Excitatory and Inhibitory Postsynaptic Currents in a Rat Model of Epileptogenic Microgyria

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Jacobs, K. M. and D. A. Prince. Excitatory and inhibitory postsynaptic currents in a rat model of epileptogenic microgyria. J Neurophysiol 93: 687–696, 2005. First published September 22, 2004; doi:10.1152/jn.00288.2004. Developmental cortical malformations are common in patients with intractable epilepsy; however, mechanisms contributing to this epileptogenesis are currently poorly understood. We previously characterized hyperexcitability in a rat model that mimics the histopathology of human 4-layered microgyria. Here we examined inhibitory and excitatory postsynaptic currents in this model to identify functional alterations that might contribute to epileptogenesis associated with microgyria. We recorded isolated whole cell excitatory postsynaptic currents and GABA<sub>A</sub> receptor-mediated inhibitory currents (EPSCs and IPSCs) from layer V pyramidal neurons in the region previously shown to be epileptogenic (paramicrogyral area) and in homotopic control cortex. Epileptiform-like activity could be evoked in 60% of paramicrogyral (PMG) cells by local stimulation. The peak conductance of both spontaneous and evoked IPSCs was significantly larger in all PMG cells compared with controls. This difference in amplitude was not present after blockade of ionotropic glutamatergic currents or for miniature (m)IPSCs, suggesting that it was due to the excitatory afferent activity driving inhibitory neurons. This conclusion was supported by the finding that glutamate receptor antagonist application resulted in a significantly greater reduction in spontaneous IPSC frequency in one PMG cell group (PMGE) compared with control cells. The frequency of both spontaneous and miniature EPSCs was significantly greater in all PMG cells, suggesting that pyramidal neurons adjacent to a microgyrus receive more excitatory input than do those in control cortex. These findings suggest that there is an increase in numbers of functional excitatory synapses on both interneurons and pyramidal cells in the PMG cortex perhaps due to hyperinnervation by cortical afferents originally destined for the microgyrus proper.

INTRODUCTION

An association between developmental malformations and neurological disorders, including epilepsy, has been known for more than a century (Crome 1952); however interest in this correlation has increased in the last 15 years. This is due to improvements in imaging techniques that have demonstrated a higher incidence of cortical malformations than previously diagnosed, particularly in patients with refractory epilepsy (Farrell et al. 1992; Hauser 1995; Lagae 2000; Palmini et al. 1991a,b). It is not currently known whether mechanisms underlying hyperexcitability in malformed cortex are unique among the epilepsies or why seizures associated with cortical malformations are particularly difficult to treat with currently available anti-epileptic medications.

A number of animal models of developmental malformations associated with epilepsy have been characterized in recent years (Chevassus-Au-Louis et al. 1999; Jacobs et al. 1999b). These models mimic various aspects of the histopathology as well as the increased propensity for cortical hyperexcitability. The form of structural defect or type of malformation created depends on the timing of the insult. Most malformations occur after the failure of a critical process in neurogenesis (resulting in microcephaly), migration (resulting in double cortex, or other heterotopias), or neuronal differentiation (resulting in focal cortical dysplasia). The malformation studied here, focal unilateral 4-layered microgyria, is distinct from others because the structural abnormality is usually formed as a result of cell death rather than an error in a developmental program. A correlation between prenatal traumatic events and the subsequent detection of polymicrogyria has been demonstrated in a number of cases (Barkovich et al. 1995; Montenegro et al. 2002; Richman et al. 1974). Because neurons of the deep layers migrate into place first, these neurons will be lost (Rosen et al. 1996), while cells of the superficial layers migrate through the area of damage, resulting in a region of dyslamination bordered by normal six-layered cortex (Dvorak and Feit 1977).

Animals with microgyral cortex show enhanced c-fos expression long after the freeze lesion is placed (Jacobs et al. 2001) and greater propensity for seizures after systemic kainic acid injections (unpublished observations). Epileptiform activity can be evoked without the addition of seizure-inducing agents in neocortical slices containing microgyri (Jacobs et al. 1996; Luhmann and Raabe 1996) after a 10- to 11-day latency after the freeze lesion (Jacobs et al. 1999a). We have previously determined that epileptiform activity is most readily evoked by stimulation within the normally laminated cortical region surrounding the microgyrus (the “paramicrogyral” zone) and that severing the connections between the microgyrus and surrounding cortex does not eliminate this hyperexcitability (Jacobs et al. 1999b). Therefore we have focused efforts on identifying the epileptogenic mechanisms in the paramicrogyral cortex.

Previous findings indicate that both inhibitory and excitatory synaptic transmission may be altered (Jacobs et al. 1999b). For example, immunocytochemical experiments showed that there were decreased numbers of parvalbumin-immunoreactive neurons adjacent to the microgyrus, suggesting that at least one population of inhibitory interneurons might be reduced in this region (Rosen et al. 1998). Also, enhanced excitatory connec-

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tivity likely results from substantial rewiring of excitatory circuits, presumably because the lesion-induced loss of neurons occurs prior to maturation of axonal projection patterns (Holopainen and Lauren 2003; Jacobs et al. 1999b; Poulter et al. 1997; Redecker et al. 2000; Rosen et al. 2000).

To test the hypothesis that enhanced excitatory and/or reduced inhibitory synaptic activity contributes to a malformation model of partial epileptogenesis, we recorded spontaneous and evoked postsynaptic currents from layer V pyramidal neurons within the region of hyperexcitability adjacent to the malformation just after the onset of epileptogenesis (13–16 days postnatally). These cells are known to have particular intrinsic characteristics and axonal projection patterns that make them likely to be involved in initiation and spread of epileptiform activity (Chagnac-Amiot et al. 1990; Connors 1984; Hoffman et al. 1994). Results suggest that there is enhanced glutamatergic excitatory innervation of both layer V pyramidal cells and interneurons in the paramicrogyral zone resulting in an increased amplitude of spontaneous and evoked inhibitory currents as well as a shift in the balance of synaptic activation of pyramidal neurons toward excitation.

METHODS

All experimental procedures were performed under protocols approved by the Stanford Administrative Panel on Laboratory Animal Care. Freeze lesions were made as previously described (Jacobs et al. 1996). Briefly, Sprague Dawley rat pups <$48$ h old were covered with ice for $<4$ min until movements and responses to tail pinch were absent. The skull was exposed through a scalp incision and a freezing probe with a rectangular tip, $5 \times 2$ mm, cooled to $-50$ to $-60^\circ$C, was placed on the skull over somatosensory cortex for $5$–$6$ s. The scalp was then sutured, and the pup warmed and returned to the dam. Thirteen to 16 days later, rats were anesthetized with pentobarbital ($55$ mg/kg ip) and decapitated and brains were removed. The site of the previous freeze lesion could be located as a small depression in the pial surface that corresponded to the site of the microsulcus (Jacobs et al. 1996). Standard techniques were used for preparing and maintaining neocortical slices. After removal, the brain was immediately placed in a sucrose-modified artificial cerebral spinal fluid (ACSF) containing (in mM) $2.5$ KCl, $10$ MgSO$_4$, $3.4$ CaCl$_2$, $1.25$ Na$_2$HPO$_4$, $234$ sucrose, $11$ glucose, and $26$ NaHCO$_3$; pH $7.4$ when saturated with $95\%$ O$_2$-$5\%$ CO$_2$. Coronal $300$-$500$ μm-thick slices through the microgyri were used. Layer V pyramidal cells from 65 rats with microgyri were used. Layer V pyramidal cells within somatosensory cortex were easily visually identified based on depth relative to the pia ($0.7$–$1.6$ mm, equivalent to $40$–$70\%$ of total cortical depth) and cell morphology (large soma with single emerging apical dendrite extending toward the pial surface) in DIC images. Pyramidal structure was also confirmed in some cells processed after intracellular biocytin labeling. In freeze-lesioned cortex, layer V cells chosen were $0.3$–$2.0$ mm from the microsulcus ($0.68 \pm 0.03$ mm for 131 cells), within the area expected to be epileptogenic (Jacobs et al. 1999a) and at an age when nearly every slice from every animal generated evoked epileptiform activity in previous experiments (Jacobs et al. 1999a). For most, but not all neurons, the response to nearby electrical stimulation was recorded. Stimulus parameters were similar to those that evoked epileptiform activities in our previous experiments. Monopolar square pulses were applied within layer V, $50$–$300$ μM from the recorded cell with a glass micropipette filled with $1$ M NaCl. A $20$-μs-duration pulse was applied at threshold intensity for evoking a just detectable IPSC. A series of graded intensity stimuli was then applied by increasing the pulse duration to $40$, $80$, $160$, and $320$ μs ($2\times$, $4\times$, $8\times$, and $16\times$ threshold). Digitized waveforms were imported into Microsoft Excel and macros used to calculate peak and area of evoked currents. Measurements of peak current were made over a $0.5$-ms time window from short-latency evoked responses.

Spontaneous events were recorded for a period of $3$–$10$ min with $\geq 50$ and typically $>500$ events for each recording condition. Currents were analyzed with event detection software (Detector, by John Huguenard). For amplitude measurements, currents uncontaminated by subsequent events were isolated and peak current over a $1$-ms window was determined. Ratios of event frequencies were made in individual cells, as follows: EPSC frequency/(EPSC frequency + IPSC frequency). Measurements are reported as means $\pm$ SE. Student’s $t$-test were used to test for significance.

RESULTS

Evoked and spontaneous IPSCs in normal ACSF

In slices from control animals, two types of response were evoked by stimuli delivered within $300$ μm of the recorded layer V pyramidal neuron in normal ACSF. Short-latency outward IPSCs that were graded in amplitude with stimulus intensity, were evoked in $65\%$ of control cells ("standard" responses, $n = 83$, Fig. 1A1). In the other $35\%$ of control cells, variable form, variable latency multiphasic events were evoked in an all-or-none manner, in addition to the graded short latency IPSC (see $\rightarrow$, Fig. 1A2). This multiphasic activity had characteristics similar to those seen in the field potentials.
evoked in unlesioned immature animals (Luhmann and Prince 1990) and the paramicrogyral cortex of mature animals (Jacobs et al. 1996; Luhmann and Raabe 1996). Parameters of sIPSCs were not significantly different in neurons that had standard versus polysynaptic responses to stimulation (Fig. 1B, 1 and 2; Table 1) and all control cells were therefore considered as a single group.

Both types of evoked response were also observed in layer V pyramidal neurons adjacent to microgyri (paramicrogyral or PMG cells). The PMG cells, however, differed from controls in the ratio of the two response types. The majority (60%) of cells in PMG cortex had late, multiphasic activity that was more consistently activated in PMG than in control cells (Fig. 1A3 →). In terms of their long and shifting latency, variable amplitude and all-or-none appearance, these responses closely resembled the evoked epileptiform field potentials and neuronal responses seen in this and other models of lesion-induced chronic focal epileptogenesis (Hoffman et al. 1994; Jacobs et al. 1996; Prince and Tseng 1993). For this reason, we have termed the neurons showing such abnormal evoked responses PMGE cells. A smaller population of PMG neurons had standard responses consisting of short-latency, smoothly decaying evoked IPSCs (termed PMGS cells in the following text). Both experimental cell groups (PMGE and PMGS) were found throughout the depth of layer V and the entire PMG cortex examined (0.3–2.0 mm from the microsulcus). The type of response in a given cell could not be altered by changes in stimulus parameters or stimulus position. In some cases, these two different response types were seen in neurons close to one another within the same slice (Fig. 1C). There was no difference in mean input resistance for the two PMG cell groups (250 ± 21 and 263 ± 23 MΩ for 70 PMGE and 49 PMGS, respectively). For measures in which the two PMG groups were not significantly different quantitatively, they are compared as a single group to the control population. On measures for which PMGE cells were significantly different from PMGS,
cells, these experimental groups were compared individually to the entire control cell group.

As previously reported (Salin and Prince 1996), characteristics of sIPSCs varied greatly between individual layer V pyramidal neurons, even within the control population. The frequency of sIPSCs ranged from 0.3 to 21.5 Hz and mean peak conductance from 0.18 to 0.59 nS. When control and PMG cell populations were compared, there was no significant difference in sIPSC rise time (1.3 ± 0.4 ms control and 1.3 ± 0.3 ms PMG) or mean frequency (see Table 1). However, as suggested from examination of raw traces (e.g., Fig. 1B, I and 2, vs. 3 and 4), the peak conductance of sIPSCs was significantly larger in the PMG group (0.38 ± 0.02 nS, n = 80) than in control cells (0.32 ± 0.01 nS, n = 72; P < 0.05; Fig. 2). This increase in the average peak conductance of sIPSCs was mainly due to a greater number of large events rather than an increase in the maximal amplitude recorded (Fig. 2C). For PMG cells, most sIPSCs (51%) had a peak conductance >0.45 nS, whereas this was true for only 33% of sIPSCs recorded in control cells (Fig. 2C, inset). The largest event recorded from control cells had a peak conductance of 3.70 nS. Only 2 of 6,000 sIPSCs recorded from PMG cells were larger than this, having peak conductances of 3.75 and 4.39 nS. When the two groups of PMG cells were separately compared with controls, only the PMGE group showed a significant increase in average sIPSC peak conductance (Table 1). IPSCs evoked in control ACSF were also significantly larger in PMG neurons, at all intensities tested (Fig. 2D). One potential mechanism for these differences in sIPSCs and evoked (e)IPSC amplitude would be an increased excitatory input onto GABAergic interneurons (see DISCUSSION). To examine this possibility, we recorded sIPSCs and eIPSCs after perfusion of slices with bathing medium containing ionotropic glutamate receptor antagonists DNQX and APV to bath AMPA/kainate and N-methyl-p-aspartate (NMDA) receptors.

eIPSCs and sIPSCs in DNQX/APV

Addition of glutamate antagonists decreased both the frequency and mean amplitude of spontaneous sIPSCs (Fig. 3, A–C). Under these conditions, there was no longer any difference in peak conductance between control and PMG cells for either the spontaneous (Table 1) or evoked IPSCs (Fig. 3F). The mean amplitude of sIPSCs is affected by the relative proportions of smaller miniature (m)IPSCs and larger action potential-driven IPSCs (Edwards et al. 1990). Thus the difference in IPSC peak conductance between these cell groups found in normal bathing medium was most likely due to a greater proportion of the large, action potential-related IPSCs resulting from enhanced glutamatergic excitatory activation of inhibitory interneurons synapsing on PMGE cells. We further assessed the proportion of glutamate-driven IPSCs within individual cells by calculating changes in the sIPSC frequency after addition of glutamate antagonists. In most cells, the direction of change in sIPSC frequency was a decrease. The average decrease in frequency was significantly greater for PMG cells than for controls (48 vs. 18%, respectively; Fig. 3D). PMG cells were not significantly different from controls on this measure (Fig. 3, D and E). PMGE cells showed less than a 30% decrease in sIPSC frequency after addition of DNQX/APV and was not simply dependent on the initial sIPSC frequency recorded in normal slice bathing medium (Fig. 3E).

After addition of the glutamate antagonists, there was no significant difference in the eIPSC amplitude between control and PMG groups at any stimulus intensity (Fig. 3F). To assess the proportion of the evoked IPSC that was due to polysynaptic activity, we calculated within individual cells the change in

### TABLE 1. Parameters of synaptic currents in layer V pyramidal neurons of control and paramicrogyral cortex

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>All PMG</th>
<th>PMGE</th>
<th>PMGS</th>
</tr>
</thead>
<tbody>
<tr>
<td>sIPSCs Conductance</td>
<td>0.32 ± 0.01 (72)</td>
<td>0.38 ± 0.02 (80)*</td>
<td>0.39 ± 0.02 (45)*</td>
<td>0.36 ± 0.02 (28) NS</td>
</tr>
<tr>
<td>sIPSCs Frequency</td>
<td>4.3 ± 0.4 (72)</td>
<td>4.8 ± 0.5 (80) NS</td>
<td>4.7 ± 0.6 (45) NS</td>
<td>5.2 ± 0.9 (28) NS</td>
</tr>
<tr>
<td>APV/DNQX IPSCs Conductance</td>
<td>0.28 ± 0.03 (16)</td>
<td>0.31 ± 0.01 (29) NS</td>
<td>0.34 ± 0.02 (14) NS</td>
<td>0.28 ± 0.02 (13) NS</td>
</tr>
<tr>
<td>APV/DNQX IPSCs Frequency</td>
<td>3.4 ± 0.7 (16)</td>
<td>3.9 ± 0.9 (29) NS</td>
<td>3.0 ± 1.0 (14) NS</td>
<td>5.5 ± 1.6 (13) NS</td>
</tr>
<tr>
<td>Percentage Change in Frequency</td>
<td>17.9% ± 9.4% (16)</td>
<td>23.3% ± 9.5% (29) NS</td>
<td>47.9% ± 6.0% (14)*</td>
<td>-7.9% ± 16.1% (13) NS</td>
</tr>
</tbody>
</table>

Results for all measures in all cell groups expressed as means ± SE (n). Conductance is given in nS, frequency in Hz. All paramicrogyral (PMG) cell group includes some cells in which response type was not determined (only spontaneous events were recorded). NS, nonsignificant; sIPSC and eIPSC, spontaneous and evoked inhibitory postsynaptic current; APV, d-2-amino-5-phosphonopentanoic acid; DNQX, 6,7-dinitroquinoxaline-2,3 (1H, 4H)-dione; mIPSC, miniature IPSC; sEPSC and mEPSC, spontaneous and miniature excitatory postsynaptic current. *P < 0.05.
peak amplitude of the eIPSC after addition of DNQX/APV. These calculations showed a decrease in eIPSC amplitude after addition of DNQX/APV for every cell recorded. The amount of decrease was significantly greater for the PMGE group relative to control cells (Fig. 3G). This again suggested a greater excitatory afferent activation of inhibitory neurons specifically synapsing on PMGE neurons.

**sEPSCs**

Characteristics of sEPSCs also varied greatly between cells within the control population, with frequencies of 0.3–19.5 Hz and peak conductances of 0.17–1.31 nS for 26 layer V neurons. There was no significant difference between control and PMG cells in sEPSC rise time (1.3 ± 0.09 and 1.3 ± 0.06 ms, respectively) or peak conductance (see Table 1). The mean frequency of sEPSCs was significantly greater for PMG cells than for controls (Fig. 4). Because the frequency of both sIPSCs and sEPSCs varied greatly between cells, we compared sIPSCs and sEPSCs within individual cells for 21 control and 23 PMG neurons. Control cells typically had a higher frequency of inhibitory currents than excitatory currents (e.g., 4.9 and 4.1 Hz, respectively, in cell of Fig. 4A). For PMG cells, sEPSCs were more frequent than sIPSCs (e.g., 3.6 and 5.1 Hz for sIPSCs and sEPSCs, respectively, in neuron of Fig. 4B). We used a ratio of EPSC and IPSC frequencies (E ratio, see METHODS) to compare these events in individual cells. The mean E ratio was slightly below 0.5 for control cells (Fig. 4E) and significantly higher for PMG cells (0.6, P < 0.05). This indicates that the relative frequencies of EPSCs and IPSCs are shifted toward excitation in individual pyramidal neurons in layer V of the PMG zone. Ratios were not significantly different for PMGE versus PMGS neurons (0.56 ± 0.05 and 0.63 ± 0.06, respectively, N.S.).

**mIPSCs and EPSCs**

To estimate relative numbers of functional synapses, miniature events were recorded with 1 μM TTX in the bathing medium. There was no difference in mIPSC frequency (Fig. 5, A–D), amplitude (Table 1), or rise time (1.1 ± 0.04 and 1.2 ± 0.04 ms, respectively) for 34 control versus 45 PMG cells. Assuming no significant differences in the probability of transmitter release at inhibitory terminals between these cell groups, results suggest that control and PMG layer V pyramidal neurons receive approximately equivalent numbers of inhibitory synapses. In contrast to this, miniature (m)EPSCs were nearly twice as frequent in PMGS cells as in control cells (Fig. 5E, Table 1), suggesting that PMGS neurons have more functional excitatory synapses. Amplitude and rise times of mEPSCs were not significantly different between groups for mEPSCs (Table 1; rise times: 1.1 ± 0.1, 1.4 ± 0.1, and 1.1 ± 0.1 ms for control, PMGE, and PMGS, respectively). The mean ratio of mEPSC and mIPSC frequencies, calculated in individual cells
as in the preceding text, was also significantly higher for PMG\textsubscript{S} than for either control or PMG\textsubscript{E} neurons (Fig. 5F).

**DISCUSSION**

Our results suggest that there is a functional increase in the excitatory afferent input to neurons of the PMG cortex. These findings are consistent with our previous suggestion that cortical afferents unable to find appropriate targets within the malformed region may instead synapse in the adjacent (PMG) region (Jacobs et al. 1999c).

**Response types in PMG neurons**

The two subgroups of PMG neurons were distinguished not only by their response to stimulation, but also by measures of...
PMGS neurons does not indicate that these cells are silent in the absence of epileptiform-like activity in the evoked IPSCs of epileptiform activity by firing action potentials. Similarly, the absence of reduction in frequency of mIPSCs in the PMG layer V could resolve this issue. The fact that these measures are not directly related suggests that the PMG subgroups reflect distinct neuronal types rather than simply different responses due to changes in stimulus location or methodological differences in the recordings. Neocortical layer V pyramidal neurons can be separated into a number of different groups based on dendritic and axonal morphology, intrinsic firing pattern, and postsynaptic targets (Chagnac-Amitai et al. 1990; Kasper et al. 1994; Larkman 1991; Mason and Larkman 1990; Tseng and Prince 1993). These characteristics have particular depth correlations within layer V. It is not clear whether the two groups of PMG cells are also differentiated by any of these other factors; however, the PMG subgroups are not segregated by depth. In fact, these response types were sometimes seen in cells within the same slice. This makes it less likely that PMG subgroups will correlate with the previously described layer V cell types.

The response of PMG cells to stimulation suggests that they receive input from inhibitory neurons that are activated during epileptiform activity; however, we do not know whether the PMG cells typically also participate in initiation of that epileptiform activity by firing action potentials. Similarly, the absence of epileptiform-like activity in the evoked IPSCs of PMG neurons does not indicate that these cells are silent during abnormal synchronization, in fact the opposite may be true because less inhibition occurs at longer latencies when epileptiform activity is typically observed in field potential recordings. Current-clamp recordings in which action potentials are not blocked could resolve this issue. Consistent differences in the amount of inhibition during epileptiform discharges have previously been observed in specific layer V cell types (Chagnac-Amitai and Connors 1989). Although we do not currently know whether these cell types have any relation to our PMG and PMG cells, this finding does suggest that inhibitory cells do not innervate layer V pyramidal neurons in a random fashion. In addition, these two cell types likely receive different amounts of excitatory innervation because significantly higher mEPSC frequencies were recorded in the PMG cells relative to both controls and PMG cells.

Changes in cortical inhibition

A reduction in functional inhibition has been observed in some epilepsy models, using measures of peak inhibitory postsynaptic potential (IPSP) conductance (Luhmann et al. 1995), IPSC amplitude (Isokawa 1996), sIPSC frequency (Li and Prince 2002), mIPSC frequency (Mangan and Bertram 1998), and response to GABA application (Whittington et al. 1995). Recent work has also suggested that function of GABAB receptors may be reduced in slices from human dysplastic cortex (D’Antuono et al. 2004). In addition, previous results in the microgyrus model have shown a decrease in the numbers of parvalbumin immunohistochemically stained interneurons throughout the hemisphere containing the microgyrus in young animals (P13-15) and focally within layers IV and V of the PMG zone (0.5 mm outside of the malformed region, but not 2.0 mm distant) in older (P21-60) animals (Rosen et al. 1998; but see Schwarz et al. 2000). While parvalbumin might be downregulated independently of loss of GABAergic neurons (Wittner et al. 2001), our previous finding raised the possibility that a reduction in numbers of inhibitory neurons and synapses in the PMG zone would lead to decreases in inhibition. Inhibitory currents due to GABA release from parvalbumin-containing interneurons that target somata and proximal dendrites of pyramidal cells should be detectable in somatic voltage-clamp recordings (Soltész et al. 1995). However, the lack of reduction in frequency of mIPSCs in the current experiments suggests that the overall number of GABAergic synapses is unchanged adjacent to the malformation, assuming that the probability of release is not altered. The idea that inhibition is functional in this model is supported by others’ findings as well (Hagemann et al. 2000). One possible explanation for these seemingly discrepant findings is compensatory sprouting of axonal arbors of surviving inhibitory interneurons with establishment of new inhibitory synapses that may be an important consequence of cortical injury (Davenport et al. 1990; Katsumaru et al. 1986; Nicoullon and Dusticier 1981). It is important to emphasize that monosynaptic eIPSCs are not altered in the PMG neurons perhaps because the degree of interneuronal loss and compensatory new inhibitory connectivity are roughly balanced. It is also possible that subtypes of GABAergic neurons in PMG cortex are differentially affected (Buckmaster and Dudek 1997; Dinocourt et al. 2003; Morin et al. 1998), leading to an increase in the contacts provided by one subgroup and a decrease in those from another group (Cossart et al. 2001). Recent demonstration of separate inhibitory cortical networks for different physiologically-defined types of GABAergic cells (Galarreta and Hestrin 1999; Gibson et al. 1999) suggests that these function independently and therefore...
their synapses are likely to be differentially regulated. GABAergic cell types distinguished by their content of peptides and calcium-binding proteins display differential changes in cell numbers after kainate-induced epilepsy (Buckmaster and Dudek 1997; Magloczky and Freund 1993). Changes in one subtype without changes in the total GABAergic cell population have also been described for human temporal lobe epilepsy patients (Mathern et al. 1995). The possibility that similar selective changes in inhibitory cell types occur in malformed cortex is currently being investigated.

A second compensatory mechanism that might maintain the strength of inhibition in the PMG zone is an increased afferent excitatory input onto inhibitory neurons. This appears to be relatively selective for inhibitory neurons that synapse on a subgroup of layer V pyramidal neurons (PMG<sub>E</sub> cells). The increase in sIPSC amplitude for the population of PMG cells is mainly due to PMG<sub>E</sub> cells (see Table 1). The significantly greater decrease in IPSC frequency after addition of glutamate antagonists is also specific to the PMG<sub>E</sub> group and supports our conclusion that increased sIPSC amplitude in this cell group is due to a greater number of large events from impulse-related GABA release (Otis et al. 1991). It is possible that the increased afferent drive onto inhibitory neurons is a compensatory change in response to hyperactivity of this cortical region; however, the timing of these alterations in sIPSC and eIPSC amplitude relative to the onset of epileptogenesis (at P12) is not currently known. Apparent increases in cortical inhibition have been described in other epilepsy models (Buhl et al. 1996; Prince et al. 1997) as well as in tissue from human epilepsy patients (Wittner et al. 2001). Enhanced inhibition may also contribute to the synchronization of excitatory cortical activity that is necessary to initiate the spread of epileptiform activity (Avoli et al. 2002; Khazipov and Holmes 2003; Michelson and Wong 1994; Troyer et al. 1992).

Hyperinnervation of PMG region

It is well known that specific thalamic afferents target layer IV neocortical neurons, even when their normal placement is altered (see for review Bolz et al. 1993). We have hypothesized that, for this reason, thalamic afferents originally destined to synapse in the malformed region may instead synapse mainly in the adjacent cortex, where layer IV neurons are present. Anatomical studies tracing thalamocortical afferents have supported this idea (Jacobs et al. 1999b; Rosen et al. 2000). Increased frequency of mEPSCs seen in the current study may reflect an increased number of excitatory synapses onto layer V pyramidal cells from these or other redirected afferents. We also cannot rule out the possibilities that the increase in mEPSC frequency is caused by an increase in release probability or a redistribution of the same number of excitatory synapses, causing more mEPSCs to be detected. Altered dendritic morphologies shown to occur in this model include longer basal dendrites of layer V neurons (Di Rocco et al. 2002), which may specifically be associated with redistribution of excitatory synapses. These dendrites may also provide increased synaptic “space” for hyperinnervation. Whatever the mechanism, the ratio of numbers of EPSCs to IPSCs was significantly increased, suggesting that these changes may help to maintain the membrane potential near the threshold for action potential firing (Bernander et al. 1991; Destexhe and Pare 1999; Fellous and Sejnowski 2003; Ho and Destexhe 2000; McCormick et al. 2003), thus increasing the overall excitability of the cortex.

The increase in mEPSC frequency was specific to the cell group that did not show changes in inhibition (PMG<sub>S</sub>). Our data suggest that if hyperinnervation occurs, it does so on selective postsynaptic targets rather than in a random fashion. One possible explanation for these differences may be in the main cortical afferents that contact these two cell groups. Thalamic afferents invade single whisker representations in somatosensory cortex (barrels) (Agmon et al. 1993), whereas callosal afferents avoid these regions and synapse mainly in the surrounding zones (septa) (Hayama and Ogawa 1997). Callosal axons have not lost all targets within the malformation because superficial layer neurons remain. We would expect then that there would be less aberrant connectivity of callosal afferents than of thalamic afferents. Rosen and colleagues indeed found that callosal axons did invade the malformed cortex, presumably finding appropriate targets there (Rosen et al. 2000). In addition, a study of cortical-cortical association projections suggests that these may be reduced rather than enhanced (Giannetti et al. 2000). Thus if the apical dendrites of PMG<sub>S</sub> cells passed through layer IV barrels, while those of PMG<sub>E</sub> cells were passed through layer IV septal regions, an increase in excitatory afferent contacts would be expected for the reorganized thalamocortical afferents synapsing in the barrels. This increase might not be observed in PMG<sub>E</sub> cells if they receive mainly callosal axons and few thalamocortical afferents. Although we have no direct evidence on changes in innervation relative to barrel location, our results do show PMG<sub>S</sub> cells receive different levels of excitatory afferent innervation than do PMG<sub>E</sub> cells.

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