Initiation of Spontaneous Epileptiform Activity in the Neocortical Slice

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Tsau, Yang, Li Guan, and Jian-Young Wu. Initiation of spontaneous epileptiform activity in the neocortical slice. *J. Neurophysiol.* 80: 978–982, 1998. Cortical local circuitry is important in epileptogenesis. Voltage-sensitive dyes and fast imaging were used to visualize the initiation of spontaneous paroxysmal events in adult rat neocortical slices. Although spontaneous paroxysmal events could start from anywhere in the preparation, optical imaging revealed that all spontaneous events started at a few confined initiation foci and propagated to the whole preparation. Multielectrode recording over hundreds of spontaneous events revealed that often two or three initiation foci coexisted in each preparation ($n = 10$). These foci took turns being dominant; the dominant focus initiated the majority of the spontaneous paroxysmal events during that period. The dominant focus and dynamic rearrangement of foci suggest that the initiation of spontaneous epileptiform events involves a local multineuronal process, perhaps with potentiated synapses.

INTRODUCTION

Intrinsic cortical mechanisms were suggested to be important in epileptogenesis (Connors 1984; McNamara 1994; Traub et al. 1994). A small piece of isolated neocortex when perfused with convulsants (e.g., bicuculline) or high [K] or low [Mg] media is enough to autonomously generate all-or-none paroxysmal events (the paroxysmal depolarization shift, PDS) involving the activation of the whole preparation (Flint and Connors 1996; Silva et al. 1991; Wong and Prince 1990). Intrinsic bursting neurons (Chagna-Amitai and Connors 1989; Connors 1984) in layers 4 and 5 were proposed as potential pacemaker cells (Silva et al. 1991). However, it is unknown whether a spontaneous PDS is initiated from an individual cell's activity or by an interaction among a group of neurons. If an individual or small cluster of pacemaker cells can start a PDS then the initiation sites might be small and randomly distributed in a preparation. Conversely, if the initiation of PDS involves distributed interactions among a large group of neurons, then the initiation of PDS would occur in a large and diffused area with no apparent individual site. In this report we attempt to distinguish these possibilities by imaging the spontaneous initiation of PDS events.

Voltage-sensitive dye imaging (Davila et al. 1974; Ross et al. 1977) provides adequate spatial and temporal resolution for these measurements. This method was used to study the spreading of paroxysmal events in neocortical slices (Albowitz and Kuhnt 1995; Sutor et al. 1994) and epileptiform activity evoked by sensory stimuli in cortex in vivo (London et al. 1989). Here we use this imaging method to address the spontaneous initiation of epileptiform events in in vitro cortical tissue. Dual-electrode measurements were also used to determine the spatial distribution of the initiation sites. With the use of different in vitro models the paroxysmal events appear different in local field potential recordings. In bicuculine and high [K] models the paroxysmal events appear as single spikes (PDS), and in the low [Mg] model a paroxysmal event is composed of a large initial spike (correlated to PDS) and a series of afterdischarges (ictal-like activity). Because in all three in vitro models paroxysmal events start with an initial population spike, we refer to the origin of this spike as the initiation site of the paroxysmal event.

METHODS

Sprague-Dawley rats (postnatal day P21–28) were deeply anesthetized with CO_2 and decapitated. Neocortical slices (400 μm, coronal section, Bregma 4–6 mm) were prepared by a Vibratome (Campden Instruments) and incubated for 2 h in 95% O_2-5% CO_2 equilibrated artificial cerebral spinal fluid (ACSF) containing (in mM) 132 NaCl, 3 KCl, 2 $MgCl_2$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 glucose, at pH 7.4. The slices were stained with 0.02 mg/ml of voltage-sensitive dye JPW1131 (RH 479) before the experiment. During recording sessions the stained preparation was perfused in a submerged chamber with one of three modified ACSF solutions: *1*) 0 [Mg]—ACSF but containing no added $MgCl_2$, *2*) bicuculine—ACSF but containing 1 mM $MgCl_2$ and 20–40 μM bicuculine (Research Biochemicals International), and *3*) high [K]—ACSF but containing 5 mM KCl and 1 mM $MgCl_2$. Dye-related absorption signals (705 nm) were imaged by a 124-element (12×12) photodiode array at a rate of 1,000 frames per second (Wu and Cohen 1993). The raw image data has a low resolution (12×12 pixels). We used the contour display in NeuroPlex (OptImaging, LLC, Fairfield, CT) to convert the raw data to pseudocolor images. A $\times 2.8$ objective lens resulted in a field of view of 4.5-mm diam. A section of the gray matter containing all cortical layers was covered by approximately five detectors (Fig. 1*A*). The dye signals were filtered at 400 Hz before digitizing. The fractional absorption change ($\Delta I/I$) for a typical initial spike was ~0.1–1% of the resting light intensity (Fig. 1*B*). Field potential recordings were made in layer II of the cortex with the use of glass microelectrodes filled with 1 M NaCl and a tip resistance of 2–5 MΩ.

RESULTS

Confined initiation foci

Spontaneous paroxysmal activity emerged 20–40 min after the preparation was perfused with a modified ACSF. The PDS was a spontaneously occurring all-or-none population spike in the field potential recordings and in optical recordings (Fig. 1*B*, PDS). The optical signal had a similar waveform and time course as the recordings from electrodes in adjacent areas (e.g., Fig. 1*B*, *top 2 traces*). To identify the initiation site of the paroxysmal events, the PDS spike from each detector was normalized to its maximum amplitude and pseudocolor images were made from the contour

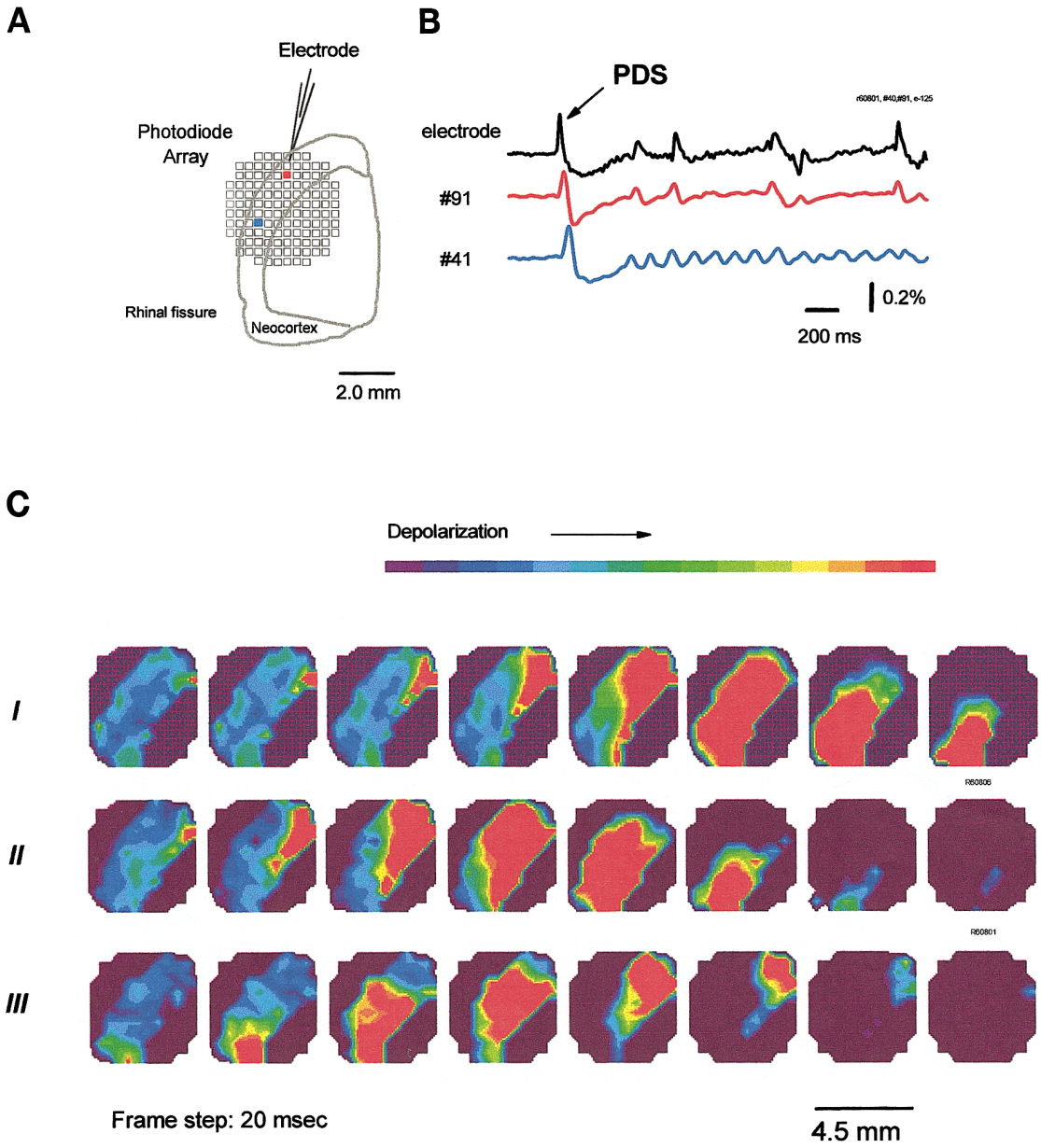

FIG. 1. Optical images of initiation focus. Initiation of paroxysmal activities in 0 [Mg] artificial cerebrospinal fluid was examined by simultaneous optical and electrical recordings. *A*: schematic illustration of microelectrodes for electrical recordings and 124-pixel photodiode array for optical recording from a neocortical slice. *B*: similarity of electrical recordings and optical signals. Optical signals obtained closer to the electrode were more similar to the electrical recordings. Note that the ictal-like activity had very different spatiotemporal patterns in different regions. *C*: image series of the onset of 3 paroxysmal depolarization shift (PDS) events from the same field of view. Each series shows 10 images from the 1st 200 ms of the events (1 image per 20 ms). *CI*: spontaneous PDS initiated from a focus located in a deep layer. *CII*: 2nd recording trial 30 min after *CI*. A spontaneous event started from the same focus. *CIII*: 1 h after *CI*, a spontaneous event started from another focus.

display. Images from 10 animals showed that the paroxysmal events always started as a confined spot smaller than one photodetector (375 × 375 μm) and propagated over the entire preparation (Fig. 1*C*). Because the voltage-sensitive dye image is a direct measurement of transmembrane potential (Cohen and Salzberg 1978), the site of origin is accurately localized and is not subject to concerns about the volume spread of extracellular currents. However, the resolution of our imaging device only allows us to set an upper limit on the actual size of the initiating region.

Initiation foci and dominant focus

Repeated optical recording trials suggested that the spontaneous paroxysmal events started from the same initiation focus (Fig. 1, *CI* and *CII*). Because prolonged optical recording is limited by dye bleaching and phototoxicity (Wu and Cohen 1993), we used two electrodes placed between the possible initiation foci to determine the locations of the initiation sites (Fig. 2*A*). Because the initiation foci were much smaller than the distance between measuring elec-

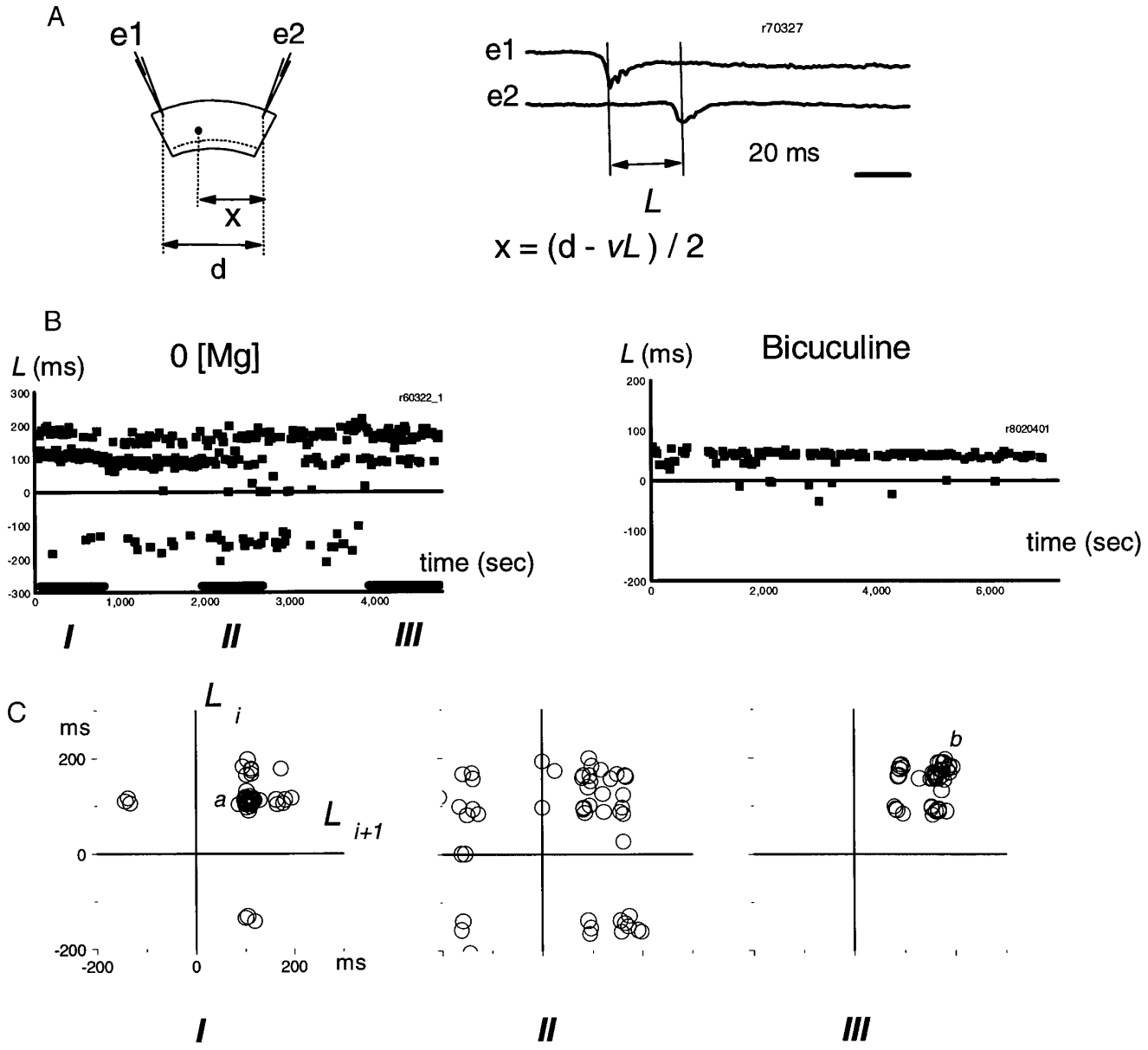

FIG. 2. Initiation foci and dominant focus. *A*: locations of initiation foci were measured by 2 local field potential electrodes (e1 and e2). The onset of a PDS event measured by the electrodes (*right panel*) and the relative location of the initiation focus (x) can be determined by measuring the distance between the electrodes (d), the time difference (latency, L) of the paroxysmal depolarization shift (PDS) onset, and the PDS propagation velocity (v) with the use of the equation. Because the width of the slice (distance between pia and white matter) is much smaller than the distance between electrodes and d is fixed, and assuming v is constant, the distance between initiation focus and the electrodes is proportional to the time latency L, which is plotted in *B*. *B*: latencies from 1 preparation in 0 [Mg] artificial cerebrospinal fluid (ACSF) (*left panel*, 384 events) and another in ACSF containing 20 mM bicuculine (*right panel*, 191 events) over a 2-h recording period are plotted against the recording time. The latencies are clustered around several values during the whole course, indicating that all PDS events were started from a few initiation foci. Variation of the latency values was probably caused by variation in the propagation velocity. Three sections (indicated by the thick bars along the x-axis) are plotted in the autocorrelation plots *I*, *II*, and *III* in *C*. *C*: autocorrelation plots showing domination among initiation foci. Three plots represent 3 sections (*I*, *II*, and *III*) of the data in *B*, *left panel*. In the plots, L_i (y-axis) is the latency of the ith event, and L_{i+1} (x-axis) is the latency of its following event. Each PDS event is represented as a circle in the plot. *CI*: a dominant focus creates a cluster of circles. The circles in ''a'' mean that if one event was initiated by the dominant focus the next one was also initiated by the same focus. *CII*: during this period there was frequent switching between initiation foci, and there was no obvious dominant center. *CIII*: later a new dominant center developed (''b'') while the old dominant focus (''a'') became less active.

trodes (Fig. 1), the location of the foci can be determined by the time difference (latency, Fig. 2*A*) for the PDS spike to propagate from its initiation focus to the two electrodes if the propagation velocities of spontaneous PDS in vertical and horizontal directions are known. These were measured directly from the optical images and were 36.1 ± 9.9

mm/s (means ± SD, $n = 14$) and 31.0 ± 13.2 mm/s ($n = 14$), respectively.

Over a 2-h recording period, usually 300–400 spontaneous PDS events can be recorded ($n = 7$). In 0 [Mg] ACSF, the latencies were clustered around a few values (Fig. 2*B*, *left panel*), consistent with the idea that the PDS events were initiated from only a few sites.

The results in Fig. 2*B* as well as the plots showing the autocorrelations of the latency (Fig. 2*C*) suggest that an initiation focus that initiates one paroxysmal event is more likely to be the initiation site for successive events. The three plots are from different time periods of data in the *left panel* of Fig. 2*B*. The latency of each event (L_i) is plotted against the latency of its successive event (L_{i+1}). If the latency of each event is correlated with that of its precedents and successors, then a cluster will be formed around the L values. The clusters of circles represent different initiation foci. Among coexisting initiation foci, one focus often became dominant for a period and initiated the majority of the spontaneous PDS events (Fig. 2*CI*, ''a''). Dominant foci were observed in all seven preparations examined. During different recording periods the dominant focus could be different. The old dominant center became nondominant with time, and another initiation focus became dominant (Fig. 2*CIII*, ''b''). Occasionally, however, there were three or four initiation foci with none obviously dominant (Fig. 2*CII*).

Dominant initiation sites also occurred in ACSF containing bicuculine (Fig. 2*B*, *right panel*) and high extracellular potassium (data not shown), suggesting that in these epilepsy models the initiation of spontaneous epileptiform events follows the same pattern as in the low-magnesium model.

DISCUSSION

The main findings of this report are listed as follows. *1*) In the deafferented and hyperexcitable neocortical slice spontaneous epileptiform events start from confined foci rather than large diffuse areas. *2*) There were only a few initiation foci in each preparation. *3*) Among coexisting foci, one focus often became dominant for a period of many minutes. The domination was not permanent; an old dominant focus was often replaced by a new one.

Initiation foci are confined to a small size and have a relatively fixed location, suggesting that even in the hyperexcitable tissue, PDS events do not start everywhere. A specialized local process may be needed for this all-or-none population event. The phenomenon of dominant foci indicate that only a few loci in the tissue execute this specialized process. We observed that the PDS events always became visible in the image from a spot smaller than one detector, suggesting that the initiation process is relatively localized. Coherent activation of a local group of neurons may be needed to initiate an all-or-none cortical event (see Traub et al. 1994). The traditional view about cortical connectivity is sparse and divergent (Douglas and Martin 1990) and not in favor of a highly localized process. However, a recent study showed that local neurons can be highly interconnected (Markram et al. 1997). In our experiments the volume of cortical tissue under each photodetector contains ~4,000 cortical neurons, which is similar to the cortical domains of coactivation demonstrated in the developing neocortex (Yuste et al. 1992). An initiation focus may also be a single pacemaker neuron or a small cluster of interactive neurons. Although it is possible to drive one neuron intracellularly to start a PDS in hippocampus (Miles and Wong 1983), similar attempts in neocortex were not successful (B. Connors 1997, personal communication). In contrast, a weak stimulus applied extracellularly to simultaneously activate a group of neurons in cortex is capable of evoking a PDS event (Silva et al. 1991), suggesting that in cortex the initiation site might be a small group of simultaneously activated neurons.

Analysis of the frequency of spontaneous PDS events suggests complex nonlinear dynamics (e.g., Schiff et al. 1994). Our data suggest that the global frequency of the whole preparation may be determined by two levels of interactions: *1*) a local process to determine the frequency of each initiation focus and *2*) the global frequency determined by the dominant focus or a competition among nondominant foci (Fig. 2*C*).

We propose that asynchronized local interactions among hyperexcitable neurons may undergo activity-dependent potentiations to become a local cluster with coherent activity. If this activity passes a certain threshold, it becomes an all-or-none PDS event. The activity of the PDS event itself, in contrast, may have an opposite effect of disrupting the formation of other potential initiation foci. This allows an initiation focus to become dominant over the whole slice. Activity-dependent facilitation (Thomson 1997) and LTP (Lee et al. 1991; Sutor and Hablitz 1989) might be candidates for the dynamically potentiating connections within a local cluster of neurons.

In conclusion, spontaneous epileptiform activity may start from a small group of cortical neurons. Potentiation among the neurons in the group may play an important role so that local interactions that start one paroxysmal event become more likely to start successive paroxysmal events.

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