

# Focal Epileptogenesis in a Rat Model of Polymicrogyria

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**Jacobs, Kimberle M., Bryan J. Hwang, and David A. Prince.** Focal epileptogenesis in a rat model of polymicrogyria. *J. Neurophysiol.* 81: 159–173, 1999. Polymicrogyria, a developmental cortical malformation associated with epilepsy, can be modeled in rats with a transcortical freeze lesion on the day of birth (P0) or P1. We have used field potential recordings to characterize the incidence, propagation patterns, and distribution of epileptiform activity in slices from rats with experimental microgyri. Interictal-like epileptiform activity was evoked in slices from 85% of freeze-lesioned rats aged P12–P118. These data show age-specific properties of epileptogenesis, including: a delay in onset, a decrease in the incidence of epileptiform activity in rats >P40 that was specific to those lesioned on P0 as opposed to P1, and a shift in the likely site of initiation to areas further from the microgyrus in mature animals. Several observations suggest that the area adjacent to the microgyrus, which appears histologically normal in Nissl stains, contains the necessary epileptogenic neuronal circuits: 1) in 78% of slices, epileptiform activity could be evoked only from a focal zone adjacent to the microgyrus (paramicrogyral zone) and not within the microgyrus proper; 2) epileptiform activity consistently originated from a particular site within this paramicrogyral zone, independent of the location of the stimulating electrode, suggesting that the generator is outside of the microgyrus; 3) evoked epileptiform activities in the paramicrogyral cortex were unaltered after separation of this zone from the microgyrus with a transcortical cut; and 4) the short-latency graded field potential evoked in the paramicrogyral zone contained an additional negativity not seen in control slices. The epileptiform activity was blocked reversibly by *N*-methyl-D-aspartate receptor antagonists in slices from mature as well as immature freeze-lesioned rats. These results suggest that aberrant synaptic connectivity develops in rat cortex surrounding the microgyrus and produces a focal epileptogenic zone whose capacity to generate epileptiform activities does not depend on connections with the malformation itself. We hypothesize that afferents, originating from cortical and extracortical sites, lose their targets in the region of the malformation and make appropriate laminar contacts in the cortex adjacent to the malformation, creating an overabundance of excitatory input to this cortical zone. Increased excitatory feedback onto specific cortical elements may be one factor involved in epileptogenesis in this model of a cortical malformation.

## INTRODUCTION

Developmental malformations of the brain make up 13–24% of cases of epilepsy in which an etiology can be identified (Annegers 1994). Recognition of the association between such lesions and epilepsy, particularly seizures resistant to antiepileptic drug (AED) therapy, has increased in the last 10–15 years, coincident with improvement in magnetic resonance imaging (MRI) techniques. The incidence of microdysgenesis, a general term for developmental aberrations in cortical laminar or gyral pattern, has been reported to be as high as 43% in patients who have undergone surgery for intractable seizures (Brannstrom et al. 1996).

Epilepsy associated with cortical malformations is often difficult to treat because currently available AEDs are often ineffective, and in  $\geq 50\%$  of surgically treated patients seizures cannot be eliminated or significantly reduced (Olivier et al. 1996; Palmini et al. 1994). An understanding of the mechanisms of aberrant development and subsequent epileptogenesis is clearly necessary for the evolution of more successful treatment of these epilepsy patients. In addition, cortical malformations also are associated with developmental delay (Gonatas and Moss 1975; Kuzniecky et al. 1994; Wisniewski 1990), dyslexia (Galaburda 1991; Humphreys et al. 1990), mental retardation (Brodtkorb 1994; Striano et al. 1996), cerebral palsy (Truwit et al. 1992), and schizophrenia (Weinberger and Lipska 1995).

An animal model of one type of cortical malformation, that of polymicrogyria, has contributed to the current understanding of the causes of microgyral formation (Larroche 1984). Dvorak and Feit (1977, 1978) have shown that a transcortical freeze-lesion, which kills most of the neurons present at the time of the lesion (those in cortical layers IV, V, and VIa on the day of birth in rat), mimics all histological aspects of human four-layered polymicrogyria. Both clinical and experimentally induced microgyri contain four layers instead of six, with the absence of layers V, VIa, and sometimes part or all of layer IV (Dvorak et al. 1978; Jacobs et al. 1996a; Levine et al. 1974; Williams et al. 1976). Although previously thought to be a migratory defect (see Larroche 1977; Robinson 1977), polymicrogyria now is believed to be due to cell death as a result of a focal hypoxia because some gliosis is evident, laminar abnormalities are primarily those of loss rather than misplacement of neurons (Richman et al. 1974; Williams et al. 1976), and there is a correlation between the presence of layered polymicrogyria and hypoxic events occurring during the late stages of cortical migration (Barkovich et al. 1995; Cohen and Roessmann 1994; McBride and Kemper 1982). Thus the rat model duplicates not only the histopathology but also the likely initiating mechanisms in that focal cell loss results in abnormal lamination.

Polymicrogyria is associated with epileptiform activity in up to 80% of cases (Barkovich and Kjos 1992); however, until recently it was not known whether the freeze lesion model of microgyria developed similar physiological abnormalities. We recently reported that neocortical field potentials evoked in vitro from brain slices of freeze-lesioned rats contain additional late multiphasic events (Jacobs et al. 1996a; see also Luhmann and Raabe 1996). These late events have been characterized as epileptiform and are most similar to interictal types of electroencephalographic (EEG) activity. This model is clearly appropriate for the study of mechanisms contributing to epileptiform activity in brains

with polymicrogyria and experiments focused on potential treatments for this form of epilepsy.

Localizing the site of initiation of epileptiform activity is essential for applying surgical treatments as well as identifying neuronal and receptor subtypes to target for drug development. Dysplastic lesions associated with epilepsy differ from tumors and vascular abnormalities in that epileptiform activity can occur within the lesion area itself, as identified with magnetic resonance imaging (MRI) or other imaging techniques (Palmini et al. 1995, 1996). However, currently there is not complete agreement as to whether the epileptiform activity typically originates within the dysgenic region and how to select the region for resection. It is unclear whether the malformation viewed on MRI is the primary area that should be surgically removed (Palmini et al. 1994), even when no abnormal physiological activity is associated with it (Olivier et al. 1996), or whether EEG can more clearly identify the extent of the area that should be resected (Munari et al. 1996). An understanding of the mechanisms initiating the epileptiform activity will help to formulate strategies for seizure treatment.

In the experiments reported here, we examined the incidence of epileptiform activity in rats with an experimentally induced microgyrus, the site of initiation of evoked interictal discharges, the role of the microgyrus per se versus the adjacent cortex in epileptogenesis, and the involvement of *N*-methyl-D-aspartate (NMDA) receptors in generating the epileptiform activity.

## METHODS

Freeze lesions were made in albino Sprague Dawley rat pups on P0 or P1, as previously described (Jacobs et al. 1996a). Pups were anesthetized with hypothermia by immersing them in ice until there were no longer responses to noxious stimuli such as tail pinch. The skull then was exposed, and a freezing probe (about  $-50^{\circ}\text{C}$ ) placed on the skull for 3–7 s. Probe tip was either circular, 2–3 mm in diameter; or rectangular,  $1 \times 5$  mm. The scalp then was sutured, and the pup warmed and returned to the dam.

Standard techniques for preparing and maintaining neocortical slices were used (Jacobs et al. 1996a). Rats aged P9–P18 were anesthetized with pentobarbital (55 mg/kg) and decapitated; animals >P29 were anesthetized with pentobarbital (55 mg/kg), cooled by immersion in an ice water bath, and then decapitated. Brains were removed and placed in a cold modified Ringer solution containing (in mM) 2.5 KCl, 10  $\text{MgSO}_4$ , 3.4  $\text{CaCl}_2$ , 1.25  $\text{NaH}_2\text{PO}_4$ , 234 sucrose, 11 glucose, and 26  $\text{NaHCO}_3$ , saturated with 95%  $\text{O}_2$ –5%  $\text{CO}_2$ , pH 7.4. Coronal slices, 400- $\mu\text{m}$  thick, were cut on a vibratome in the modified Ringer solution and incubated at  $32^{\circ}\text{C}$  in normal gassed Ringer solution (ACSF) containing (in mM) 126 NaCl, 3.0 KCl, 2.0  $\text{MgCl}_2$ , 2.0  $\text{CaCl}_2$ , 1.25  $\text{NaH}_2\text{PO}_4$ , 10 glucose, and 26  $\text{NaHCO}_3$ . Recordings were made in a modified interface chamber in which slices were maintained at  $34^{\circ}\text{C}$  and superfused with a layer of normal Ringer solution gassed with 95%  $\text{O}_2$ –5%  $\text{CO}_2$  (pH 7.4). The NMDA antagonists, D,L-2-amino-5-phosphonopentanoic acid (AP5, Sigma), and ( $\pm$ )-2-amino-5-phosphonopentanoic acid (AP5, Research Biochemicals International) were bath-applied at concentrations of 50 or 100  $\mu\text{M}$  in normal Ringer solution in some experiments.

The microsulcus was visualized readily at the pial surface in two or three slices from animals lesioned with the circular-tipped freezing-probes and in up to eight slices from lesions produced by the rectangular probe. Distances over the surface of the slice were measured using a calibrated reticule in the dissecting microscope.

In 228 slices from 90 freeze-lesioned rats and 16 slices from 5 control rats, field potential recordings (DC–5 kHz, low-pass filtered at 1 kHz) were obtained either using glass micropipettes (2–8  $\text{M}\Omega$ ) filled with 1 M NaCl or multielectrode arrays made of 14–16 stainless steel wires aligned in a single row and spaced 107  $\mu\text{m}$  apart (NeuroScientific Laboratories). Data were either stored on magnetic tape and later digitized or digitized on-line (2–5 kHz) using software from Axon Instruments. Electrical stimuli were delivered through concentric bipolar electrodes (FHC). Typically, single 20- $\mu\text{s}$  square current pulses were applied at increasing current intensities (5–100  $\mu\text{A}$ ) until a short-latency negative field potential with an amplitude of 50  $\mu\text{V}$  was observed. This was considered the threshold level of stimulation. A series of stimuli of increasing duration (threshold current for 20, 40, 80, 160, and 320  $\mu\text{s}$ ) then was applied (1, 2, 4, 8, and 16 times threshold) at 0.1 Hz. The lowest current intensity required to evoke epileptiform activity (the “epi-threshold”) was sometimes different from the threshold current required to evoke a 50- $\mu\text{V}$  short-latency response. The epi-threshold was determined by applying 20- $\mu\text{s}$  square current pulses at increasing current intensities (5–100  $\mu\text{A}$ ) until epileptiform activity was observed. The epi-threshold was measured at various distances from the microsulcus to find the most sensitive epileptogenic region within a particular slice (Fig. 4D). The stimulus threshold for the short-latency event was used for all other comparisons between slices. During pharmacological manipulations, stimuli were delivered at 0.033 Hz.

In 13 slices, the microgyrus was visualized with a dissecting microscope and separated from the adjacent cortex on one side by making a single cut with a hand-held piece of razor blade. The cut was made 0.4–0.7 mm from the microsulcus, between the microgyrus proper and the recording site in layer III from which epileptiform activity had been evoked before the cut, 0.6–1.5 mm from the microsulcus. Stimuli were delivered directly beneath the recording electrode, within layer VI. The incidence of epileptiform activity was assessed in some slices by applying groups of 10 stimuli at twice threshold before and after the microdissection.

After the electrophysiological recording, slices were immediately fixed in 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.4, cryoprotected by immersion in 30% sucrose in phosphate-buffered saline until they sank ( $\sim 15$ –30 min) and then resectioned on a freezing microtome at 50  $\mu\text{m}$ . Sections were Nissl-stained so that the histology of the lesion could be related to the electrophysiological results.

## Calculations

The incidence of epileptiform activity was measured in one to five slices from each rat (Fig. 1). The number of slices examined varied because of the time required for different subsequent experimental procedures and, in some cases, because a limited number of slices contained a microgyrus. The average number of slices per rat however was similar for different age groups (3.0, 2.4, and 2.9 slices per rat for age groups P9–P10, P12–P40, and >P40, respectively, *not* significantly different, by *t*-tests). Only slices in which stimuli evoked graded, short-latency field potentials of  $\geq 0.6$  mV in amplitude were used. In these slices, at least four sites on either side of the microgyrus were tested at a number of stimulus intensities.

To determine the laminar site of origin of epileptiform activity, latency to onset and peak amplitude were measured using an array of  $\leq 16$  electrodes spaced at 107- $\mu\text{m}$  intervals. Epileptiform activity onset was identified in single stimulus presentations as the first point after the evoked, graded, short-latency field potential that was 1 SD below (more negative than) the mean voltage of the baseline period. Only slices in which there was a clear time difference between the end of the graded short-latency field potential and the onset of epileptiform activity were used for these calculations.

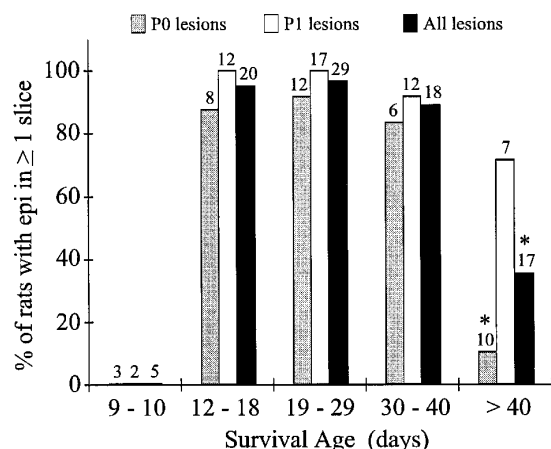


FIG. 1. Incidence of epileptiform activity in rats receiving a transcranial freeze lesion on either P0 (day of birth) or P1. Survival age: age of the rat at the time of in vitro field potential recordings. No epileptiform activity was evoked in slices from P9 to P10 rats. For rats >P40, significantly less epileptiform activity was evoked in P0 and All lesions groups, relative to rats P12–P40. Significantly less epileptiform activity also was evoked for P0 lesions relative to P1 lesions, in rats >P40. \*, *z* tests,  $P < 0.05$ . Number of rats in each group indicated above bars.

tions (8 of 10 slices examined). The minimum latency within layers II/III, V, and VI was measured for each slice, and the values averaged to obtain mean minimum latencies for each layer. The peak amplitude of epileptiform activity was determined at each recording site from the largest negative deflection following the graded short-latency field potential. The peak values for layers II/III, V, and VI were calculated for each slice and then the values averaged to obtain the mean peak epileptiform activity for each layer. Nissl-stained sections from each slice were used to identify the laminar location of each electrode. The laminar profile for amplitude of epileptiform activity did not vary from one stimulus presentation to the next, therefore all final calculations were made from a single sweep for each slice.

The time course of the AP5 effect was measured for each slice, by calculating the integrated area of the epileptiform activity (microvolts  $\times$  milliseconds) occurring after the short-latency response and then normalizing to the average area of epileptiform activity recorded before AP5 application. Before AP5 application, epileptiform activity occurred on every stimulus presentation (0.033 Hz) for all slices. The integrated area of each response, before, during, and after AP5 perfusion slices was averaged across seven slices (Fig. 10).

## RESULTS

### Incidence

We have previously shown that late multiphasic discharge, characterized as interictal-like epileptiform activity, can be evoked in slices from freeze-lesioned rats (FL slices) (Jacobs et al. 1996a). Overall, 77.8% of 90 rats, aged P9–P171, had evoked epileptiform activity in at least one slice. Spontaneous epileptiform events were observed occasionally; however, the incidence of such activity was not examined systematically. The incidence of evoked epileptiform activity varied according to the rat's age at recording time. Although the microgyrus is developed fully by P10, as assessed histologically (Dvorak and Feit 1977), we never observed epileptiform activity in slices from rats aged P9 or

P10 (Figs. 1 and 2A). In these slices, typical graded, short-latency field potentials were evoked by a series of incrementing stimuli (Fig. 2A, 1–3). The amplitude of these field potentials was similar to that of field potentials evoked in older rats (Fig. 2). The incidence of epileptiform activity was 82.3% in rats aged P12–P171 and 94% in rats aged P12–P40. There was significantly less epileptiform activity in rats >P40 (35%, Fig. 1, *z* test,  $P < 0.05$ ) compared with rats P12–P40. This was primarily due to a significantly lower incidence of epileptogenesis for rats lesioned on P0 versus P1. The incidence of epileptiform activity in rats studied at >P40 was 10 and 71% for groups lesioned at P0 and P1, respectively (*z*-test,  $P < 0.05$ ).

There was not a clear correlation between the incidence of epileptiform activity within a particular slice and the histological characteristics of the microgyrus, observed from Nissl-stained sections (however, cytoarchitecture of the microgyrus may contribute to whether epileptiform activity is observed within the microgyrus itself, see following text and Fig. 6). Slices that clearly contained the typical form of a microgyrus did not always generate epileptiform activity (Fig. 3, A and D), whereas some slices in which the microgyrus was less distinct were epileptogenic (Fig. 3B). The aberrant lamination typical for the microgyrus was present in P9–P10 rats as well as those older than P40 that were lesioned on P0 (Fig. 3, A and D); however, these two groups were ones that had a significantly lower incidence rate, despite their histological similarity to slices from rats aged P12–P40 (compare Fig. 3, A and D with C). In addition to aberrant lamination, the neocortex of freeze-lesioned rats occasionally contains ectopic nests of cells in layer I adjacent to or within the microgyrus (Jacobs et al. 1996a). In 48 slices from which field potential recordings were made, we examined the Nissl-stained sections for the presence of ectopic nests in layer I. Fourteen of 48 slices contained ectopia, and in 7 of the 14 epileptiform activity could be evoked adjacent to the microgyrus, whereas no abnormal field potentials were recorded in the other 7 slices.

Characteristics of the epileptiform activity evoked at different ages were quite similar. As previously reported (Jacobs et al. 1996a), the abnormal discharges varied in form, latency, and duration and typically were evoked only by threshold and just above threshold stimuli. In rats aged P20–P22, however, epileptiform activity often could be evoked by higher stimulus intensities (example shown in Fig. 2C). In 69% of slices from rats aged P20–P22, epileptiform activity was evoked by stimuli that were 16 times threshold ( $n = 15$ ); such stimuli were effective in only 31% of slices from P12 to P14 rats ( $n = 16$ ) and 7% of slices from rats aged P30–P35 ( $n = 15$ ).

### Region of epileptogenesis

The region from which epileptiform activity could be evoked was mapped by moving a pair of electrodes together to sites at various distances from the microsulcus. The pair consisted of a single recording electrode within layer III, situated directly above (on-column with) a single stimulating electrode within layer VI. Previous results had suggested that the epileptogenic zone was focal and located within a few millimeters of the microgyrus but not within the micro-



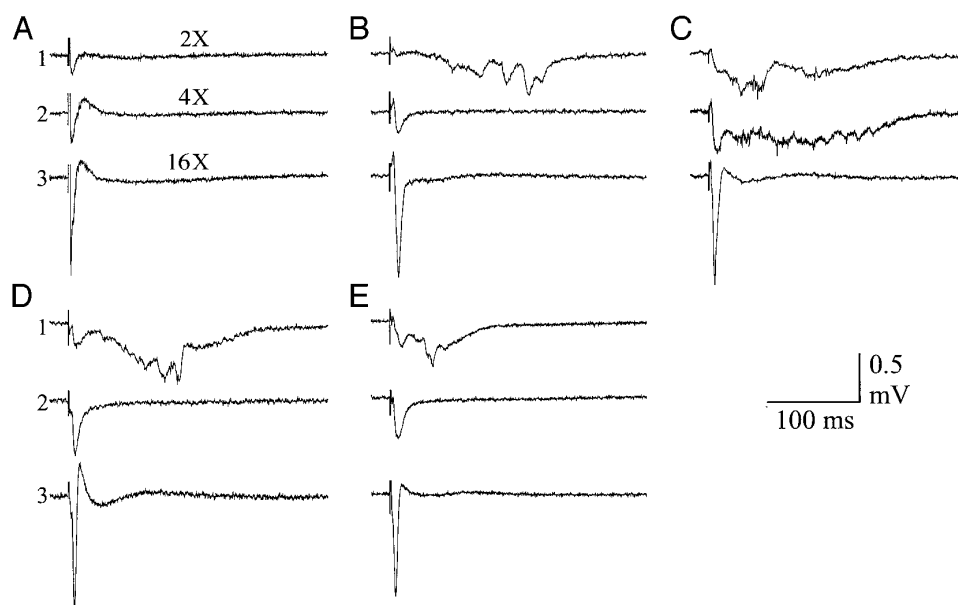


FIG. 2. Field potentials evoked 1.0 mm from the microsulcus in slices from freeze-lesioned rats. Traces 1–3 in A–E show responses to individual stimulus presentations of increasing stimulus intensity: 2, 4, and 16 times threshold. A: P9 rat. Slice shows evoked responses typical of those from rats <P12. No epileptiform activity was evoked even though the stimulus intensity series evoked short-latency field potentials with an amplitude similar to that seen in slices from older rats. B–E: long latency epileptiform activities evoked in rats P12 and older by 2 times (B–E) and 4 times (C) but not 16 times threshold stimuli. B: P16; C: P21; D: P34; E: P53.

gyrus itself (Jacobs et al. 1996a). In the larger sample, obtained from the current experiments, epileptiform activity was evoked *focally* in 82% ( $n = 45$ ) of slices mapped in this way (see, for example, Fig. 4, A and B). In most of these slices, epileptiform activity could be evoked at sites 0.5–1.4 mm (Fig. 4C) but not at sites  $\geq 2.5$  mm from the microsulcus. In 8 of 45 slices, epileptiform activity was evoked at all sites tested outside of the microgyrus,  $\leq 4$  mm from the microsulcus (“nonfocal” slices). Seven of the eight nonfocal slices were from rats aged P20–P21, and one was from a rat aged P14. This suggested that the cortical area from which epileptiform activity could be evoked might vary with age. To test this, we examined the percentage of slices with epileptiform activity at various distances from the microsulcus for different age groups. The *focal* P20–P21 group was not different from the *focal* P12–P16 group; both had the highest incidence of epileptiform activity 0.5–1.4 mm from the microsulcus (Fig. 4C). Slices from rats  $\geq P27$ , however, had the highest incidence of epileptiform activity at more distant sites (1.5–1.9 mm from the microsulcus, Fig. 4C). The incidence of epileptiform activity at 1.5–1.9 mm was significantly different for the older rats relative to the focal P12–P16 and P20–P21 age groups ( $z$  tests,  $P < 0.05$ ). We measured the extent of the microgyrus itself in the slices from older rats, to test whether typical brain growth might account for these age-dependent differences in the epileptogenic zone. Measurements were made from the microsulcus (identified at the pia) to the furthest border of the microgyrus, which was in layer 3 of the malformation (see Fig. 3C, arrowheads), and thus covered one side or half of the microgyrus. Measurements were made over the half of the microgyrus from which field potential recordings were made. This cortical distance was similar for the three age groups:  $0.35 \pm 0.03$ ,  $0.34 \pm 0.04$ , and  $0.38 \pm 0.04$  mm for P12–P16 ( $n = 12$ ), P20–P21 ( $n = 10$ ), and  $\geq P27$  ( $n = 10$ ), respectively. Epileptiform activity rarely was evoked within the microgyrus itself at any age. When both stimulating and recording electrodes were placed within the microgyrus only short-latency graded field potentials, without epi-

leptiform activity were evoked in 83.3% of 30 slices tested. Under these recording conditions, we did not determine whether epileptiform activity was evoked at sites outside of the microgyrus during stimulation within the microgyrus (see following paragraphs).

It previously has been reported that slices from normal immature rats have long-latency, multiphasic, NMDA-dependent, all-or-none field potentials similar to those found adjacent to the microgyrus (Luhmann and Prince 1990b). We mapped the distribution of such responses in eight slices from three control rats, aged P13–P14, in the same way that we mapped the epileptiform activity adjacent to the microgyrus: a single recording electrode within layer III and a single stimulating electrode within layer VI directly beneath the recording site were moved in steps of 0.5–1.0 mm along lines parallel to the pia, within somatosensory neocortex. In five slices in which we tested four to eight sites and three slices in which two distant sites were stimulated, the long-latency multiphasic field potentials were present at all locations and thus were never focal.

To identify the cortical region with the lowest threshold for initiating the epileptiform activity, the latency of onset and the stimulus intensity required to produce the epileptiform activity (epi-threshold, see METHODS) were measured at various distances from the microsulcus. Because the onset latency may vary from one stimulus presentation to the next, we used a multiple electrode array to record simultaneous field potentials from 14 different sites (see METHODS). The array was placed within layer III, parallel to the pia, with the first two to three electrodes within the microgyrus and the remaining recording points extending within this lamina to  $\sim 1.5$  mm from the microsulcus (see Fig. 5A). The stimulating electrode always was placed within layer VI. Both single recording electrode experiments and those with the electrode array were used to map the epi-threshold for evoking epileptiform field potentials relative to the distance of the stimulating electrode from the microsulcus. Epi-threshold stimulus intensity (microseconds  $\times$  microamperes) was normalized for each slice to the largest value for any stimulus

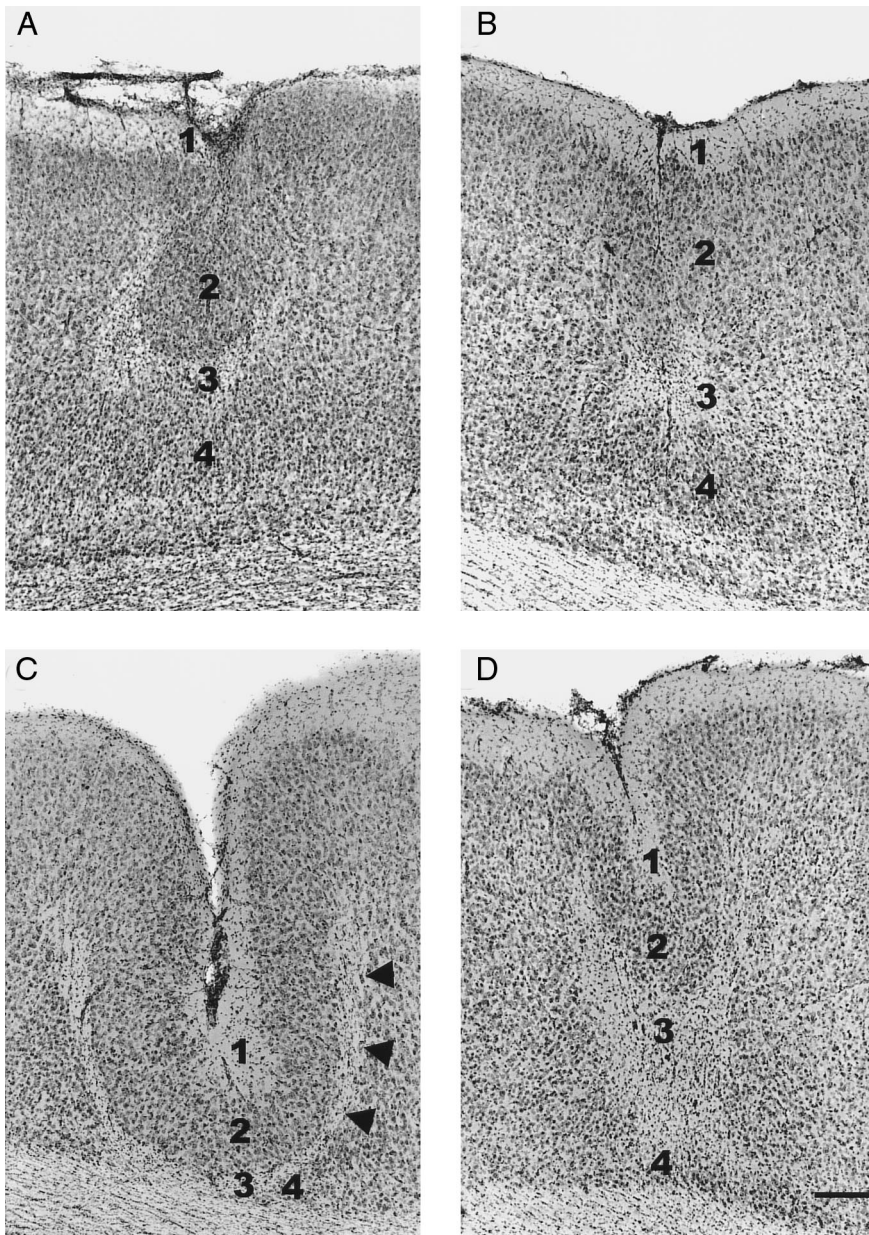


FIG. 3. Examples of Nissl-stained sections from rats of different survival ages. Slices were fixed and resectioned after evoked field potential recordings were made. Numbers identify microgyral layers. *A*: section from a P9 neocortical slice in which short-latency field potentials but no epileptiform activity was evoked. *B*: section from a P30 slice in which epileptiform as well as short-latency fields were evoked. Appearance is relatively atypical in that there is only a small region of laminar disruption and the layers are not "garlanded" (see text). *C*: section from a P36 rat, lesioned on P0. Lamination pattern is somewhat different from that shown in *B*, but epileptiform activity similar to that recorded from the slice represented in *B*, was evoked adjacent to the microgyrus. Arrowheads show abrupt border between layer 3 of the microgyrus and the adjacent 6-layered cortex. *D*: section from a P113 rat, lesioned on P0. Although lamination pattern is similar to that in *C*, no epileptiform activity was evoked in any of the slices from this rat. Scale bar = 0.2 mm, for *A–D*. *A–D* are representative sections from each microgyrus.

location within that slice. The location of the site with the lowest epi-threshold for epileptogenesis was dependent on the age of the rat. In rats  $\leq$ P25, the site with the lowest epi-threshold was 0.5 mm from the microsulcus, whereas in rats  $\geq$ P30, it was 2.0 mm from the microsulcus (Fig. 4*D*). These age groups showed significant differences in epi-threshold at 0.5, 1.0, and 2.0 mm from the microsulcus (*t*-tests,  $P < 0.05$ ).

Simultaneous recordings with the electrode array placed horizontally also allowed for identification of the probable region of initiation of epileptiform activity. The shortest latency, largest amplitude, and fastest epileptiform activity typically occurred simultaneously at 2–4 electrodes (Fig. 5, *D–G*; electrodes 10–13). When the stimulating electrode was moved to different distances from the microsulcus, the actual latency to epileptiform activity varied but was shortest

at the same 2–4 electrodes (initiation zone, Fig. 5, *D–G*) in 10 of 12 slices. For rats aged P12–14, the center of this initiation zone was  $\leq 0.6$  mm from the microsulcus ( $n = 3$ ), whereas in slices from older rats (P21–40), the initiation zone was centered  $\geq 1.4$  mm from the microsulcus ( $n = 6$ ). The mean distance from the center of the initiation zone in which the shortest latency epileptiform activity was evoked to the microsulcus was significantly greater in slices from the older age group ( $1.58 \pm 0.05$  mm) than in the P12–P14 age group ( $0.48 \pm 0.09$  mm; *t*-test,  $P < 0.05$ ).

The direction of propagation was not always consistent even within a particular slice. In some cases, "reflections" of activity back to the same location, similar to those reported for bicuculline-induced epileptiform activity (Chagnac-Amitai and Connors 1989a), were observed (Fig. 5, *D* and *F*, electrodes 8–13). Corresponding peaks in the



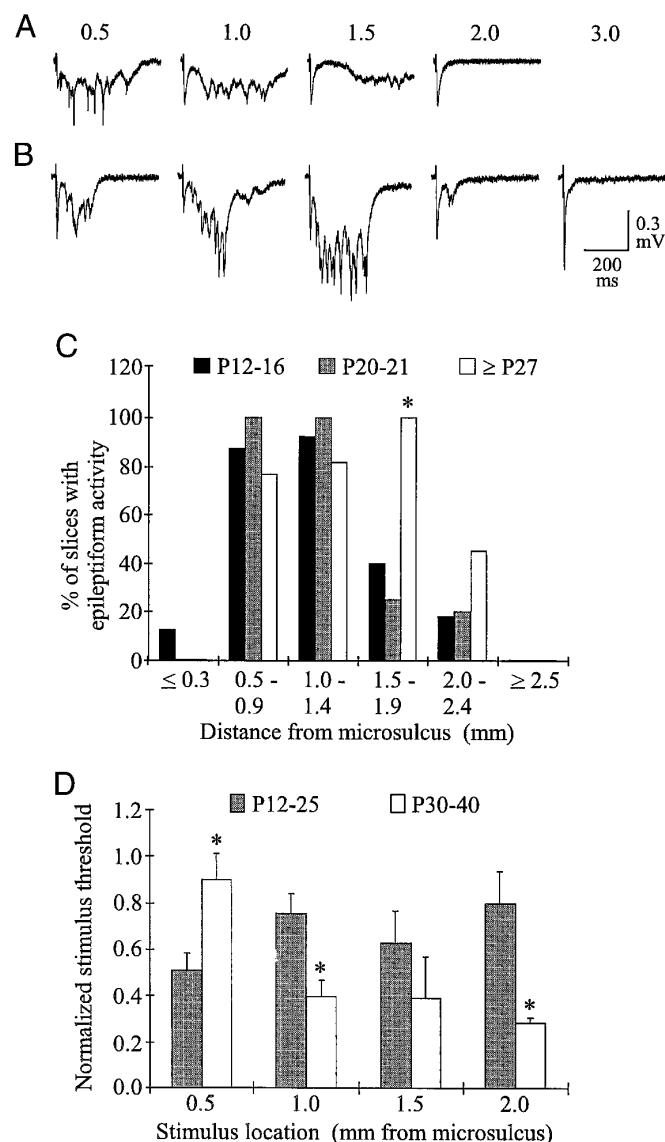


FIG. 4. Focal nature of epileptogenesis in freeze-lesioned rats. *A*: responses to stimulation at 2 times threshold in a slice from a P14 rat. Numbers above traces indicate recording distance in millimeters from microsulcus for *A* and *B*. *B*: responses to stimulation at 2 times threshold in a slice from a P30 rat. *C*: bar graph shows percentage of slices in which epileptiform fields were evoked at various distances from the microsulcus. Data shown are for the 37 of 45 slices with focal epileptiform activity. At distances of 1.5–1.9 mm, slices from rats  $\geq$ P27 had significantly more epileptiform activity than slices from younger rats. \*,  $z$  tests,  $P < 0.05$ . For P12–16, P20–21, and  $\geq$ P27,  $n = 15, 9$ , and  $13$ , respectively. *D*: lowest stimulus intensity able to evoke epileptiform activity (epi-threshold) at different stimulus locations within layer VI. Epi-threshold stimulus intensity ( $\mu s \times \mu A$ ) normalized for each slice, to the maximum value for that slice. Epi-thresholds for slices from rats P12–P25 were significantly lower at 0.5 mm and significantly higher at 1.0 and 2.0 mm from the microsulcus than for slices from rats P30–P40,  $t$ -tests,  $P < 0.05$ .

epileptiform activity were identified in different recording channels on the basis of their shape and position. To calculate the speed of propagation, latency measurements between peaks were divided by the distance between the electrodes at which the peaks were observed. Mean speed of propagation for each slice was calculated from two to three stimulus presentations. The number of calculations that could be made

was limited by the number of clearly identifiable peaks at different electrodes. Propagation velocity, measured in six slices, varied from 9.8 to 78.5 mm/s and by as much as 62 mm/s between stimulus presentations within a particular slice. The overall mean for the six slices was  $23.6 \pm 5.8$  (SE) mm/s. Although slow compared with conduction velocities for both myelinated and unmyelinated axons, these values are in the range of those reported in other models of in vitro epileptiform activity (see Connors and Amitai 1993; Wong and Prince 1990).

Stimulation within the microgyrus evoked epileptiform activity outside of the microgyrus in 8 of 13 slices tested (Fig. 5, *B* and *D*), whereas epileptiform activity initiated by stimuli outside the microgyrus propagated into the microgyrus in 6 of 13 slices (Fig. 5, *E* and *G*). Because the specific histological pattern of the microgyrus also varied in slices from different rats, we determined whether a particular histological characteristic was associated with the presence or absence of epileptiform activity within 0.3 mm of the microsulcus (i.e., within the microgyrus proper). For all of the slices in which epileptiform activity did not propagate into the microgyrus ( $n = 7$ ), there was a striking interruption of the normal laminar pattern, and a U shape to the layers wrapping around the microsulcus (Fig. 6*A*), similar to a pattern in human specimens in which this wrapping of the layers has been called “garlanded” (Crome 1952). In slices where epileptiform activity was observed  $<0.3$  mm from the microsulcus, the normal lamination pattern appeared to continue up to the microsulcus itself (Fig. 6*B*), the microgyrus was less well demarcated and did not have a garlanded appearance.

#### Separation of the microgyrus from the epileptogenic zone

We previously have hypothesized that the epileptogenic zone around the microgyrus (or paramicrogyral generation zone) develops in part as a result of aberrant connections that are formed when axons that ordinarily would synapse on neurons in the missing layers IV–VI lose their targets (Jacobs et al. 1996a). If these afferents maintain laminar specificity and synapse in the region adjacent to the microgyrus (see, for example, callosal afferents depicted in Fig. 11), this zone would be hyperinnervated and, by the time cortical synaptogenesis is complete, might be capable of initiating epileptiform activity independent of its synaptic connections with the microgyrus. To test this possibility, we recorded evoked field potentials in the paramicrogyral epileptogenic zone of slices before and after separating the microgyrus from the recording site with a cut through the depth of cortex (Fig. 7*A*; see METHODS). In 10 of 13 slices from rats as young as P12 and as old as P34, the epileptiform activity returned in an average of  $22.6 \pm 5.6$  min after the cortical transection (Fig. 7). The stimulus intensities at which the epileptiform activity could be evoked were not different before and after the cut. The mean  $\pm$  SE incidence of evoked epileptiform activity per 10 stimulus presentations at twice threshold for all slices tested was  $86.2 \pm 5.7\%$  before and  $72.5 \pm 9.8\%$  after the cortical cut. Sites distant to the microgyrus that had short-latency graded field potentials without epileptiform activity before the transection, had similar fields, without epileptiform activity afterwards. In 3 of

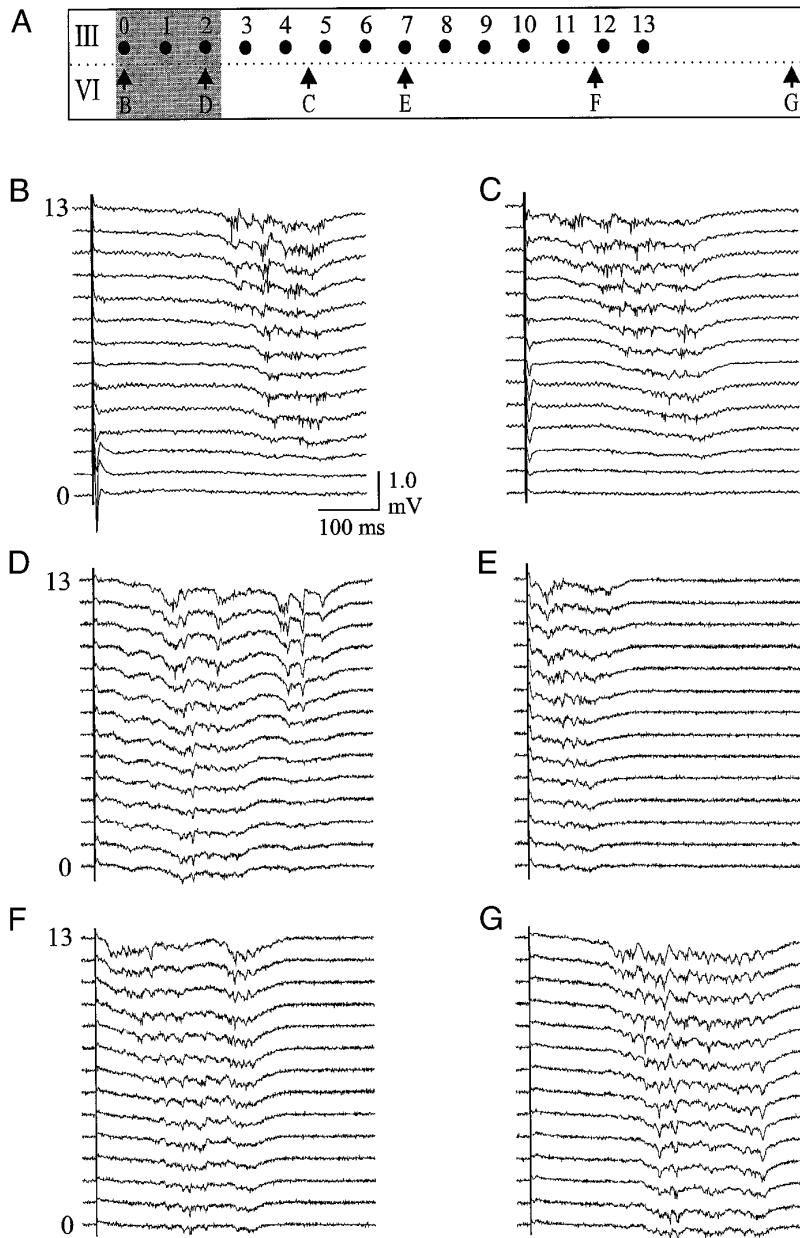


FIG. 5. Zone for initiation of epileptiform activity is distant to the lesion. A: schematic showing electrode positions. Gray area indicates region of microgyrus; numbered circles are sites recorded simultaneously with a 14-channel electrode within layer III; lettered arrows represent independent stimulus locations within layer VI. B–G: responses evoked by stimuli delivered at points B–G in diagram of A. B and C from one P21 slice, D–G from a P25 slice. B: stimulation within the microgyrus evokes epileptiform activity outside of the microgyrus that does not propagate into the microgyrus at electrodes 0–2. C: stimulation outside the microgyrus produces a pattern of epileptiform activity similar to that in B. D–G: shortest latency and largest amplitude epileptiform activity occurs at the recording sites furthest from the microsulcus (electrodes 11–13) for stimuli at sites within, near, or far from the microgyrus (positions D–G).

13 slices the epileptiform activity did not return  $\geq 45$  min after the transection. It is possible that these three slices, all from rats  $>30$  days old, would have recovered if more time had elapsed because the recovery of epileptiform activity required significantly more time in slices from older rats ( $13.7 \pm 6.6$  min in slices from P12–P22 rats,  $n = 7$ , and  $43.3 \pm 15.3$  min in slices from P29–P34 rats,  $n = 3$ ,  $t$ -test,  $P < 0.005$ ). On the basis of these results, it may be that epileptiform activity is more labile in older animals or that microgyral connections become essential to the initiation of epileptiform activity in older animals.

#### Laminar pattern of epileptiform and short-latency field potentials

To examine the laminar pattern of epileptiform activity, the multicontact electrode was placed perpendicular to the

pia (vertically, Fig. 8A) at a distance of 0.5–1.5 mm from to the microgyrus, and simultaneous recordings were made from 16 sites spanning the cortical layers in 8 slices. The stimulating electrode was placed within layer VI, either below the electrode array or, when the array spanned the entire depth of cortex, 100  $\mu$ m from the array within layer VI. Epileptiform activity appeared to occur nearly simultaneously in all layers (Fig. 8B), and quantitative analyses showed that there were only small laminar differences in latency and amplitude (see Table 1). The lamina with the shortest latency and that with the largest amplitude epileptiform activity also varied in different slices, but the difference in time of onset of epileptiform activity within different layers in an individual slice was small (see Table 2). Fast ( $< 2$  ms) sharp waves occurred in layer V in 62% of slices ( $n = 8$ ) and were simultaneous with slower epileptiform activity in other layers (Fig. 8C).

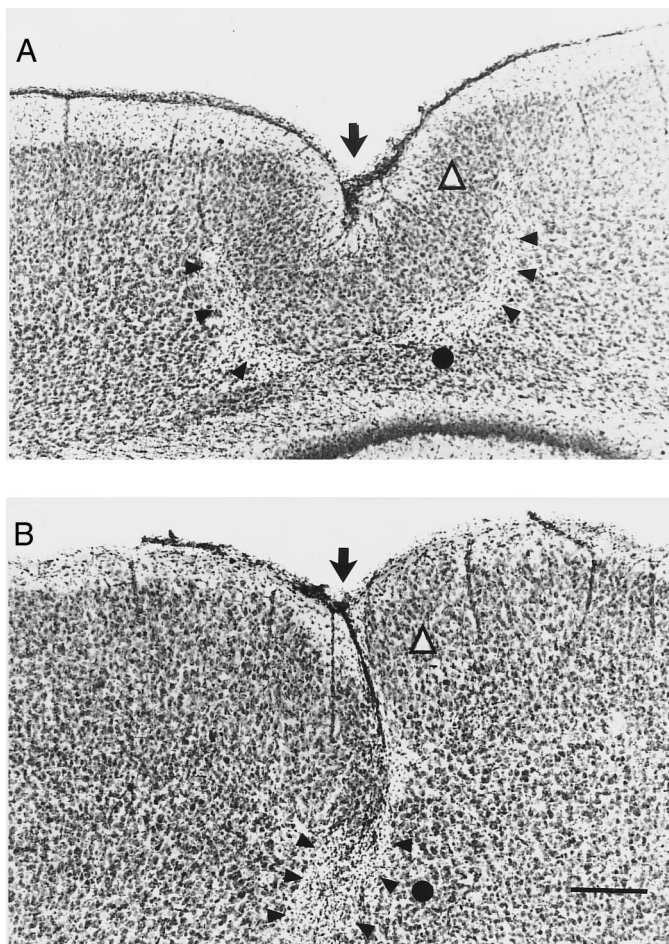


FIG. 6. Typical patterns of lamination for slices with only short-latency field potentials and no epileptiform activity  $\leq 0.3$  mm from the microsulcus (A) and for slices with epileptiform activity  $\leq 0.3$  mm from the microsulcus (B). A: 4-layered microgyrus can be seen interrupting the normal laminar pattern. Epileptiform activity evoked with stimulation outside of the microgyrus did not propagate into the microgyrus. B: slice contains little interruption of normal laminar pattern. Arrow, microsulcus; arrowheads, border of microgyral layer 3; circle, stimulation site; white triangle outlined in black, 1 recording site close to microsulcus. Scale bar = 0.3 mm for A and B.

Examination of laminar profiles of epileptiform activity revealed that the short-latency field potential was also abnormal. Electrodes were placed in FL slices as shown in Fig. 8A and in a homotopic region of cortex for slices from control rats. The initial event in field potentials from layers II/III and V recorded in control slices was a short-latency graded negativity (Fig. 9A). In 10 of 13 FL slices, the field negativity evoked in layers V and VI contained two individual components that were separated by a return of the field potential to near baseline (Fig. 9, B and D). Typically at threshold and twice threshold, only the second of the two negativity peaks ( $FL_{p2}$ ) was observed, whereas at stimulus intensities of 4–16 times threshold, both components were present. In two of six control slices, a small negative “notch” was observed before the peak negativity at stimulus intensities of 4–16 times (Fig. 9C). In the other four control slices, there was no similar separation of components at any stimulus intensity. The peak amplitudes of the control negativity and  $FL_{p2}$  were not significantly different ( $-0.70 \pm$

$0.18$  and  $-0.79 \pm 0.17$  mV, respectively), but  $FL_{p2}$  occurred significantly earlier than the peak negativity of control slices ( $4.15 \pm 0.28$  and  $5.28 \pm 0.45$  ms, *t*-test,  $P < 0.05$ ). In addition, the duration of the field negativities was much shorter in FL slices, lasting 4–5 ms for both components, compared with the control negativity, which lasted 6–10 ms (compare Fig. 9, C with D). The presence of a second peak negativity in FL slices was not related to the age of the rat at the time slices were made because it was observed in slices from rats aged P15–P38.

#### NMDA antagonists

We previously have reported that bath application of the NMDA antagonist, AP5, blocked the epileptiform activity evoked adjacent to the microgyrus. Because a conflicting report was published (Luhmann and Raabe 1996), we examined this issue further. In 10 slices from rats aged P13–39, AP5 always completely eliminated the evoked epileptiform activity (Fig. 10). With low intensity stimulation, epileptiform activity was evoked in each slice by every stimulus before AP5 application. Epileptiform responses were entirely eliminated after 10 min of AP5 perfusion (fig. 10A). However, in 6 of 10 experiments we found that higher intensity stimulation was still able to evoke the epileptiform activity. Increasing the concentration of AP5 to  $100 \mu\text{M}$  blocked the epileptiform activity at all stimulus intensities. Responses monitored during reperfusion with normal ACSF showed that epileptiform activity returned after 65–115 min of AP5 washout ( $n = 4$ , Fig. 10, A and C).

#### DISCUSSION

The rat freeze-lesion model of microgyria consistently shows epileptiform activity and is therefore appropriate for the study of mechanisms contributing to epileptogenesis associated with human polymicrogyria. The principal findings of these experiments include age-dependent incidence, time-dependent changes in the underlying epileptogenic processes, location of an epileptogenic zone adjacent to the microgyrus and support for the concept that the malformation itself is the inciting lesion but not the generator of epileptiform activity, abnormal graded short-latency field potentials in the paramicrogyral zone, and involvement of NMDA receptors in maintaining the epileptiform activity.

#### Age-dependent characteristics

The ease with which the experimental cortical malformation can be induced in a high percentage of lesioned animals and the similarity in histopathology to human microgyria provide an opportunity to explore some important phenomenology similar to that found after epileptogenic lesions in man. The overall incidence of epileptiform activity in the microgyrus model is 78%, a value similar to that estimated for the clinical incidence of epilepsy in patients with polymicrogyria (70–80%) (Barkovich and Kjos 1992). This, of course, may be pure coincidence, although there are other features that the experimental and clinical disorders have in common, including the abnormal laminar pattern and a latency to the appearance of epileptogenic activity. The onset of seizures associated with polymicrogyria in humans typi-



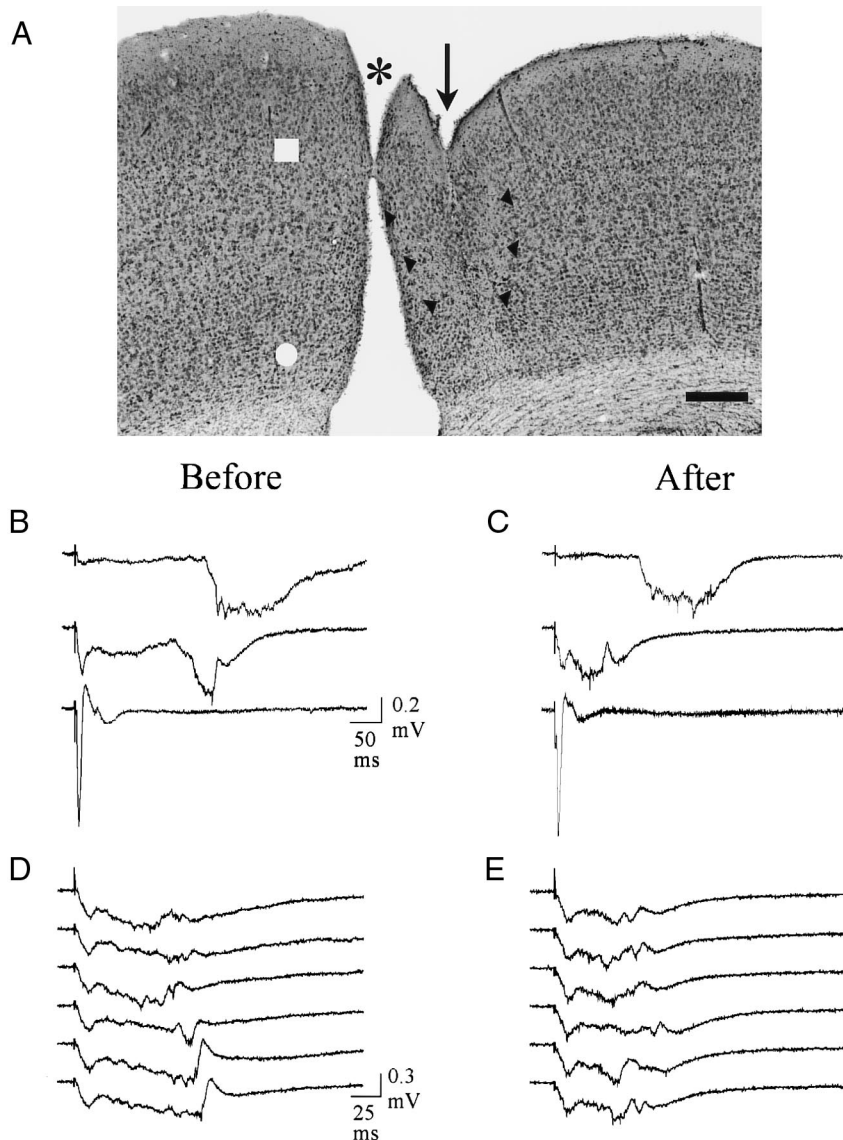


FIG. 7. Effect of separating the microgyrus from the region of epileptogenesis. *A*: Nissl-stained 40- $\mu$ m section from a P24 slice in which a transcortical cut was made from pia to white matter between the recording site and the microgyrus (arrowheads). Arrow, microsulcus; arrowheads, microgyrus; square, recording site; circle, stimulation site; asterisk, transcortical cut. Scale bar = 0.25 mm. *B* and *C*: responses from slice of *A* to on-column stimulation of layer VI at threshold, 2 times, and 16 times threshold intensities before (*B*) and 25 min after the microdissection (*C*). *D* and *E*: 6 consecutive stimuli (0.05 Hz) at 2 times threshold before (*D*) and 28 min after the microdissection (*E*).

cally is in early childhood (Barkovich et al. 1992; Mischel et al. 1995) but can be delayed for years and may not be present until adulthood (for example, see Raymond et al. 1995).

The expression of epileptiform activity in the rat microgyrus model, and in certain types of clinical seizure disorders occurring during infancy and childhood, such as infantile spasms (Koo et al. 1993), may require that a particular stage of brain maturation be reached. It is currently not known what specific process(es) correlates with this timing and would serve as an enabling factor for the occurrence of epileptogenesis. There are ongoing changes in synaptogenesis, spine density (Wise et al. 1979), spine distribution (Miller 1981), neuronal somal size (Miller 1986), and refinement of axonal arbor distributions (Uozumi et al. 1988). It is possible that quantitative rather than qualitative changes in some developmental parameter are required. For instance, a correlation has been demonstrated between the number of synaptic contacts per neuron and the development of the placing reflex (see Eayrs and Goodhead 1959). Although

the specific contributions of each of these developmental processes to the evolution of interictal spiking in neocortex is uncertain, there are two functional developmental events that occur in normal rat cortex during the second week of life that might contribute to the abrupt onset of epileptogenesis in the area around the microgyrus. First, intrinsically bursting (IB) neurons, which have been proposed as critical elements in generating and propagating epileptiform activity within neocortex (Amitai et al. 1993; Chagnac-Amitai and Connors 1989b; Connors 1984; Gutnick et al. 1982) are absent in P8–P10 cortex (Franceschetti et al. 1993; Hoffman and Prince 1995) and appear abruptly at about P14 (Franceschetti et al. 1993). Although epileptiform discharges in P8–P10 cortex can be elicited by a convulsant drug before the development of burst-generating capacities in layer V neurons (Hablitz 1987; Hoffman and Prince 1995), burst generation may well be a contributing factor under other circumstances. The second functional developmental event is the strong expression of polysynaptic activity mediated by NMDA receptors, beginning about P11 in rat cortex

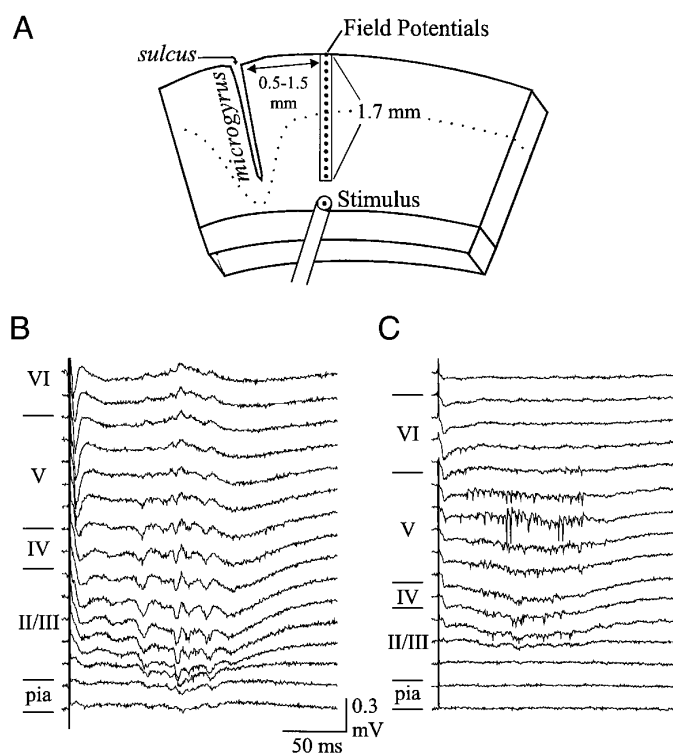


FIG. 8. Pattern of epileptiform activity recorded simultaneously at 16 sites through the cortex in 2 slices (B and C). A: schematic diagram illustrating location of stimulating electrode, and multichannel recording electrode. Array was positioned 0.5–1.5 mm from the microsulcus. B: epileptiform activity appeared to occur simultaneously throughout layers but with a larger amplitude in layer II/III. C: sharp waves are seen within layer V, simultaneous with slow negative fields in superficial layers.

(Luhmann and Prince 1990a,b). Changes in subunit composition of NMDA or other types of glutamate receptors during development, or changes in receptor density (Monyer et al. 1994, 1991; Sheng et al. 1994) also could be a contributing factor to the sudden onset of epileptogenesis in this model. Epileptiform events can be eliminated with NMDA receptor blockade (Fig. 10), suggesting that activation of these receptors may mediate the abnormal field potentials. Using the same rat microgyrus model with multiple rather than single freeze lesions, Luhmann and Raabe (1996) found that 30  $\mu$ M AP5 was ineffective in blocking the epileptiform activity. The difference between their results and ours may be due to different underlying pathologies, the concentration of antagonist applied, or the intensity of stimulation used to activate the epileptiform activity.

TABLE 1. Laminar differences in epileptiform field latency and amplitude

Layer	Mean Minimum Latency, ms	Mean Peak Amplitude, mV
II/III	63.7 $\pm$ 18.8	-0.32 $\pm$ 0.15
V	62.3 $\pm$ 15.1	-0.26 $\pm$ 0.10
VI	69.4 $\pm$ 19.1	-0.20 $\pm$ 0.10

Measured in seven of eight slices. One slice had epileptiform activity only within layer V. No significant differences between lamina (1-way analysis of variance). Values are means  $\pm$  SE.

TABLE 2. Laminar detection of epileptiform activity

Slice Number	Largest Amplitude	Shortest Latency	Latency Difference Between Lamina
134913	II/III	V	1.92
234438	II/III	V	2.88
135024	II/III	V	0.4
134438	II/III	VI	0.96
134991	II/III	VI	0.48
135313	V	II/III	0.48
135307	V	II/III	40.8
235255	V*	V*	*
Mean $\pm$ SE	—	—	6.85 $\pm$ 5.67

\* Epileptiform activity only occurred within layer V in this slice.

### Time-dependent alterations in epileptogenic characteristics

Aside from the rather sudden maturation of a capacity to generate epileptiform discharge at P12 discussed above in *Age-dependent characteristics*, there are several other indications of an ongoing functional cortical reorganization or continual adaptation that occurs over time in the area of the microgyrus. These findings include changes in the site with the lowest threshold for evoking epileptiform activity and in the site(s) with the highest incidence of epileptiform activity across all slices mapped. For both of these measures, there is a shift to areas further from the microgyrus in older rats (Fig. 4, C and D). There are two indications that this is not simply due to brain growth and expansion of the microgyrus: measurements within the electrophysiologically

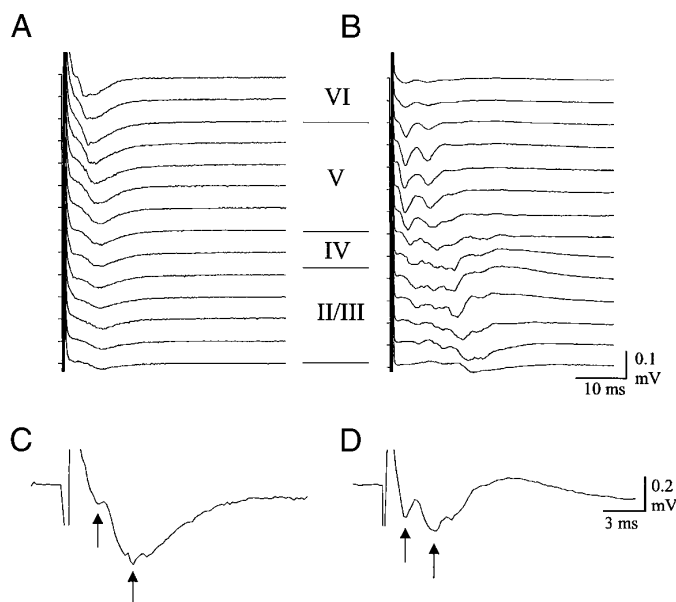


FIG. 9. Effect of freeze lesion on the short-latency field potential. Field potentials were recorded simultaneously, with multichannel electrode array positioned as in Fig. 8A. Each trace in A–D is the average response to 3 stimulus presentations. A: field potentials recorded in a control slice. B: field potentials recorded in a slice from a freeze-lesioned rat. C: control field potential recorded in layer VI contains small negative ‘notch’ (1st arrow) before peak negativity (2nd arrow) but without a return to baseline between these 2 components. D: field potential recorded in layer VI of epileptogenic slice shows return of field potential toward baseline between peaks shown with arrows.

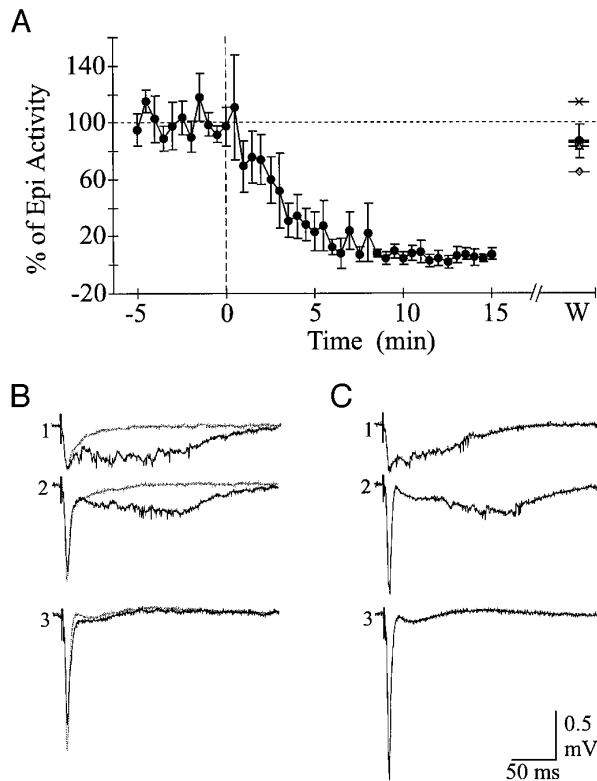


FIG. 10. Effect of *N*-methyl-D-aspartate (NMDA) antagonists on epileptiform activity evoked in slices from freeze-lesioned rats. *A*: time course of the effect of NMDA antagonist D,L-2-amino-5-phosphonovaleric acid (AP5) on the epileptiform activity evoked at threshold or 2 times threshold in 7 slices. AP5 (50  $\mu$ M) was bath applied for 10–30 min beginning at time 0. Recovery of the epileptiform activity after bath application of control artificial cerebrospinal fluid (ACSF) was monitored in 4 of the 7 slices and occurred at variable rates. Gray symbols above W (wash) indicate the amount of recovery for each of those 4 slices, and the black symbol indicates the average recovery. Percentage of epi activity: [Average integrated area of epileptiform activity ( $\text{mV} \times \text{ms}$ ) during AP5 perfusion/average area before AP5 perfusion ( $\text{mV} \times \text{ms}$ )]  $\times 100$  (see METHODS). *B* and *C*: series of 2, 4, and 8 times threshold stimulus intensities applied before (dark lines, *B*, 1–3, respectively), and during AP5 perfusion (gray lines, *B*, 1–3), and after washout of AP5 (*C*, 1–3). Responses during AP5 application were measured 27 min after adding AP5 to the bathing medium. No epileptiform activity was evoked at any stimulus intensity, after 15 min of AP5 application. Responses in *C* were recorded after 65 min of ACSF wash.

mapped hemispheres demonstrated that the average distance from microsulcus to the onset of typical six-layered cortex was not significantly different in younger and older age groups and in both the oldest and younger age groups, epileptiform activity was never evoked at sites  $\geq 2.5$  mm from the microsulcus (Fig. 4C). The shifts within the epileptogenic zone, although not likely due to a shift in microgyral size, still could be related to normal growth of cell processes and increasing connectivity between the initially affected cortical area and normal cortical and extracortical afferents.

An additional factor that suggests ongoing functional reorganization is the significant decrease in incidence of evoked epileptiform activity in rats  $>P40$  that were lesioned on P0 (Fig. 1). This may be due to the influence of normal cortical developmental processes such as pruning of excess excitatory connectivity (Jones and Schallert 1992; O'Leary 1992; O'Leary and Stanfield 1985) or maturation of GABAergic circuits (Chronwall and Wolff 1980; Luhmann and Prince

1991; Oh et al. 1995). Other results suggest that inhibitory neurons in the epileptogenic zone around the microgyrus may be hyperinnervated by fibers contacting glutamatergic receptors, thus enhancing inhibition (Jacobs et al. 1996b). Sprouting of GABAergic terminals (Prince et al. 1997) or other types of upregulation of GABAergic neurons (Feldblum et al. 1990) also could enhance functional inhibitory circuitry over time and depress epileptogenesis. This temporal decrease in the occurrence of epileptiform activity has been reported in other chronic models such as freeze lesions made in the mature cortex (Lewin 1972), cobalt foci (Dow et al. 1962; Kusske et al. 1972), and some cases of human partial epilepsy (Hauser and Kurland 1975).

The difference in incidence of epileptogenesis in mature ( $>P40$ ) rats that is dependent on whether pups were lesioned at P0 or P1 emphasizes the fact that similar brain lesions may have strikingly different consequences if they occur at different stages of cortical development. Because the cortical plate is not fully developed at birth, fewer neurons are present within it on P0 than on P1, so that a lesion is likely to cause more cell injury and loss on P1. It is also possible that the later lesion affects some critical group of neurons not in place on P0. For example, a lesion on P1 would affect a greater number of layer IV cells than that on P0, perhaps leading to more extensive retargeting of thalamocortical fibers into the epileptogenic zone.

### Epileptogenic zone

In the majority of slices, stimuli could evoke abnormal activity only from a focal cortical area, suggesting that specific epileptogenic mechanisms are present in a discrete region of neocortex, as is perhaps the case in human epilepsy associated with a focal region of polymicrogyria (Barkovich and Kjos 1992). Except for a few slices in which epileptiform activity could be evoked from all sites tested, the paramicrogyral generation zone ended abruptly  $\sim 2.5$  mm from the microsulcus. The extent of this zone may reflect the spatial extent of abnormal afferents or intracortical connectivity.

The paramicrogyral generation zone may be different from the region over which the epileptiform activity propagates once triggered. Using the same rat microgyrus model, Luhmann and Raabe (1996) have shown that epileptiform activity can be observed  $\leq 3.6$  mm from the stimulation site. We did not specifically examine the spatial extent of propagation; however, it was common for evoked epileptiform activity to be observed in all channels of our multielectrode array, which spanned a distance of 1.7 mm horizontally (parallel to the pia) within layer II/III. The epileptogenic zone was always adjacent to the malformation and sometimes included the dyslaminated cortex itself. Even when epileptiform activity did propagate to cortex within a few hundred micrometers of the microsulcus, it appeared to be initiated in the adjacent cortical zone because the onset latency was shorter and the epi-threshold stimulus level lower in this paramicrogyral region. Indeed, the fact that the microgyrus need not be connected to the adjacent cortex for the epileptiform activity to occur (Fig. 7) further supports the idea that the paramicrogyral zone contains the maldeveloped structures or connections that initiate the interictal activity.



Thus it is not surprising that variations in histopathology of the microgyral region did not correlate with the likelihood of evoking epileptiform activity within the paramicrogyral zone (see Fig. 3).

In some human patients with microgyral malformations, EEG abnormalities arise from the region adjacent to the most severely malformed cortex (Leblanc et al. 1996). Palmini et al. (1991) have found that the most important factor in predicting a favorable outcome of epilepsy surgery for microdysgenesis is the amount of cortex resected. Resecting an area that contains ictal activity on the electrocorticogram and is larger than the extent of the lesion observable on MRI dramatically improves the outcome, as measured by frequency of seizures (Palmini et al. 1994). No study specific to polymicrogyria has been reported thus far; however, the surgical technique necessary for elimination of seizures is likely to be highly dependent on the type of malformation. Many malformations lumped under the term microdysgenesis are thought to be due to abnormal neuronal migration. Pathologies such as displaced and abnormally differentiated neurons, and variations in the topography of involved brain structures likely result in underlying seizure mechanisms that are different from those found in developmental lesions, such as microgyria, where the principle abnormality is elimination of subsets of neurons before cortical neuronal migration is complete. For example, the “balloon” and giant, undifferentiated neurons common in most forms of microdysgenesis, such as those identified by Taylor and colleagues (1971), are absent from most cases of polymicrogyria (Williams et al. 1976). Displaced or abnormally formed neurons might possess abnormal burst firing properties that would contribute to epileptogenesis (Baraban and Schwartzkroin 1995; Sancini et al. 1998). The neurons present within the microgyria are morphologically similar to normal layer II/III neurons, although the cell bodies may be smaller and they may be misoriented (Dvorak et al. 1978; Friede 1989; Williams et al. 1976; see also McBride and Kemper 1982). Ectopic nests of neurons occasionally are found in layer I adjacent to or within the microgyrus, suggesting that aberrations in migration sometimes occur in addition to the primary lesion that causes the microgyrus. It is possible that these displaced neurons contribute to abnormal neuronal activity; however, we found no correlation between the presence of layer I ectopia and occurrence of epileptiform activity in microgyric slices (see RESULTS). Our data suggest that for microgyria the region that produces the epileptiform activity adjacent to the experimental microgyrus may appear histologically normal in cell stains and if the region of the malformation alone is removed, the epileptiform activity will remain.

#### *Hypothetical mechanism of epileptogenesis and aberrant inputs*

Although normal neocortical field potentials may contain several short-latency negative components (Aizenman et al. 1996; Mitzdorf and Singer 1978; Vakhin et al. 1988), the appearance of two sharp negativities separated by a return of the potential toward baseline in FL slices is unusual. The short-duration and sharp peak of the individual components suggests that the underlying synaptic excitation is highly synchronous for each component. The additional field nega-

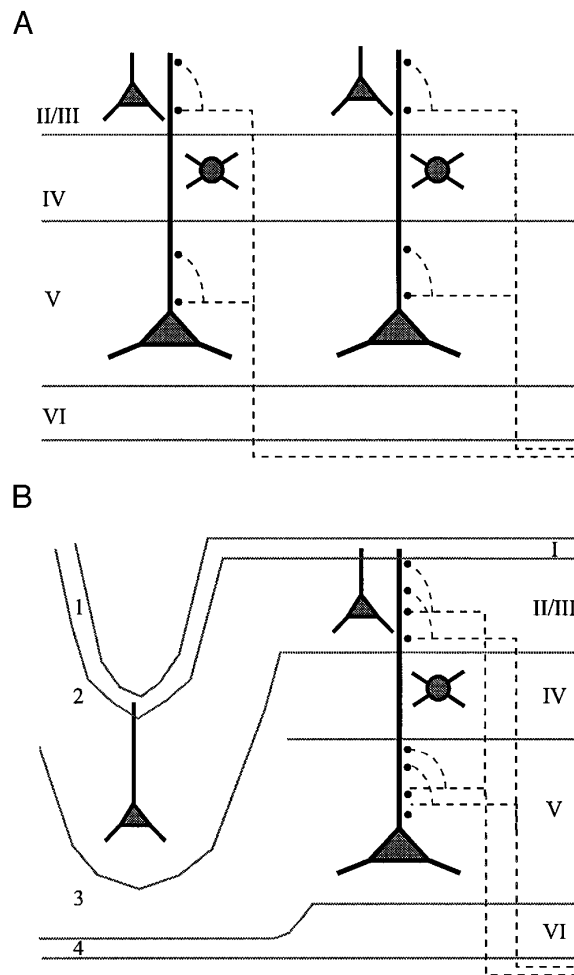


FIG. 11. Cartoon showing hypothetical pattern of aberrant synaptic connectivity occurring during development in response to cortical damage. *A*: normal cortex. — — —, callosal afferents. *B*: cortex containing a microgyrus. After loss of targets normally present in layers IV–VI, axons destined for the microgyrus make contacts with neurons in adjacent cortex, resulting in hyperinnervation.  $\Delta$ , pyramidal neurons;  $\bullet$ , stellate neurons of layer IV. This pattern shown for callosal axons also might occur for other cortical afferents, such as thalamic (in layer IV) and local intracortical axons (in layers II/III and V).

tivity evoked in the paramicrogyral zone suggests that abnormalities in synaptic connectivity occur in this region. An increase in excitatory afferents within the paramicrogyral area, such as that depicted in Fig. 11, especially one leading to an increase in recurrent excitatory activity, could produce this type of synchronous abnormal field potential pattern as well as contribute to the generation of epileptiform activity.

These data suggest that altered development due to focal loss of a subset of neocortical layers results in abnormal synaptic connectivity and the capacity of adjacent cortex to generate epileptiform activity independent of the lesional area itself. We hypothesize that callosal, thalamic, and intracortical afferents, that would normally synapse on the absent neurons, find laminar-appropriate targets in the adjacent region of cortex (Fig. 11). Experiments with cocultures of thalamic and cortical neurons have suggested that thalamic axons grow into the appropriate lamina, independent of the orientation of the two explants (see Bolz et al. 1993; O’Leary

and Koester 1993), and that lamina-specific molecules play an important role in targeting of axons to particular laminae (Castellani and Bolz 1997). If reorganizing afferents select appropriate lamina (or elements of the missing laminae, such as apical dendrites of layer V neurons within layers II/III, see Fig. 11) over originally destined cortical position, an overabundance of excitatory input or a disorganization of afferents in the paramicrogyral zone would result. Available anatomic data support this suggestion. Even small microgyral lesions can give rise to a widespread abnormality in the development of whisker barrels in adjacent somatosensory cortex (Jacobs et al. 1997). Humphreys et al. (1991) have demonstrated disorganized glutamatergic immunoreactive processes in the region adjacent to the microgyrus, a decrease in neurofilament staining within the microgyrus itself, and an increase in the adjacent cortex. Abnormalities in callosal afferents to microgyral cortex have been observed in a spontaneously occurring microgyrus in the rat (Rosen et al. 1989). In addition, thalamic afferents appear to be decreased within the microgyrus (Rosen and Galaburda 1996; V. N. Kharazia, K. M. Jacobs, and D. M. Prince, unpublished observations) and may be increased within the paramicrogyral zone (Jacobs et al. 1997). These findings suggest that afferents that normally would have synapsed on neurons in the missing laminae are directed to the surrounding zone.

The circuits necessary for the generation and propagation of epileptiform activity are present in the neocortex, and small shifts in the normal balance between excitation and inhibition toward excitation can result in epileptiform activity (Chagnac-Amitai and Connors 1989a; Empson et al. 1993; Gutnick et al. 1982; Mattia et al. 1993; Straub et al. 1992). However, simply increasing the strength of normally present afferent connections might not be expected to initiate epileptiform activity, if the enhanced excitatory inputs were onto both pyramidal cells and inhibitory neurons. For example, increasing the intensity of single electrical stimuli increases both excitation and inhibition and does not evoke epileptiform discharge. In fact such stimuli may block interictal discharges triggered by lower intensity stimuli in chronically epileptogenic brain slices (e.g., Fig. 6 of Jacobs et al. 1996a; and Fig. 8 of Prince and Tseng 1993). Thus increased excitatory afferents would have to be selective to certain neurons or certain components of the circuit for excitation to overcome inhibition as might occur with enhanced recurrent excitation among pyramidal neurons (Dichter and Spencer 1969; McKinney et al. 1997; Salin et al. 1995; Traub and Wong 1982). The hypothesis that enhanced connectivity between layer V pyramidal neurons adjacent to the microgyrus develops because of loss of layer V targets in the microgyral area can be tested with additional anatomic and electrophysiological experiments. In particular, it will be of interest to determine whether intrinsically bursting neurons are involved in this circuit reorganization, because they are predominantly located in deep layers, are important sources of horizontal intracortical axons (Chagnac-Amitai et al. 1990), and are thought to be crucial elements for initiation and propagation of epileptiform activity (Chagnac-Amitai and Connors 1989b).

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