral subiculum. One caveat is that the neocortex was not sampled systematically, so any seizures starting somewhere in this large structure would be missed. Five of the off-target recordings were from neocortex and none initiated seizures; unfortunately, where in the neocortex is not stated. More systematic recordings from the rat equivalent of human lateral temporal neocortex will be needed to determine whether the pilocarpine model does sometimes replicate clinical lateral temporal lobe epilepsy.

The question of where seizures start in the pilocarpine model is not new. Previous anatomical methods (e.g., fos immunohistochemistry) argued for extrahippocampal onsets (3, 4). Electrophysiology is better suited to determining the first recording site to generate seizure activity, but samples a small fraction of the brain, while anatomical methods do have high spatial coverage and resolution but little or no temporal resolution. That said, fos histochemistry and regular neuropathology have implicated extrahippocampal structures in pilocarpine seizures, and Toyoda et al. argue that such sites—including substantia nigra and dorsomedial thalamus—consistently were recruited late in the seizures and therefore could not be implicated in their initiation. That does not exclude the possibility that one or more of these structures may be necessary components of the circuitry required for seizure generation (5), nor that they may be relevant targets for surgical intervention.

Reassuringly electrographic signs proceeded behavioral in almost all seizures (91 of the 94 seizures with suitable video recordings). On average, the delay was 10 seconds and was longer for nonconvulsive seizures, which otherwise were similar to convulsive seizures in terms of seizure initiation and propagation. Nearly all the seizures (95/100) spread to all recording sites.

There were differences between rats and people with epilepsy. Propagation was faster in rats, probably because of their smaller size and substantially fewer numbers of neurons and, related, their stronger connectivity. In most rats, both seizure onsets and lesions were equally likely in left and right hemispheres, whereas humans with temporal lobe epilepsy usually have lateralized foci and lesions (fortunately for epilepsy surgery). Variability in onset sites is reported in the clinical literature, but it is to a lesser degree than in rats.

Overall, this is a rigorous study of a difficult but important question raised by one of the most commonly used rodent models of temporal lobe epilepsy. The conclusion that the seizures recorded in this model actually do start in the hippocampal formation should be rather welcome for most researchers using the model.

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References

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