Hyperexcitability in a Model of Cortical Maldevelopment

The presence of developmental cortical malformations has been associated with the occurrence of epilepsy, and correlative anatomic-clinical electrophysiological studies suggest that microdysgenetic lesions may actually initiate epileptiform activity. We have investigated the electrophysiological properties of an animal model of polymicrogyria created by making cortical freeze lesions in rat pups at P0 or P1. Such lesions create microgyri with histological features similar to those of human polymicrogyria. We have determined that there is a focal region of hyperexcitability around the lesion in this rat microgyrus. Field potentials evoked by stimulation within a few millimeters of the microgyrus have characteristics typical of epileptiform activity. This aberrant activity is seen as early as 12 d after the lesion, as well as in animals as old as 118 d. Immunohistochemical staining for the calcium binding protein, parvalbumin, shows a decrease in neuronal and neuropil staining within the microgyrus. These findings suggest that inhibition might be decreased within the lesion, which may contribute to generation of the adjacent hyperexcitable region. These results demonstrate that this animal model is appropriate for examining the mechanisms contributing to epileptogenesis associated with a cortical malformation.

Cortical malformations, such as heterotopia and polymicrogyria, are structural abnormalities thought to result from aberrant development due to cell death or disruption of neuroblast migration (Barkovich et al., 1992). Malformations, or microdysgenetic lesions, have been commonly observed during autopsy in the brains of patients with epilepsy (de Leon, 1972; also see Courville, 1958; Friede, 1975), in brain tissue removed at surgery (Taylor et al., 1971), and in vitro, with modern imaging techniques (Curatolo et al., 1989; Palmini et al., 1991b; Brodtkorb et al., 1992; Guerrini et al., 1992). Barkovich and Kjos (1992) report that 70–80% of patients with polymicrogyria have seizure disorders. This association has been made stronger by recent clinical experiments combining electrophographic recordings in vivo with histological examination of excised tissue, which suggest that epileptogenesis may originate from the site of the microdysgenic lesion (Moreland et al., 1988; Palmini et al., 1991a, 1994). It is likely that such developmental lesions create interruptions or irregularities in synaptic connectivity and also disrupt neuronal differentiation and maturation. Abnormalities may be localized to discrete regions or specific sets of neurons, depending on the type and timing of the insult. For instance, ischemic lesions occurring during migration of superficial layer neurons will likely primarily damage the neurons already present in the cortical plate, those of layers IV–VI (Bayer and Altman, 1987).

The cerebral cortex develops in the well known "inside-out" sequence, in which neurogenesis and migration into the cortical plate is begun first for neurons that will ultimately form layers V and VI (Rakic, 1974; Bayer and Altman, 1987). The final laminar position of a particular neuron (laminar fate) is thus temporally specified, being determined during the S phase of cell division (McConnell and Kaznowski, 1991). Because neurons in different layers send their axons and collaterals to different sites (see Jones, 1984) temporal specificity of laminar fate means that sites of cortical synaptic connectivity are also temporally determined. Loss of neurons in particular layers therefore suggests a loss of specific sets of cortical connections. Synaptic connectivity in the central nervous system has been shown to be significantly altered in situations where targets or inputs are lost (see Easter et al., 1985).

Ischemic insults during neuronal migration have been proposed as a cause of four-layered polymicrogyria (Barkovich et al., 1992). Lesions of this sort, which disrupt the development of cortical circuitry, could induce changes in the balance between excitation and inhibition that would lead to initiation and spread of synchronized excitatory activity across the neocortex (Chagnac-Amitat and Connors, 1989a), and perhaps to the development of a chronic epileptogenic focus. However, mechanisms contributing to epileptogenesis associated with cortical malformations have not been explored.

An animal model in which the normally lissencephalic rat develops a microgyrus after a freeze lesion made through the skull on the day of birth (postnatal day zero, P0) was introduced by Dvorak and Feit (1977). By P10, a microgyrus consisting of a four-layered cortex, resembling that seen in human polymicrogyria, is present (Dvorak and Feit, 1977). The development of this pattern is due to death of cells in the neocortical layers present at P0 (layers IV–VI) and subsequent continued migration of neurons to positions in layers II and III (Dvorak and Feit, 1977). Layers I and Vlb, though present at birth, are retained in the microgyrus. This lamination pattern and gyral abnormality do not develop if the transcortical freeze lesion is made after the end of neuronal migration (P4 in rats; Dvorak et al., 1978).

We performed experiments to determine whether microgyri, produced by transcortical freeze lesions in neonatal rats, become chronically epileptogenic and might serve as a model to explore the mechanisms that underlie epileptogenesis associated with cortical malformations.

Portions of these results have been published in abstracts (Jacobs et al., 1994a,b).

Materials and Methods

Freeze Lesions

Freeze lesions were made in albino rat pups 3–30 h after birth (P0 or P1), using the method of Dvorak and Feit (1977). Pups were anesthetized with hypothermia (2–5 min on ice), a scalp incision was made, and the skull exposed. Initially, a freeze-probe with a circular tip that had a diameter of 2 mm was used. In later experiments, the freeze-probe consisted of a rectangular tipped (5 × 1 mm) copper bar that protruded from inside a 20 cc syringe filled with isobutane cooled to between −40°C and −70°C with dry ice. The probe was placed on the skull for 3–7 sec, approximately 2 mm from the midline, between the coronal and lambdoidal sutures (see Fig. 1 for probe position). This placement created a lesion 2–4 mm lateral to the midline, at a location consistent with motor or somatosensory cortex.

Kimberle M. Jacobs, Michael J. Gutnick, and David A. Prince

1Department of Neurology and Neurological Sciences, Stanford University Medical Center, Stanford, California 94305 and 2Department of Physiology, Faculty of Health Sciences, Ben Gurion University of the Negev, Beersheva, Israel.

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P0 or P1

P9 to P118

sulcus

Field Potential

Stimulus

Figure 1. Placement of freeze probe, recording, and stimulating electrodes. Upper diagram shows surface of rat pup skull. Gray bar represents freeze probe placement between sutures on skull. Lower diagram represents 400 μm coronal slice and recording and stimulating sites relative to the microgyrus and sulcus.

Immunocytochemistry

Eight pups (four experimental and four control) at P12 or P13 were anesthetized with pentobarbital (55 mg/kg) and perfused with saline followed by 4% paraformaldehyde for ~20 min. Brains were removed and fixed overnight in 4% paraformaldehyde at 4°C. Sections were made at 40 μm on a vibratome. For each experimental animal, a littermate was used as a control. Pairs of control and experimental sections from the same anterior-posterior level were incubated together. Standard techniques were used to process tissue sections for parvalbumin immunoreactivity (Kubota and Jones, 1993). Adjacent sections were stained for parvalbumin and Nissl. Primary rabbit antiserum for parvalbumin was kindly provided by Dr. K. G. Baimbridge.

Results

Histology

Freeze lesions at P0 or P1 resulted in cortical injury histologically similar to that previously reported (Dvorak and Feit, 1977; Ferrer, 1993; Fitch et al., 1994; see Figs. 2, 3A). An infolding of the microgyrus that created a "microsulcus" and surrounding microgyrus, and disrupted the normal neocortical laminar pattern, was observed in rats surviving to P9-18. The nature of the abnormal laminar pattern beneath the microsulcus depended partly on the temperature of the freeze probe and the duration of exposure of the skull to the freeze-probe. In the most severe lesions, produced by a 7 sec freeze at -70°C, the microsulcus extended either to the subcortical white matter or to a depth equivalent to that of layer VI in the adjacent neocortex, with only a thin layer of cells remaining between the microsulcus and the underlying white matter. The microsulcus sometimes simply divided the neocortex into two separate parts, without creating layers that wrapped around the sulcus. The microsulcus for less severe lesions extended to a depth of layers III to superficial layer V of the adjacent cortex and was sometimes "v" or bowl shaped. Typically, the layer of the microgyrus wrapped around the microsulcus (Fig. 2A).

The most common lesion, resulting from exposure to a -70°C freeze-probe for 3-5 sec, or -50°C for 4-6 sec, was a microgyrus that extended to the depth of layer III in adjacent cortex, and contained four distinguishable layers (Fig. 2A). (1) A molecular layer contiguous with layer I of adjacent neocortex was present just below the pia, and wrapped around the microsulcus. (2) The second layer contained small cells, appearing in Nissl-stained sections to be morphologically similar to those in layers II/III of normal cortex (Fig. 2B,E). In some cases this layer appeared to be an expansion of the most superficial part of the adjacent layer II. (3) The third layer contained some glia and few neurons (Fig. 2C). (4) The deepest layer was contiguous with layer VII of the adjacent neocortex. In some microgyri there was a partial preservation of layer VIa as well (Fig. 2D).

There was some additional variability in the shape and depth of the microsulcus and the extent of individual layers within the microgyrus, even for lesions of the same temperature and duration. For instance, occasionally there would be an asymmetry in the cytoarchitecture on either side of the microsulcus (Fig. 2D). In sections where layer I wrapped around the sulcus, some pyramidal shaped neurons appeared to have apical dendrites running toward the sulcus (Fig. 2E), while others maintained an orientation similar to neurons outside of the microgyrus (arrowhead, Fig. 2D). Some glia was present in the cortex beneath or adjacent to the microsulcus in all cases. Ectopic neurons were sometimes seen in layer I adjacent to the microsulcus (Fig. 2F). Typically, the normal six-layered pattern of neocortex resumed at distances of 300 μm or more from the microsulcus. No alteration in cortical structure of the contralateral hemisphere could be detected in Nissl-stained sections.

Immunocytochemistry

Since decreases in GABAergic inhibition have been proposed as one mechanism for generation of epileptiform field potentials and cellular activities both in models of chronic epilep-
Figure 2. Nissl-stained coronal sections through microgyri of two different rats. A, Example of the most common result of a moderate lesion. Lesion made on P0, sections from a P113 rat. B, High-power picture of area indicated in A, showing layers 1 and 2 of the microgyrus. C, High-power picture, displaying layers 3 and 4 of the microgyrus shown in A. D, Asymmetrical microgyrus from a P63 rat. E, Pyramidal-shaped neurons with apicals running toward the microsulcus (arrows point to some examples). Some neurons were oriented in same direction as neurons outside of the microgyrus (arrowhead). F, Ectopic group of neurons (arrowed) in layer I, in section anterior to that shown in D and E. Scale bars: A, 300 μm; B and C, 100 μm; D, 107 μm; E, 38 μm; F, 108 μm.

togenesis (Sloviter, 1987, 1991; Franck et al., 1988; Haglund et al., 1992; Empson and Jefferys, 1993) and in normal immature cortex (Luhmann and Prince, 1990), we performed experiments in 14 animals to examine one major group of GABA-containing interneurons within the area of the microgyrus, namely those that contain the calcium-binding protein parvalbumin (Hendry et al., 1989; Ren et al., 1992). Parvalbumin immunoreactivity had a distinct laminar pattern in sections of neocortex from control animals. The heaviest labeling of neurons and neuropil was in layers IV and Vb, with very light labeling in layer Va and I. There was neuronal labeling, but little neuropil labeling in layers II/III and VI. This same pattern was also seen in sections from all of the experimental animals, except in the area of the microgyrus (Fig. 3B), where there was an almost complete absence of parvalbumin neuronal labeling and little neuropil labeling in neocortex below and within several hundred micrometers on either side of the microsulcus. Adjacent Nissl-stained sections revealed that the region lacking parvalbumin-labeled neurons contained a dense layer of Nissl-stained neurons that appeared similar to those of the adjacent layer II/III (Fig. 3A). While differences in parvalbumin immunoreactivity between experimental and control animals are not necessarily directly related to altered inhibitory function, they do further illustrate the abnormalities associated with this lesion and potential developmental changes in this subpopulation of GABAergic neurons.

**Electrophysiology**

Field potentials evoked by electrical stimulation within 1–2 mm of the microsulcus contained long, variable latency, mul-
Figure 3. Coronal sections obtained at P13, through an experimentally produced microgyrus (A1, B1) and a homologous region from a sibling control rat (A2, B2). Arrows indicate location of microsulcus. A, Nissl-stained sections. Normal neocortical layers are indicated distant to the sulcus (I-VI) and layers within the microgyrus are labeled 1-4. Layer II/III neurons are present in abundance around the microsulcus, in layer 2 of the microgyrus. B, Parvalbumin immunoreactivity in sections, from the animals of A. Parvalbumin-labeled neurons and neuropil staining are nearly absent within the microgyrus. Scale bar, 300 μm.

tiphasic events (Fig. 4B), in addition to an early stereotyped component that was typical of control neocortex (Fig. 4A). The early event in control and experimental tissue consisted of a negativity that had a latency of 2-5 msec, a duration of 10-15 msec, and an amplitude that was graded with the stimulus intensity. The late multiphasic activity (LMA) was evoked as an all-or-none response at threshold stimulus intensity (Fig. 5B) and had a variable latency, even following stimuli of the same intensity and low frequency (0.1 Hz; compare first and third responses in Fig. 5B, and Fig. 6E i and iii). Overall, latencies varied between 20 and 150 msec and the duration of the LMA was also variable from one stimulus to the next, and between animals, ranging from 50 to 600 msec. Increases in stimulus intensity often changed the form of the response and sometimes decreased the latency to onset of the LMA (Fig. 6A-C). At high stimulus intensities the LMA was completely abolished and the short latency event became triphasic (Fig. 6D). Stimulus repetition rate also had a clear effect on the probability of evoking the LMA. At stimulation frequencies of 0.067 Hz or lower, the LMA was present on every trial (0.067 Hz traces of Fig. 7). The variability in the form and latency of the LMA, at the same stimulus intensity and several different stimulus frequencies, can be seen in Figure 7. Failures of the LMA typically began to occur at stimulus repetition rates between 0.067 and 0.2 Hz, and in all cases tested (n = 5) the LMA could not be evoked regularly at a stimulus frequency of 0.5 Hz or higher (1.6 Hz, Fig. 7). The early event was unaffected at any of these frequencies. The time period after the first stimulus, over which a second LMA could not be evoked (the refractory period of the LMA) was far longer than known refractory periods for activity in individual neurons in normal tissue (compare 1.6 Hz, Fig. 7), but similar to that found for evoking epileptiform events in other models of chronic epileptogenesis (e.g., Prince and Tseng, 1993). In addition the refractory period of the LMA was variable, depending on stimulus frequency; in Figure 7, the LMA occurs on consecutive trials with 0.4 Hz stimulation, but is refractory for more than 5 sec at a stimulus frequency of 1.6 Hz.

In four preliminary experiments we used sharp microelectrodes to record from layer II/III neurons, 0.5-1.0 mm from the microsulcus, to verify that neurons in the region of the
Figure 5. All-or-none nature of late multiphasic activity. At threshold stimulus intensity. Three consecutive sweeps are shown at each stimulus intensity. A, No field response occurs at 15 mA. B, Threshold stimulation, at 20 mA, evokes a variable latency multiphasic response. C, Suprathreshold stimuli (25 mA) resulted in occasional failures. All responses were evoked in superficial layers by extracolumn, 0.1 Hz stimulation of layer VI, 300 μm lateral to the microsulcus, in a slice from a P13 rat.

abnormal field potentials showed long latency excitatory postsynaptic potentials and burst discharges typical of cells involved in epileptogenesis. Intracellular responses consisted of two components: a short latency depolarizing potential that was graded with stimulus intensity (Fig. 6B, arrow) and coincident with the early field potential response; and a later multiphasic all-or-none component (Fig. 6B, arrowheads) that occurred during the LMA. Increasing stimulus intensity shortened the latency of the multiphasic event and ultimately abolished it (Fig. 6D), so that, at the highest stimulus intensities, only the short latency event was present. The single-unit extracellular recording of Figure 6E is from a neuron near the microgyrus that generated bursts of action potentials in response to the extracellular stimulus. These bursts appeared to correspond temporally to the multiphasic postsynaptic potentials and LMA of the field potentials. A burst of action potentials activated from a large amplitude, long latency synaptic depolarization was seen in another neuron in response to stimulation near the microgyrus (Fig. 6F).

The occurrence of LMA in microgyri was a consistent finding. In all, 81 slices from 37 freeze-lesioned rats, aged 9-118 d, at the time of recording, were tested. In 81.1% of these animals, LMA could be evoked in at least one slice. The LMA was evoked in rats as young as 12 and as old as 118 d. The characteristics of the LMA, as described above, were the same in young and mature animals. In four slices from two animals aged P9 and P10, epileptiform activity could not be evoked at any site, although a microgyrus was present.

In order to determine the distribution of abnormal evoked field potentials within the region of the microgyrus, a multi-electrode array, oriented parallel to the pial surface, was used to record from 16 sites simultaneously, within layer II/III in four slices. The stimulating electrode was placed within layer VI, 0.5-1.0 mm from the microsulcus (Fig. 8). Stimuli at this site evoked short latency events that were similar in form at distances of up to 650 μm lateral to the stimulus (electrodes 1-7 of Fig. 8). Between 650 and 1070 μm horizontally distant from the point of stimulation at recording sites in layer II/III, the amplitude of the early event was reduced but the duration of this event was increased (electrodes 8-10 of Fig. 8); beyond 1070 μm lateral to the stimulating electrode the early event was barely discernible (electrodes 11-16). The amplitude and presence of the LMA was independent of the amplitude of the early event. Because of the irregular nature and multiple peaks of the long latency paroxysmal event, it was difficult to study the speed and direction of its propagation over the slice. For example, in Figure 8, the evoked LMA is seen at all 16 electrodes, but the variability in the shape of the peaks at different electrodes prevents clear determination of the site at which the LMA had the shortest latency.

By moving the stimulating electrode in layer VI away from the microsulcus in steps of 0.5 or 1.0 mm, it was possible to...
Figure 7. Effect of stimulus frequency on occurrence of the LMA in a P15 rat. The first six responses (of 10) to stimulus trains delivered at the frequencies indicated above the first sweep are shown for each set. All responses were recorded from the same cortical site, in superficial layers, 1.0 mm lateral to the microgyrus. Stimulus was on-column, in deep layers. At 1.6 Hz, LMA was not seen in any of the nine trials after the initial stimulus presentation.

Figure 8. Distribution of evoked LMA across layer II/III in a slice from a P23 rat, lesioned at P8. Recordings at points 1–16 made simultaneously, in response to a single stimulus. Interelectrode distance, 107 μm. Dashed line on slice schematic represents ventral border of layer III.

Discussion

These results show that a zone adjacent to microgyri produced by focal cortical freeze lesions at P0 or P1 in neonatal rats acquires capacities to generate synchronous abnormal activities that propagate across the cortex. This hyperexcitability appears within 12 d, and persists in rats that develop to maturity. A number of the electrophysiological observations made in neocortical slices provide strong evidence that the cortex in the area of the lesion becomes epileptogenic. The prolonged, multiphasic, variable latency field potentials that are evoked at threshold as all-or-none events closely resemble those in other chronic epilepsy models (Prince and Tseng, 1993; Hoffman et al., 1994) and are also similar to the epileptiform activities induced by threshold doses of bicuculline (Chagnac-Amitai and Connors, 1989a). In addition, the available cellular recordings (Fig. 6E,F) suggest that neurons generate orthodromic bursts of spikes during the field potential events, a characteristic of most experimental models of partial
epilepsy (Prince, 1978; Dichter and Ayala, 1987). It will be useful to obtain EEG recordings in vivo from animals with microgyri to confirm the presence of focal epileptiform activity and further validate the microgyrus as a model of focal cortical hyperexcitability and epileptogenesis due to a developmental cortical malformation.

Although there are several animal models of cortical malformations that display histological similarities to clinical pathologies, this is the first one in which epileptogenesis has been demonstrated. Microgyri induced in neonatal rats by injection of the glutamate agonist, ibotenic acid, into neocortex (Innocenti and Berbel, 1991), or with other techniques (Rosen et al., 1992; Ferrer et al., 1995) have not been examined electrophysiologically. Exposure of E13–E17 fetuses to ionizing radiation or the alkylating agent methylazoxymethanol acetate (MAM) causes cortical ectopias and gross abnormalities of cortical lamination in the offspring (Cowan and Geller, 1960; Jones and Gardner, 1976; Tamaru et al., 1988). Although the affected rat pups develop learning disorders, hyperactivity, and other behavioral abnormalities (Cannon-Spoor and Freed, 1984; Vorhees et al., 1984; Mohammed et al., 1986), to date seizures have not been reported in these animals. MAM rats with severe histological abnormalities do, however, have a lower seizure threshold when given convulsant drugs (Germano and Sperber, 1994). We have examined neocortical slices from MAM animals and found that evoked epileptiform activities of the sort reported here are not present (Jacobs and Prince, unpublished observations).

The histological features of the typical experimental microgyrus induced by freeze lesions are nearly identical to those observed in human four-layered polymicrogyria (Dvorak and Feit, 1977; Ferrer et al., 1993). Dvorak et al. (1978) have shown that layer 2 of the microgyrus contains small pyramidal and stellate neurons that are typical of layer II/III in normal neocortex, except for being misoriented (see also Fig. 2E), and have a birthdate appropriate for layer II/III neurons. The glial scar present in layer 3 of the microgyrus is also seen in clinical specimens (Williams et al., 1976). Our data confirm the reliability of this model in reproducing histological features similar to those found in a human developmental malformation, and also show that the significant incidence of hyperexcitability found in human polymicrogyria (Barkovich and Kjos, 1992) persists in slices of rat microgyri.

A relatively narrow range of stimulus intensities and frequencies were required in order to generate epileptiform activity. The suppression of epileptiform activity with high stimulus intensities observed here has also been demonstrated in other models of epileptogenesis (Prince and Tseng, 1993). Intracellular recordings suggest that, at low stimulus intensities, excitatory synaptic events can be evoked in isolation, while increasing stimulus intensity augments the influence of inhibition over excitation (Chagnac-Amitai and Connors, 1989a; Sutor and Hablitz, 1989; Prince and Tseng, 1993). At high stimulus intensities the recruitment of inhibitory events likely blocks the initiation of the LMA. In our model, epileptiform activity was seen at intensities that were just threshold for the early event, intensities that may not have evoked inhibition in normal neocortex. This suggests that alteration of excitatory connectivity alone may account for the ability of the freeze-lesioned cortex to initiate epileptiform activity at these stimulus intensities. Increases in stimulation frequency also reduced the probability of occurrence of late events, a finding typical for polysynaptic epileptiform activity (e.g., Prince, 1966). A variety of membrane conductances that tend to reduce excitability are activated in neurons of the epileptogenic neuronal aggregate following an interictal epileptiform discharge (Witte et al., 1989; Dorn and Witte, 1995), perhaps accounting for reductions in numbers of neurons brought to spike threshold by subsequent stimuli, and loss of the capacity to generate closely spaced LMA. However, it is unclear why the refractory period for the epileptiform activity varies with the stimulus frequency (e.g., Fig. 7), or why such long refractory periods are seen in some models of epileptogenesis (Kriegstein et al., 1987; Prince and Tseng, 1993).
Epileptiform activity could only be generated in a cortical zone extending a few millimeters from the microgyrus, but not within the microgyrus itself (Fig. 9A). This seems surprising, because the immunocytochemical results discussed below might suggest that disinhibition, a prime mechanism for inducing epileptiform discharge (Schwartzkroin and Prince, 1980; Chagnac-Amitai and Connors, 1989a), would be present particularly within the microgyrus. It is possible that the synaptic inputs that trigger the abnormal responses in the region adjacent to the microgyrus are absent or not properly activated in the region below the microsulcus. For example, if particular cell types present in deep layers, such as intrinsically bursting neurons, are required to initiate epileptiform activity (e.g., Chagnac-Amitai and Connors, 1989b; but see Hoffman and Prince, 1995), the absence of layers IV–VI in the microgyrus might render it incapable of such abnormal responses. However, it is known that layer II/III, isolated from deeper layers can generate epileptiform activity when treated with high concentrations of bicuculline (Gutnick et al., 1982).

Another potential explanation for susceptibility to epileptogenesis adjacent to, but not within the microgyrus, involves enhanced excitatory circuitry in the paramicrocgyral cortex, discussed below.

Reductions in the efficacy of inhibitory electrogenesis are a well-studied mechanism for initiating epileptiform activity (Schwartzkroin and Prince, 1977, 1980; Chamberlin and Dingledine, 1988; Bekenstein et al., 1993). We observed a near absence of parvalbumin-labeled neurons in all layers beneath the microsulcus in P13 rat pups. This decrease in immunocytochemical staining for parvalbumin, which is colocalized in 30–50% of GABAergic cells (Hendry et al., 1989; Ren et al., 1992), suggests that the experimentally produced microgyrus may contain a decreased number of GABAergic neurons, or some other abnormality affecting these elements; however, analysis of GABAergic immunocytochemical staining will be necessary to confirm this. Our results are similar to those reported by Ferrer et al. (1993), who found a reduction in the number of parvalbumin-labeled neurons in layers II–IV within the microgyrus. Counts of the number of parvalbumin and GABA immunoreactive neurons in the freeze-lesioned animals relative to those of controls and to the total number of neurons in the area of the microgyrus will be required to quantitate what appears to be a clear effect (Fig. 3). Reduction of parvalbumin labeling has been demonstrated in two models of temporal lobe epilepsy (Sloviter, 1991; Best et al., 1993), and also in dysplastic regions of human neocortical tissue removed for surgical treatment of intractable epilepsy (Ferrer et al., 1992), suggesting that this may be a common feature of chronically epileptogenic tissue.

Synaptic connectivity, at least within the area of the lesion, must be disrupted since certain classes of neurons are focally eliminated. Layer II/III neurons within layer 2 of the microgyrus and layer V neurons adjacent to the microgyrus have lost their normal targets (Ghosh and Porter, 1988; Burkhalter, 1989) in layers V and VI beneath the microsulcus. Subcortical afferents destined for the damaged area would be similarly affected. We speculate that one potential mechanism for the generation of hyperexcitability would be aberrant innervation by intra- and subcortical axons to produce an increase in excitatory connections in the region adjacent to the microgyrus. One possibility is that afferents destined for the lesioned area are unable to find appropriate targets within this region and therefore synapse in the adjacent region. This idea is supported by neuroanatomic findings, which show that neurofibrillament staining and glutamate-immunoreactive (IR) fibers are disrupted in adult rats that have sustained neonatal freeze lesions (Humphreys et al., 1991). The glutamate-IR fibers are specifically abnormal in layer V adjacent to the microgyrus, where Nissl staining shows histologically normal neurons, raising the possibility of abnormalities in excitatory connectivity at that site. Only small shifts in the balance between excitation and inhibition are required to produce hyperexcitability and epileptiform activity (Chagnac-Amiatii and Connors, 1989b). Thus, either a reduction of GABAergic inhibition or enhanced excitatory connectivity within the region adjacent to the microgyrus could produce the epileptogenesis observed with this animal model. In addition, GABAergic inhibition may be decreased due to a specific decrease in excitatory connections onto inhibitory neurons, as has been suggested in a model of temporal lobe epilepsy (Goodman and Sloviter, 1992).

From these results, we conclude that the neonatally freeze-lesioned rat is a promising model in which to examine the mechanisms contributing to epileptogenesis associated with cortical malformations.

References


