CURRENT LITERATURE

SITES OF INTERICTAL SPIKE GENERATION IN NEOCORTEX

The Initiation of Spontaneous Epileptiform Events in the Rat Neocortex, In Vivo

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We used voltage-sensitive dye imaging to visualize the distribution of initiation sites of the spontaneous interictal-like spikes (sISs) in rat neocortex, in vivo, induced by bicuculline or picrotoxin over the exposed cortex. The initiation site was small (~200 μm in diameter). On average, each initiation site initiated 2.0 $\pm$ 0.8 sISs (nine animals, 499 sISs, 251 sites). This is significantly different from that in neocortical slices, where each initiation site initiated 30 to 100 sISs. The initiation sites were not randomly distributed. The distance between two consecutive sites tended to be either $<$800 or $>$1,200 μm, suggesting a temporal "suppression annulus" surrounding each initiation site. Within the annulus, the likelihood for initiating the next sIS was reduced. Suppression annulus did not have a noticeable change in the presence of γ-aminobutyric acid (GABA)-b antagonist, suggesting that it did not depend on the GABA-b inhibition. We also applied bicuculline locally to a spot of 800 $\times$ 800 μm^2 for ~45 minutes. During this period, ~1,000 sISs occurred within the spot. Bicuculline or picrotoxin was then applied to the entire craniotomy window. The pretreatment created an obvious cluster of initiation sites. Around this cluster, the suppression annulus became obvious in individual animals. Our results suggest that in disinhibited cortex, epileptiform events were initiated from small sites. The initiation sites may cluster in an area with increased local activity. Surrounding each initiation site, a temporal suppression annulus may appear.

COMMENTARY

Interictal spikes are often assumed to be a marker for the epileptogenic zone, the area of cortex that is necessary and sufficient to generate seizures. However, the specific spatial and temporal relation between interictal spikes and seizure generation are uncertain (1,2). To localize epileptogenic cortex properly (e.g., when planning resective surgery), it is crucial to understand where interictal spikes develop and how they propagate. Even normal brain, if deprived of its usual inhibition, can generate interictal spikes. Certain brain regions possess circuitry that is highly susceptible to epileptic discharges (for example, hippocampal subfield CA3). In neocortex, the site of many epilepsies, areas of heightened epileptogenicity are found, despite the relatively uniform laminar structure (3).

This report by Ma and colleagues addresses the question of where interictal spikes are initiated in adult rat neocortex. The underlying hypothesis is that neocortex is not homogeneous, either structurally or physiologically, and that areas of neocortex could preferentially generate interictal spikes. The investigation of these questions in an intact, whole-animal preparation adds to their clinical relevance.

Previous studies, by using neocortical slices, showed that there are numerous zones of interictal spike generation, each producing a *large* number of interictal spikes. The present study used a voltage-sensitive dye to image neuronal activity. A γ-aminobutyric acid $(GABA)_A$-receptor blocker—bicuculline or picrotoxin—was applied to exposed neocortex to disinhibit the area and produce interictal spikes. The voltage changes sensed by the dye represented membrane potential changes, correlating with hypersynchronous firing in small groups of neurons. In contrast to in vitro studies, the present study found multiple sites of interictal spike initiation in neocortex in vivo, but each site produced *only a few* spikes. Interictal spikes began in discrete areas throughout the disinhibited neocortex, rather than in a uniform distributed pattern. Therefore the entire cortex is capable of hypersynchronous firing, but only small, discrete populations of neurons fire synchronously at any given time. Initiation sites tend to cluster together within a limited area, suggesting that they could act synergistically as an epileptic focus.

Furthermore, after a given interictal spike, the next interictal spike begins either close to the previous one ($<$800 μm away) or else distant from it ($>$1,200 μm away). That is, in a zone from 800 to 1,200 μm away from the interictal spike initiation zone, a *subsequent* interictal spike will not occur. This distinctive spatial and temporal pattern is termed the "suppression annulus." At first glance, this zone might be reminiscent of the well-described "inhibitory surround" adjacent to an epileptic

focus, but the authors stress that these phenomena are distinct. As opposed to the inhibitory surround, the suppression annulus is transient (seconds) and does not prevent the spread of excitation beyond its limits (propagation proceeds through it). Preliminary data reported in the article suggest that the suppression annulus is not mediated by $GABA_B$ receptors or calcium-activated potassium channels. The physiology and anatomic substrate of the suppression annulus remains to be determined, but it is likely a reflection of local circuit properties, perhaps related to particular axonal or dendritic ramifications.

In neocortical epilepsy, it is unknown whether analogous multiple, spatially distinct interictal spike initiation zones exist. This intriguing set of experiments opens new conceptual views as to how interictal spikes are generated in neocortex. The suppression annulus is a novel finding with an unclear significance and relation to neocortical circuitry. It would be fascinating to apply this technique to naturally epileptogenic cortex, for example, a model of cortical dysgenesis, to determine whether discrete interictal (and ictal) spike-initiation sites also are found in pathological epileptic tissue. Eventually these techniques could have relevance during surgical evaluation of potentially resectable cortical areas (4).

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