Experimental Microgyri Disrupt the Barrel Field Pattern in Rat Somatosensory Cortex

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Transcranial freeze lesions in neonatal rat pups produce microgyri and adjacent epileptogenic regions of neocortex that can be used to model human polymicrogyria. The hypothesis that the presence of microgyri is associated with abnormal cortical organization occurring within as well as adjacent to the microgyri was tested by creating microgyri within the face representation of somatosensory cortex. Microgyri were associated with a widespread disruption of the stereotypic whisker barrel field pattern delineated with cytochrome oxidase (CO) staining. CO-stained patches resembling barrel hollows were absent within the microgyrus, and were abnormally shaped and distributed outside of the microgyrus. Adjacent Nissl- or acetylcholinesterase-stained sections demonstrated that both cell clusters and thalamocortical afferents contributed to the abnormally organized paramicrogyral zone identified in CO-stained sections. Field potential recordings showed that this region of heavy CO staining corresponded to the epileptogenic zone adjacent to the microgyrus. Results support our hypothesis that the epileptogenic paramicrogyral zone develops an abnormal organization of cell clusters and thalamocortical projections that could contribute to epileptogenesis in the paramicrogyral zone.

Introduction
Structural malformations that occur as a result of aberrant cortical development in humans are associated with a variety of neurological problems, including epilepsy, mental retardation and dyslexia (Taylor et al., 1971; Crome, 1972; Wisniewski, 1990; Galaburda, 1991; Brannstrom et al., 1996). Treatments effective in other forms of epilepsy often fail in patients with a developmental cortical abnormality (Cieuta et al., 1996; Olivier et al., 1996), and the link between the aberrant structure and the mechanisms producing the neurological abnormalities is unknown. We have used a rat model of polymicrogyria to study epileptogenic mechanisms associated with this malformation (Jacobs et al., 1996a, 1999a).

Neocortical freeze lesions made transcranially within 48 h of birth in rat pups create a hypoxic–ischemic lesion similar to those thought to produce human polymicrogyria (Dvorak and Feit, 1977; Jacobs et al., 1996a). The result is a loss of deep layers present in the cortex at the time of the lesion with subsequent normal migration of neurons into superficial layers. By postnatal (P) day 10 in the rat the complete four-layered form of the microgyrus, identical to the human histopathology, can be seen (Dvorak and Feit, 1977). This aberrant lamination of the experimental microgyrus typically extends over a cortical distance of ~0.35 mm on either side of a microsulcus, with six-layered cortex commonly beginning at the border of the microgyrus (Jacobs et al., 1996a, 1999a). Epileptiform activity is selectively activated within this six-layered cortex surrounding the microgyrus [paramicrogyral zone (Jacobs et al., 1996a, 1999a)]. Although this region appears normal in Nissl stains, we hypothesize that aberrant development of connections within the paramicrogyral zone is one mechanism that gives rise to the structural–functional substrate underlying epileptogenesis, since separation of the microgyrus from the adjacent cortex does not prevent or suppress evoked epileptiform activity within the paramicrogyral zone (Jacobs et al., 1999a). Recordings from layer V pyramidal neurons adjacent to the microgyrus, and homotopic control cells suggest that there may be an increase in excitatory afferents onto both inhibitory interneurons and cortical pyramidal neurons (Jacobs et al., 1996b, 1999b; Prince et al., 1997). Thus, the paramicrogyral zone may contain an overabundance of excitatory afferents, due to a reorientation of circuitry initially fated for the microgyral region. Afferents originally destined to synapse in layers IV–V1 of the microgyral region have lost their targets, but can find laminar-appropriate structures in the nearby paramicrogyral area. Supporting this idea are a number of studies which have suggested that afferents choose specific targets based on laminar-specific molecular cues (Shatz, 1992; Bolz et al., 1996; Castellani and Bolz, 1997).

The stereotyped structural organization of the face representation in the somatosensory system of rodents provides a unique opportunity to test these ideas, because of the specificity of the pattern of excitatory thalamic afferents to the cortex (Killackey, 1973; Agmon et al., 1995) and the ease with which the anatomical organization of the representation can be visualized. Neocortical neurons representing an individual whisker are grouped into a barrel-like structure that can be recognized within layer IV in Nissl stains (Woolsey and van der Loos, 1970; Welker and Woolsey, 1974). The organization of these barrel structures in neocortex matches the pattern of whiskers on the rodent snout, and is stereotypical for individual species and strain (Woolsey et al., 1975; Rice et al., 1985; Welker and van der Loos, 1986). The vibrissae-related pattern can be visualized not only in cortex, but at each level of the somatosensory system using a metabolic stain, such as cytochrome oxidase (CO). In rats, patches of CO staining in neocortex are coincident with the centers of the architectonically defined barrels (Land and Simons, 1985), identifying both somata and dendrites of cortical neurons as well thalamocortical afferent axons (Wong-Riley and Welt, 1980).

There is much evidence to suggest that the development of the stereotyped vibrissae-related pattern in neocortex is dependent on the organization of the specific thalamic afferents from the ventral posterior nucleus (Jensen and Killackey, 1987; Senft and Woolsey, 1991; Schlaggar and O’Leary, 1993; Catalano et al., 1995). Individual barrels receive nearly all of their thalamic afferents from the specific representation of the same whisker (Agmon et al., 1995). Thalamic afferents appear to be arranged in the barrel-specific pattern before this pattern is formed in cortex (Killackey and Leshin, 1975; Jeannond et al., 1981; Erzurumlu and Jhaiveri, 1990; Senft and Woolsey, 1991; Schlaggar and O’Leary, 1994). Experimental manipulations which change the morphology of individual thalamic axons or...
the functional pattern of thalamocortical afferents also modifies the vibrissae-related pattern in neocortex (Simons et al., 1984; Jensen and Killackey, 1987). In addition, occipital neocortex, which typically contains a continuous band of CO staining in layer IV, can be made to produce the barrel structures when transplanted into the region above somatosensory thalamic afferents (Schlaggar and O’Leary, 1993). These studies suggest therefore that a disruption in the organization of thalamic afferents will produce an abnormal vibrissa-related pattern of metabolic staining.

Accordingly, we expected that a microgyrus occurring within the cortical whisker somatosensory representation would result in a disrupted CO staining pattern due to the loss of afferents to the microgyral cortex, as well as to an increase of afferents to the adjacent cortical region. We tested this idea by creating freeze lesions within somatosensory cortex of P0–P1 rat pups and adjacent cortical region. We tested this idea by creating freeze lesions within somatosensory cortex of P0–P1 rat pups and examining the thalamic and cortical CO staining patterns at ages after the formation of the microgyrus (≥P12).

Materials and Methods
All procedures followed in this study have been approved by the Stanford University animal care and use committee and conform to NIH guidelines.

Freeze Lesions
Freeze lesions were made as previously described (Jacobs et al., 1996a, 1999a), on the day of birth (P0) or on P1 in albino Sprague-Dawley rat pups of either gender. Freeze-probes consisted of those creating a large lesion (tip = circular, 3.0 mm diameter or rectangular, 2 × 5 mm) or a small lesion (tip = circular, 0.5 mm diameter). For measurements reported here, there was neither a qualitative nor a quantitative difference in results when pups were lesioned on P0 versus P1 or with the large circular versus rectangular tipped freeze probe (t-tests on number of CO-barrels, NS), so data from these groups were combined.

Fixation, Sectioning and CO Staining
For these histochemical studies, 11 control (naïve) and 29 freeze-lesioned Sprague-Dawley rats were perfused on postnatal day 12, 22, 28–37 or 105–110. Rats were deeply anesthetized with a lethal dose of Nembutal (200 mg/kg) and perfused transcardially with 40 ml of 0.9% saline, followed by a phosphate-buffered aldehyde fixative, and then by 10% sucrose in 0.1 M phosphate buffer. Lower concentrations of glutaraldehyde (range = 0.1–2.5%) yielded better staining, and 4% paraformaldehyde without any glutaraldehyde produced staining with the lowest background and highest contrast, and was therefore used in the majority of cases.

After removal, brains were cryoprotected in 30% sucrose at 4°C for ≥24 h, and subsequently sectioned on a freezing microtome, at either 50 or 80 μm. For 36 (of 40) brains, the cortices were prepared for tangential sectioning prior to cryoprotection (Welker and Woolsey, 1976). For each hemisphere the cortex was separated from the diencephalon and brainstem, and then flattened between clean microscope slides with 2.0 mm spacers. In four freeze-lesioned animals, the entire brain was cryoprotected and then sectioned coronally.

Cortical sections were treated with a cobalt chloride intensification (13.75 mg CoCl2, 5 g sucrose per 50 ml 50 mM Tris buffer) prior to CO staining. Cortical sections were treated with cytochrome c and diamino-benzidine (DAB) according to the method described by Wong-Riley (Wong-Riley, 1979). In all four coronally sectioned brains, and in five control and eight experimental tangentially sectioned brains, alternate sections were stained for Nissl and CO.

AChE Staining
Two additional P12 freeze-lesioned animals were used for acetylcholinesterase (AChE) staining, following the protocol of Bear et al. (Bear et al., 1985). Adjacent sections were stained for AChE, and for CO as described above.

Reconstructions of the Vibrissae-related CO Staining Pattern
In the rat somatosensory cortex, barrels are grouped into six discrete somatic representations: the whisker pad or postero medial barrel subfield (PMBSF), the snout/upper lip (SN), the furry buccal pad (FBP), the lower jaw (LJ), the forelimb (FL) and the hindlimb (HL) (Welker, 1971, 1976; Dawson and Killackey, 1987). CO-stained patches (CO-barrels) for all six somatic representations were typically confined within four to ten 80 μm sections (320–800 μm), depending in part on survival age. In order to compare the pattern of CO-barrels in separate animals all of the sections containing CO-barrels within a single hemisphere were reconstructed into a two-dimensional drawing. This was accomplished by aligning acetate drawings of each of the individual sections for a single hemisphere. These drawings contained the outlines of the hemisphere: regions of intense CO staining, including barrel-like structures; some blood-vessel artifacts; and for experimental sections, the microsulcus. After aligning the drawings, the border of each CO-barrel was drawn as the largest area generated by the combined outlines for that CO-barrel.

In order to estimate the expected location of the PMBSF in lesioned hemispheres, a control composite drawing outlining the FL, LJ, FBP, SN and PMBSF representations was made from five controls aged P22 (gray outlines in Fig. 1B,D,F and H). The composite also included the small group of barrels lateral to the PMBSF (arrow in Fig. 1B). The outline for each representation was created by drawing the border around the barrels. Drawings from the five control hemispheres were then aligned and the composite was drawn from the outer border of the overlapping outlines. In order to show the variability between control hemispheres for the PMBSF region, the inner border of the overlapping outlines was also drawn for this representation (dashed outlines in Fig. 1). The number of CO-barrels falling within the composite outline (outer border) PMBSF is reported as mean ± SEM. To compare the number of CO-barrels within freeze lesion versus control PMBSF, CO-barrels were counted in the contralateral hemispheres of four of the five animals used to create the composite outline (the contralateral hemisphere in one of the five animals was not processed), as well as in left and right hemispheres of four control animals that were not used to create the composite outline. The distance over which CO-barrels were lost in the experimental hemispheres was measured from the edge of the microsulcus and from the edge of the pale-stained region (presumed microgyrus, see below) to the most distal point on the outer border of the control composite PMBSF outline (see vertical scalebar in Fig. 1F).

Electrophysiological Mapping
In six slices from three adult microgyral rats, the presence or absence of epileptiform activity in the evoked field potential was assessed at various distances from the microsulcus. Standard techniques for preparing and maintaining neocortical slices were used (Jacobs et al., 1996a). Recordings were made in artificial cerebral spinal fluid containing (in mM): 126 NaCl, 3.0 KCl, 2.0 MgCl2, 2.0 CaCl2, 1.25 NaH2PO4, 10 glucose and 26 NaHCO3 at 34°C. Field potential recordings (DC-5 kHz, low pass filtered at 5 kHz) were obtained using glass micropipettes (2–8 MΩ) filled with

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Figuro 1. Pattern of CO-stained patches in tangential cortical sections from P22 rats. (A) Photomicrograph of stereotypical pattern of control CO barrel field. (B) Drawing of all barrels present from all sections of hemisphere shown in (A). Gray outlines indicate area of composite derived from the left hemisphere of five control rats. Dashed gray outline shows the variability in the whisker area (see Materials and Methods). PMBSF, posterior medial barrel subfield; FBP, furry buccal pad; LJ, lower jaw; FL, forelimb; HL, hindlimb. Arrow shows small group of unmarked barrels lateral to PMBSF. (C) CO-stained tangential section from a P22 rat that received a freeze lesion on P0, interrupting the CO barrel field. Arrows point to pale zone surrounding microsulcus (asterix). (D) Drawing of all barrels from all sections for hemisphere shown in (C), with control composite (gray outlines) overlaid. Black diagonally lined area: microsulcus in (D) and (F). Dark continuous line around microsulcus shows extent of pale stained region surrounding microsulcus in (D) and (F). (E) CO-stained tangential section from a P22 rat that was lesioned on P1, showing the absence of barrels in the PMBSF region. Long horizontal slit: microsulcus. (F) Drawing for hemisphere shown in (F). Each division on vertical scalebar: 500 μm. (G) CO-stained tangential section from hemisphere contralateral to that shown in (E) and (F). PMBSF barrels are undisturbed contralateral to the microgyrus. (H) Drawing for hemisphere shown in (G). Scalebar in (G): 800 μm for (A)–(H). Left is anterior, top is medial.
Results

Microgyri produced by P0 or P1 freeze lesions were similar to those previously described, and contained a region of abnormal lamination (microgyrus) on either side of a microsulcus, identified in coronal sections. The microgyrus extended 0.2–0.6 mm from the edge of the microsulcus, and the acellular third layer of the microgyrus formed a border between the abnormally layered microgyrus and the adjacent six-layered region (Jacobs et al., 1996a, 1999a) (see Fig. 3B). In tangential sections from control cortices, patches of intense CO staining, typical of CO-stained barrels, could clearly be distinguished from background staining (Fig. 1A). When the entire hemisphere was reconstructed in a two-dimensional drawing (see Materials and Methods), we observed the stereotypical pattern of CO-barrels (Fig. 1B) (Welker, 1971, 1976). Typically, 36 barrels are found in the PMBSF (Rice and van der Loos, 1977; Dawson and Killackey, 1987; Zheng and Purves, 1995), which we identified using a composite outline created from five control animals (see Materials and Methods, and gray outlines in Fig. 1B,D,F,H). In control hemispheres contralateral to those used to create the composite outlines, an average of 36.3 ± 0.5 CO-barrels were located within the PMBSF outline. An average of 36.6 ± 0.7 CO-barrels fell within the control composite PMBSF in the left and right hemispheres of four additional control animals not used to create the composite outline. One to five CO-stained patches were observed posterior to the PMBSF and were not included in the outlined representations.

CO-barrel Field Pattern in Lesioned Brains

The extent of the microsulcus (asterix in Fig. 1C, dark shaded area with diagonal lines in Fig. 1D,F) was evident in sections from freeze-lesioned rats. Surrounding the microsulcus was a region of pale CO staining evident in both tangential and coronal sections (arrows in Figs 1C and 3A). Heavy but diffuse CO staining sometimes containing segmented CO staining occurred in regions just adjacent to the pale zone (see Figs 1C, 2A,B). The extent of the diffuse staining is shown as the gray shaded area in Figure 2B (and see further description below). The individual segments of heavy CO staining (or CO-patches) were shaped abnormally relative to control CO-barrels, particularly adjacent to the pale zone (see below). The microsulcus and these surrounding zones disrupted the pattern of the PMBSF CO-barrel field. From a qualitative assessment it was clear that experimental hemispheres contained fewer CO-patches within the PMBSF region than controls (Fig. 1D,F). In contrast, the pattern of snout/upper lip, furry buccal pad, jaw and forepaw CO-barrels ipsilateral to the microgyrus was similar to controls. These representations were therefore used to align the control composite outline to drawings of freeze-lesioned cortex. This allowed a more quantitative determination of the number of CO-barrels (or patches) remaining within the expected PMBSF region.

In all 12 cortices lesioned with a large probe tip and examined at age P22, there were fewer than 36 CO-barrels within the expected PMBSF region, with an average of 21.4 ± 2.4 CO-barrels. The cortical distance over which CO-barrels were lost, measured from the microsulcus to the edge of the composite PMBSF outline, was between 1.0 and 2.0 mm (see scale in Fig. 1F), and the distance from the medial or lateral margins of the pale-stained area was between 0.7 and 1.8 mm (n = 12). The location of this zone of abnormal staining was similar to that over which epileptiform activity could be evoked (see Fig. 3 and description below). In all cases the disruption of the CO-barrel pattern extended beyond the region of pale staining surrounding the microsulcus.

In cortex contralateral to the freeze lesion, the pattern of all CO-barrels, including those of the PMBSF representation, was similar to control hemispheres (Fig. 1G,H). Even when the majority of ipsilateral PMBSF CO-barrels were missing, the contralateral cortex contained five rows of PMBSF CO-barrels, whose organization and shape was similar to controls (Fig. 1G is contralateral to 1E).

Distinguishing Microgyral and Paramicrogyral Cortex

The border between the microgyral region and the adjacent six-layered paramicrogyral zone could be distinguished in tangential Nissl-stained sections by the presence of the cell-sparse third layer of the microgyrus (arrow in Fig. 2H). From adjacent CO-stained sections, it was clear that this border also corresponded to the border between the pale zone surrounding the microsulcus and the adjacent heavy CO staining that was either diffuse or contained abnormally shaped barrels (arrow in Fig. 2G). Thus, the microgyral cortex coincided with the pale CO staining, although Nissl sections demonstrated that there was not a paucity of neurons in this region. Cell density within the microgyrus, in fact, appeared to be higher than that of adjacent layer II/III in superficial tangential sections (not shown), a fact which has been previously noted (Dvorak and Feit, 1977; Jacobs et al., 1996a). The region of heavy, diffuse CO staining and abnormally shaped CO-barrels surrounding the pale area clearly corresponded to the six-layered paramicrogyral zone adjacent to the microgyrus.

Much of the heavily stained paramicrogyral zone was not differentiated into CO-barrel structures (arrowheads in Fig. 24, and outlined gray-shaded area in Fig. 2B). The reconstructions of CO staining from serial sections demonstrated that this area of heavy diffuse CO staining overlapped the area of the expected...
lesioned rats (Jacobs et al., 1996a, 1999a). Here we sought to determine whether there is a relationship between the location of the epileptogenic zone and regions of pale and heavy CO staining surrounding the microsulcus. Field potential recordings were made in layer III directly above (in a plane orthogonal to the pi) the stimulation site at the layer VI/white matter border. This pair of electrodes was moved together to different distances from the microsulcus. In confirmation of our previous findings, no epileptiform activity was evoked within 0.2 mm of the microsulcus, while at sites 0.5–3.0 mm away epileptiform activity (Fig. 3D, asterisks) followed the typical short latency field potential. The distance over which epileptiform activity could be activated varied between slices; in most slices a typical short latency field potential without epileptiform activity was evoked when stimuli were applied 2.5 mm or more medial to the microsulcus. Mapping of the region from which epileptiform activity could be evoked was done for eight paramicrogyral zones either medial or lateral to the microsulcus in five slices. Subsequent CO staining of these sections showed similar patterns to other coronally stained sections, with heavy CO staining in layers III, IV, Vh and VI extending for 2–3 mm adjacent to the microgyrus. Comparison of the location of this heavy CO staining with the evoked field potentials showed that in seven of the eight paramicrogyral zones mapped, sites at which epileptiform activity was evoked were located in regions of heavy CO staining, whereas typical short latency field potentials without epileptiform activity were elicited within regions of relatively light CO staining (Fig. 3C,D, e.g. compare CO staining and responses at points 2 and 3 with those at 8 and 12). There was no correlation between intensity of CO staining and amplitude or form of the epileptiform activity. Thus, the presence of CO only correlates with the capacity to initiate epileptiform activity. In one of eight cases epileptiform activity was absent in a region which was heavily CO stained, but in none of the sections was epileptiform activity present in a region of pale or light CO staining.

**Survival Age**

While the overall size of the CO-barrel field varied with survival age, the pattern and the number of PMBSF CO-barrels was consistent in control rats aged P12, P22 and P37. The effect of the freeze lesion was similarly unchanged with age (Fig. 4A–D). In freeze-lesioned rats examined after a survival of 12–106 days of age, the PMBSF pattern was always disrupted and the number of PMBSF CO-barrels decreased. The pattern of light staining surrounding the microsulcus and an adjacent zone of heavy undifferentiated CO staining was also observed at all ages examined.

**Smaller Lesions**

Because our results with large lesions differed from those previously described for the effect of small electrolytic lesions on the pattern of the succinic dehydrogenase-identified barrel field (Ito and Seo, 1983; Seo and Ito, 1987), we examined the effect of smaller freeze lesions (probe tip: circular, 0.5 mm diameter) on the PMBSF pattern in three rats aged P22. The pattern of the CO staining in the PMBSF was still disrupted with these smaller
lesions, and a pale-stained region surrounded the microsulcus with numerous abnormally small CO-barrels bordering this region, similar to the results with larger lesions (Fig. 4E,F). The number of CO-barrels disturbed, however, and the area affected was much smaller than when the larger freeze-probes were used. The typical CO-barrel pattern was also present within 1 mm of the microsulcus. In addition, the lesion tended to be nearer to the border of the PMBSF in animals examined at P22 relative to those examined at P5–P6 (not shown), a finding that may be similar to the shift in CO-barrel field location that Seo and Ito observed.

Discussion
These results demonstrate that the development of a cortical microgyrus within the whisker representation of somatosensory cortex disrupts the overall pattern of metabolic staining within the barrel field as well as the formation of individual barrels. The microgyrus contains reduced CO staining while the region just outside of the microgyrus shows heavy and abnormally patterned staining extending over several millimeters and corresponding to the epileptogenic region. The organization of both cortical cell aggregates and thalamic afferents appears to be affected by the presence of the microgyrus and this disruption.

Figure 4. Aberrant CO staining patterns present at various survival ages and for different lesion sizes. (A) Pattern of CO staining in a rat surviving to P12 after a P1 freeze lesion with a circular 3.0 mm diameter probe tip. (B) Drawing of barrels in all sections of hemisphere shown in (A). (C) Pattern of CO staining in a P106 rat after a P1 freeze lesion with a rectangular 2 × 5 mm probe tip. (D) Barrel field drawing for hemisphere shown in (C). (E) Pattern of CO staining after a small lesion in a rat surviving to P22. Freeze probe was circular with a diameter of 0.5 mm. (F) Barrel field drawing for hemisphere shown in (E). In (A), (C) and (E), a pale area surrounds the microsulcus with heavy CO staining in the adjacent cortex. Less disruption of the pattern occurred with the smaller lesions. In (B), (D) and (F), black shaded area with diagonal lines represents area of microsulcus, and black outline around microsulcus shows extent of light CO staining in the surrounding microgyrus. Scalebar in (C): 400 µm for (A) and (C); scalebar in (E): 600 µm. Note that orientation and scale of drawings in (B), (D) and (F) are different from sections in (A), (C) and (E).
Cortical Folding and Barrel Shape

Was the disruption of the barrel field caused by a simple displacement during changes in the gross structure of the cortex? The formation of a microgyrus in the lissencephalic rat brain may occur through a mechanical process related to a greater growth of superficial layers relative to deep layers (Richman et al., 1975) or variations in tension along axons, dendrites and glia, as described by Van Essen (Van Essen, 1997). While current experiments cannot differentiate between these theories, the variable and abnormal shapes of the microsulcus and microgyrus (Jacobs et al., 1999a) suggest that tensile forces might shape these structures. These processes, although not yet fully understood, are likely to be comparable to those that occur in human polymicrogyria since the initiating factor of a hypoxic-ischemic lesion is the same (Dvorak and Feit, 1977; Rosen et al., 1995). Cortical folding might cause CO-barrels to appear to have abnormal shapes when sectioned tangentially, if they were present within the microgyrus. This was not the case, since heavy CO staining was never found within the microgyrus in the PMSBF region of cortex. Outside of the microgyrus, mechanical displacement may have contributed to some of the abnormally shaped CO-barrels, since layer IV was apparently located deeper than usual at the borders of the microgyrus in some animals. However, even if the microgyrus had been unfolded, the spacing and circumference of many of the irregularly shaped CO-barrels would not have been normal. In addition, the loss of CO-barrels, especially at sites distant to the microgyrus, cannot be explained by obscuration due to cortical folding.

Metabolic Activity

It has been repeatedly shown that peripheral deafferentation produced by plucking whiskers, cauterizing follicles or cutting the sensory nerve from the whiskers disrupts the architecture and reduces the intensity of staining within the corresponding cortical barrel (van der Loos and Woolsey, 1973; Killackey et al., 1978b; Wong-Riley and Welt, 1980; Dietrich et al., 1981). Associated with this decrease in metabolic staining is a decrease in neuronal activity (Durham and Woolsey, 1978; Simons et al., 1984). The light CO staining within the microgyrus likely reflects a reduction in neuronal activity. Wong-Riley has shown that the CO with which the barrel regions are visualized is located in both dendrites of cortical neurons as well asafferent axons (Wong-Riley, 1980). Thus the light CO staining within the microgyrus could reflect less activity in both neurons and afferent axons, while the heavy, undifferentiated staining in the paramicrogyral zone could be the result of a combination of the following factors: (i) an increased neuronal activity in both cells and axons, such as might occur during epileptogenesis; (ii) an increase in the number of cells and axons in this region; and (iii) a redistribution of cells and axons across the paramicrogyral zone. It is possible that thalamocortical afferents are present within the microgyrus, but not observed because of a reduction of AChE staining as reported after peripheral deafferentation (Chiaia et al., 1995). However, the fact that intense AChE staining, which is preferentially localized within the thalamic axons, was found with a pattern nearly identical to the aberrant CO staining (Fig. 2LJ) suggests that the thalamic afferents project in a wholly different pattern than in normal cortex, with an increased innervation of the paramicrogyral zone. Additionally, we believe that thalamic afferents are abnormal in microgyral cortex, since cortical architecture of the barrels appears to be dependent on the pattern of thalamic projections into cortex (see references cited in the Introduction). Manipulations which change this pattern also result in altered metabolic staining (Jensen and Killackey, 1987). Further, preliminary evidence using neuronal tracers shows that very few, if any thalamocortical afferents invade the microgyrus (Rosen and Galaburda, 1998; Jacobs et al., 1999b). These results also suggest that for thalamocortical connections made after small cortical lesions, laminar specificity is preferred over prototypical spatial organization within the residual laminae of the microgyrus.

The Paramicrogyral Zone

Analysis of adjacent Nissl- and CO-stained coronal and tangential sections made it clear that the area of the microgyrus contains little CO staining, and that the borders of the pale-stained region correspond to the boundary between microgyral layer 3 and the adjacent six-layered paramicrogyral cortex. These results are a further indication that although lamination is normal within the paramicrogyral zone, neurons and afferents in this region are abnormally organized. This idea is also supported by the finding that glutamate-immunoreactive processes travel in disorganized paths adjacent to the microgyrus (Humphreys et al., 1991), and that neurofilament immunostaining is abnormally intense at the microgyral border (Rosen et al., 1992) (K.M. Jacobs, I. Parada and D.A. Prince, unpublished observations). Changes in some glutamate receptors may also be affected throughout the cortical hemisphere containing a microgyrus (Zilles et al., 1998). Thus, the effect of these relatively small cortical malformations is more widespread than the structural lesions themselves.

Detailed electrophysiological mapping studies have shown that the microgyrus itself seldom exhibits epileptiform activity, while the epileptogenic region extends 0.5–2.5 mm from the microsulcus [see Fig. 9 in Jacobs et al. (Jacobs et al., 1996a) and detailed mapping in Jacobs et al. (Jacobs et al., 1999a)]. Current results confirm the focal nature of the epileptiform activity and show that it is evoked within the CO-dense zone outside of the microgyrus. The restructuring of thalamocortical innervation suggested here, together with potential changes within intra-
Cortical Lesions

After P0 cortical electrolytic lesions centered on the PMBSF, Ito and Seo found that the entire barrel field pattern emerged intact in mature animals (Ito and Seo, 1983; Seo and Ito, 1987). In contrast to this, even our small freeze lesions disrupted the pattern of the PMBSF, although the number of CO-barrels was not necessarily reduced. These differences may be related to the type, size and depth of the lesions in the present study relative to those of Seo and Ito. Electrolytic lesions applied at a depth of 0.8 mm in P0 rats (Seo and Ito, 1987) would be centered within the subplate (Rice and van der Loos, 1977), rather than the cortical plate, perhaps killing neurons yet to migrate into the cortex (those of layer II/III), while leaving intact neurons of layers IV–VI within the cortical plate. Seo and Ito report that, when examined in adult rats, their lesions could be seen primarily superficially, rather than at the expected level of the barrels [within layer IV (Seo and Ito, 1987)]. The consequences of transcranial freeze lesions are different, in that neurons within the cortical plate at the time of the lesion die (layer IV–VI neurons), while layer II/III cells that continue to migrate through the region of necrosis (Dvorak and Feit, 1977; Ferrer et al., 1993; Rosen et al., 1996) survive. Thus, the normal target of thalamocortical neurons is disrupted by the freeze lesions, and an interruption in the final pattern of barrel distribution is more likely to result. Our results are similar to those of Finger et al., who also found the barrel field pattern disrupted and barrels missing after lesions in which layer IV was lost [P0 aspiration of the cortical plate (Finger et al., 1978)].

Cortical Rewiring

Assuming that the reorganization of somatosensory afferents to the region of the microgyrus and its surround shown here is prototypic for other axonal systems, a major ‘rewiring’ of cortical circuits would be expected as a consequence of the early postnatal lesion. Callosal, recurrent intracortical, as well as other extracortical afferent connections are expected to be complete at birth in rat (Wise and Jones, 1976; Killackey et al., 1978a; Krist, 1978; Wise and Jones, 1978). In addition, callosal afferents show exuberance and are refined postnatally (Innocenti and Caminiti, 1980; Elberger, 1994). Loss of infragranular neuronal targets in a focal cortical area (e.g. the microgyrus) could foster an aberrant excessive invasion pattern within the adjacent cortex for all of these systems. In cat microgyri, a maintenance of exuberant cortical afferents occurs (Innocenti and Berbel, 1991). Abnormal terminations of callosal axons have also been reported in a spontaneously occurring microgyrus in rat (Rosen et al., 1989), and a preliminary report of experiments in rat suggests that intracortical afferents and efferents are abnormal in the hemisphere containing a microgyrus (Rosen and Galaburda, 1996). An overabundance of excitatory afferents originating within and outside of the cortex adjacent to the microgyrus may contribute to the generation of epileptiform activity which is initiated from the parain microgyral zone (Jacobs et al., 1999a).

Notes

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