Synaptic Activity in Chronically Injured, Epileptogenic Sensory-Motor Neocortex

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Li, Huifang, and David A. Prince. Synaptic activity in chronically injured, epileptogenic sensory-motor neocortex. J Neurophysiol 88: 2–12, 2002; 10.1152/jn.00507.2001. We recorded spontaneous and evoked synaptic currents in pyramidal neurons of layer V in chronically injured, epileptogenic neocortex to assess changes in the efficacy of excitatory and inhibitory neurotransmission that might promote cortical hyperexcitability. Partial sensory-motor neocortical isolations with intact blood supply (“undercuts”) were made in 20 rats on postnatal day 21–25 and examined 2–6 wk later in standard brain slice preparations using whole cell patch-clamp techniques. Age-matched, uninjured naive rats (n = 20) were used as controls. Spontaneous and miniature excitatory and inhibitory postsynaptic currents (s- and mEPSCs; s- and mIPSCs) were recorded using patch-clamp techniques. The average frequency of s- and mEPSCs was significantly higher, while that of s- and mIPSCs was significantly lower in neurons of undercuts versus controls. The increased frequency of excitatory events was due to an increase in both s- and mEPSC frequency, suggesting an increased number of excitatory contacts and/or increased release probability at excitatory terminals. No significant difference was observed in 10–90% rise time of these events. The input-output slopes of fast, short-latency, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid/kainate (AMPA/KA) receptor-mediated components of evoked EPSCs were steeper in undercuts than in controls. The peak amplitude of the AMPA/KA component of EPSCs evoked by supra-threshold stimuli was significantly greater in the partially isolated neocortex. In contrast, the N-methyl-D-aspartate receptor-mediated component of evoked EPSCs was not significantly different in neurons of injured versus control cortex, suggesting that the increased AMPA/KA component was due to postsynaptic alterations. Results support the conclusion that layer V pyramidal neurons receive increased AMPA/KA receptor-mediated excitatory synaptic drive and decreased GABA<sub>A</sub> receptor-mediated inhibition in this chronically injured, epileptogenic cortex. This shift in the balance of excitatory and inhibitory synaptic activation of layer V pyramidal cells toward excitation might be maladaptive and play a critical role in epileptogenesis.

INTRODUCTION

The incidence of posttraumatic epilepsy following penetrating cortical wounds is >50% (Salazar 1985). However, the mechanisms underlying epileptogenesis that results from traumatic brain injuries are largely unknown. Neocortical islands, chronically isolated from surrounding cortex are a well-established in vivo model of injury-induced cortical hyperexcitability in cat and monkey (Echlin and Battista 1963; Halpern 1972; Sharpless 1969) and in man (Echlin et al. 1952). After isolation, the injured neocortex becomes progressively more excitable and can develop prolonged evoked ictal events within several weeks (Sharpless and Halpern 1962). Various pathological mechanisms have been proposed, including selective loss of inhibitory synapses (Ribak and Reiffenstein 1982); and axonal sprouting of injured neurons with formation of new recurrent excitatory synapses (Purpura and Houser 1961). We have previously used a standard brain slice preparation to study the cellular mechanisms of posttraumatic epileptogenesis in this model (Hoffman et al. 1994; Prince and Tseng 1993; Salin et al. 1995). Epileptiform activities, including spontaneous and evoked synchronous interictal polysympathetic field potentials and associated excitatory and inhibitory polysynaptic potentials/currents in pyramidal neurons, persist in neocortical slices from the partially isolated cortex maintained in vitro (Hoffman et al. 1994; Prince and Tseng 1993; Salin et al. 1995). Abnormal activities are present beginning ~10 days to 2 wk after the initial lesion, originate in layer V and spread to other lamina and across the slice (Hoffman et al. 1994; Prince and Tseng 1993). By using intracellular biocytin labeling techniques, we have also found that layer V pyramidal neurons from injured neocortex sprout extensive axonal collaterals that arborize principally within layer V, where they presumably establish new excitatory connections (Salin et al. 1995). A variety of other anatomical findings, discussed in the following text, suggest that a reorganization of both excitatory and/or inhibitory synaptic circuits, as well as alterations in the receptor properties of individual neurons, might occur in injured cortex. The possible functional consequences of these anatomical changes have not been thoroughly examined. We therefore quantitatively compared various features of spontaneous and evoked synaptic activities in control neurons and those of injured, partially isolated cortex. Results support the conclusion that alterations in the intracortical synaptic network result in a significant shift of the balance between excitatory and inhibitory inputs on pyramidal neurons toward increased excitation. Such alterations may play an important role in the pathogenesis of focal epileptiform activity and seizures following cortical injury. Portions of this work have been reported in an abstract (Li and Prince 1999).

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METHODS

All experiments were carried out according to protocols approved by the Stanford Institutional Animal Care and Use Committee. A total of 40 Sprague-Dawley rats aged P36–63 (P0 = date of birth) were used for in vitro recordings. Twenty rats (referred to as “undercut animals” in the following text) had neocortical lesions placed at ages P21–25. They were deeply anesthetized with ketamine (80 mg/kg ip) and xylazine (Rompun 8 mg/kg ip), and a ~3 × 5-mm bone window centered on the coronal suture removed, leaving the dura intact, exposing a portion of the frontoparietal cortex unilaterally. A partial isolation of an island of sensory-motor cortex (Fr1–2, HL, Par1) (Zilles 1985) was made as previously described (Hoffman et al. 1994). A 30-gauge needle, bent at approximately a right angle 2.5–3 mm from the tip, was inserted tangentially through the dura, just beneath the pial vessels, parasagittally ~1–2 mm from the interhemispheric sulcus, and lowered to a depth of 2 mm. The needle then was rotated through 120–135° to produce a contiguous white matter lesion, elevated to a position just under the pia, making a second transcortical cut, and removed. An additional transcortical lesion was placed ~2 mm lateral and parallel to the parasagittal cut in a similar manner. The skull opening was then covered with sterile plastic wrap (Saran Wrap), and the skin was sutured. Lesioned animals recovered uneventfully, and were reanesthetized for slice experiments 2–6 wk later.

Techniques for preparing and maintaining brain slices in vitro were as previously described (Fukuda and Prince 1992). Animals were deeply anesthetized with pentobarbital sodium (55 mg/kg ip), decapitated, and the brain was rapidly removed and placed in cold (4°C) oxygenated cutting solution containing (in mM) 230 sucrose, 2.5 KCl, 1.25 NaH2PO4, 10 MgSO4·7H2O, 10 glucose, 0.5 CaCl2·2H2O, and 26 NaHCO3. A block of brain containing the injured area was fastened to the stage of a vibratome (Lancer Series 1000) with cyanoacrylate C), which contained (in mM) 126 NaCl, 2.5 KCl, 1.25 NaH2PO4, 10 MgSO4·7H2O, 10 glucose, 0.5 CaCl2·2H2O, and 26 NaHCO3. A block of brain containing the injured area was fastened to the stage of a vibratome (Lancer Series 1000) with cyanoacrylate (Krazy Glue), and 350-μm-thick coronal slices were cut in the preceding solution. Slices were then incubated in standard perfusion solution (32°C), which contained (in mM) 126 NaCl, 2.5 KCl, 1.25 NaH2PO4·2 CaCl2, 2 MgSO4·7H2O, 26 NaHCO3, and 10 glucose; pH 7.4 when saturated with 95% O2-5% CO2.

After ≥1 h of incubation, single slices were transferred to a recording chamber where they were minimally submerged and maintained at 32 ± 1°C. Patch electrodes were pulled from borosilicate glass tubing (1.5 mm OD) and had an impedance of 2–3 MΩ when filled with intracellular solution containing (in mM) 115 CsGluconate, 10 CsCl, 5 ethylene glycolglycolis (β-aminooxyethyl)-N,N,N',N'-tetraacetic acid (EGTA), 0.5 CaCl2·2H2O, 2 MgCl2·6H2O, 10 N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 1 lidocaine N-ethyl bromide (QX-314), 3 ATP·Na, 0.2 GTP; pH 7.3 adjusted with 1 M CsOH. The osmolarity of the pipette solution was adjusted to 275–285 mosM. Whole cell voltage-clamp recordings were made from visually identified layer V pyramidal cells using infrared video microscopy and a ×63 water-immersion lens with differential interference contrast optics (Zeiss Axiostar) and an Axopatch 200 amplifier (Axon Instruments). An estimated liquid junction potential of 7 mV was subtracted from the recorded membrane potentials. The estimated EL was ~50 mV based on the Nernst equation, taking into account the permeability of glucose through Cl- channels (Barker and Harrison 1988). Under these recording conditions, inhibitory postsynaptic currents (IPSCs) were outward at a holding potential (Vh) of 0 to +5 mV and spontaneous excitatory postsynaptic currents (sEPSCs) were inward at Vh of −50 to −55 mV. Access resistance was measured in voltage-clamp mode from responses to 2 mV hyperpolarizing voltage pulses using software provided by Dr. J. Huguenard. The series resistance (Rs) was not compensated. The maximum Rs in our experiments was 14 MΩ, and the maximum Iw was <200 pA holding at +5 mV and <100 pA holding at −55 mV, so that maximum error in measured membrane potential was <2.8 mV. Only recordings with access resistance of 7–14 MΩ and without significant (>25%) changes during the recording were used for data analysis.

Spontaneous IPSCs (sIPSCs) and sEPSCs were low-pass filtered at 2 kHz. Evoked EPSCs (eEPSCs) were obtained in perfusate containing bicuculline methiodide (BMI) using 0.12-Hz monopolar focal extracellular stimulation delivered with an artificial cerebrospinal fluid (ACSF)-filled patch pipette that was placed 80 ± 10 μm from the recorded soma in the same lamina. Under these conditions, epileptiform responses that were occasionally evoked at higher stimulus intensities were discarded. In some experiments, we blocked N-methyl-D-aspartate (NMDA) or α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid/kainate (AMPA/KA) receptor-mediated components of eEPSCs by adding 2-amino-5-phosphonovaleric acid (APV, 50 μM) or 6-cyano-7-nitroquinolinoxide-2,3-dione (CNQX, 10 μM), respectively, to the perfusate. All drugs were obtained from Sigma or RBI.

Analysis of sEPSCs and sIPSCs was performed using event detector programs (provided by Dr. J. Huguenard) on a 486 IBM computer. The amplitude of events had to exceed a detection threshold set at three times the SD of baseline noise. Only those spontaneous currents with falling phases that reached baseline before the onset of the succeeding event were analyzed to determine decay time constants. Amplitudes of overlapping events were measured as previously described (see Fig. 10B in Salin & Prince 1996). Rise time was measured from 10 to 90% of peak amplitude. Statistical significance was determined with a two-tailed student’s t-test (P < 0.05) or Kolmogorov-Smirnov (KS) test (P < 0.0001; software provided by Dr. I. Mody). Data are expressed as means ± SE.

The amplitude of action potential-dependent EPSCs (Table 2) was calculated as follows

\[
\text{Amp. AP-EPSCs} = \frac{[(\text{Amp. sEPSCs}) \times (\text{Freq. sEPSCs})] - (\text{Amp. mEPSCs}) \times (\text{Freq. mEPSCs})}{(\text{Freq. sEPSCs} - \text{Freq. mEPSCs})}
\]

where: Amp. and Freq. = mean amplitude (pA) and frequency (Hz); sEPSCs = spontaneous EPSCs in control solution; mEPSCs = miniature EPSCs in TTX-containing solution; AP-EPSCs = action potential-dependent EPSCs; and Freq. AP-EPSCs = frequency of sEPSCs – frequency of mEPSCs.

RESULTS

The data were obtained from 137 layer V pyramidal neurons of adult rat sensory-motor neocortex. Sixty-two neurons were from control slices and 75 from the chronically injured partially isolated cortex. The transcortical and undercutting lesions in slices were easily seen with a low power objective (>2.5), and cellular recordings were obtained under direct visualization from neurons located ~500–2000 μm from a transcortical cut within the partially isolated area. Layer V neurons, located within ~100 μm of the slice surface, which had a typical pyramidal-shaped soma and a single emerging apical dendrite, were visually selected for recordings.

Properties of sEPSCs and sIPSCs in layer V pyramidal neurons of isolated cortex

sEPSCs were recorded under voltage clamp at a holding potential (Vh) of −55 mV in control ACSF solution (Fig. 1A). At this membrane potential, sIPSCs were not detectable. Under these recording conditions, sEPSCs were unaffected during perfusion with ACSF containing 50 μM APV but were completely blocked when the perfusate contained 10 μM CNQX (n = 8, Fig. 1A), indicating that they were mediated by activation of
mV, close to the equilibrium potential for excitatory currents \( E \) (Fig. 2A). Abnormal evoked responses recorded from a layer V pyramidal neuron from an undercut slice. \( V_h = -55 \) mV. Bath perfusion of artificial cerebrospinal fluid (ACSF) containing 50 \( \mu M \) 2-amino-5-phosphonovaleric acid (APV) did not affect the sEPSCs (APV). Inclusion of both of 10 \( \mu M \) 6-cyano-7-nitroquinolinyl-2,3-dione (CNQX) and 50 \( \mu M \) APV in the perfusate completely blocked the sEPSCs (APV+CNQX). B: miniature inhibitory postsynaptic currents (mIPSCs) recorded in a layer V pyramidal neuron from an undercut slice in the presence of 1 \( \mu M \) TTX (control). \( V_h = 0 \) mV. Addition of 10 \( \mu M \) bicuculline to the perfusate completely blocked the mIPSCs (Bicu). Note different current calibrations in A and B.

AMPA/KA receptors. sIPSCs were recorded as outward currents at \( V_h = 0 \) to +5 mV and were completely blocked by 10 \( \mu M \) BMI (n = 10, Fig. 1B), suggesting that they were mediated by gamma-aminobutyric acid type A (GABAA) receptors.

Abnormal synaptic activities were evoked by extracellular stimulation in some slices from undercut cortex in standard perfusion solution. Stimuli elicited variable latency, all-or-none, polysynaptic potentials following the initial response (Fig. 2). These evoked currents were large in amplitude, outward at \( V_h = 0 \) to +5 mV, close to the equilibrium potential for excitatory currents (\( E_{EPSC} \); Fig. 2A), and inward at \( V_h = -55 \) mV (Fig. 2B), which was close to the calculated equilibrium potential for chloride (\( E_C \)), indicating that they contained both inhibitory and excitatory components (see also Salin et al. 1995). We did not record simultaneous field potentials in these cases, so it is unclear whether the late events occurred synchronously in a large population of neurons. In other experiments in which evoked extracellular field potentials were recorded, we found that 12/12 rats, with lesions made using techniques similar to those employed here, had at least one neocortical slice that generated evoked interictal epileptiform activity (Graber and Prince 1999). However, only 58% of 36 slices examined from cortical lesions in these previous experiments had these abnormal field potentials. In the present experiments, we did not systematically survey slices by stimulating at multiple sites (cf. Graber and Prince, 1999), and so the incidence of such abnormal events in slices from which cellular recordings were obtained is unknown.

**Increase in the frequency of sEPSCs in chronically injured cortex**

Figure 3 shows representative recordings of sEPSCs and sIPSCs from neurons in control (A1 and A2) and lesioned cortex (B1 and B2). Although the frequency of sEPSCs in individual neurons was variable (2–18 Hz), analysis of a large sample showed that there was a significant \( \sim 26\% \) increase in neurons from the partially isolated cortex (9.3 ± 0.7 vs.7.4 ± 0.6 Hz in lesioned and control cortex, respectively; \( P < 0.05 \); Table 1). A cumulative probability plot of 3,300 interevent intervals from 33 neurons (100 sequential intervals from each neuron) confirmed the shift toward higher sEPSC frequencies in lesioned slices (K-S test \( P < 0.0001 \), Fig. 4A).

Higher sEPSC frequencies might reflect an increased rate of spike discharge of presynaptic pyramidal cells, a larger number of contacts by presynaptic excitatory neurons, or enhanced spontaneous (nonimpulse-related) transmitter release. To further examine the contributions of impulse-related and spontaneous transmitter release to the increased sEPSC frequency, we recorded mEPSCs in control (Fig. 3C1) and lesioned (Fig. 3D1) slices bathed in ACSF containing 1 \( \mu M \) tetrodotoxin (TTX). For the purposes of this analysis, we approximated the contribution of miniature currents and impulse-related events to the frequency of spontaneous events in control solutions by subtracting the frequencies in TTX from those in control. Potential underestimates of the frequency of miniature currents in normal solution may have resulted from this approach if action potentials induced delayed, transient increases in mEPSC frequency (see Cummings et al. 1996). However, the frequency of action potential-dependent release was quite low, judging from changes in sEPSC frequency after TTX perfusion (Table 2), making a sizable contribution from this effect unlikely. There was a significantly higher mEPSC frequency in neurons from lesioned cortex (43% increase; Table 1), confirmed with a cumulative probability plot of interevent intervals, which were significantly shorter in the undercut group (Fig. 5A; K-S: \( P < 0.0001 \)). The data of Table 2 were obtained from a subpopulation of 13 control and 12 undercut neurons from Table 1 in which the frequency and amplitude of s- and mEPSCs and s- and mIPSCs could be measured in each cell. In this group of neurons, mEPSCs accounted for \( \sim 83\% \) of sEPSC frequency in control and \( \sim 85\% \) in undercut cells, with the balance due to impulse-related transmitter release. Data from the whole population of neurons (Table 1) showed similar large proportions of miniature events in the sEPSC totals. The amplitude of sEPSCs was significantly larger in the undercut group (\( P < 0.05 \)). The frequency of s- and mEPSCs in the subgroups of neurons in Table 2 tended to be greater in

**FIG. 1. Pharmacological properties of spontaneous postsynaptic currents.** A: spontaneous excitatory postsynaptic currents (sEPSCs) recorded in a layer V pyramidal neuron from control slice. \( V_h = -55 \) mV. Bath perfusion of artificial cerebrospinal fluid (ACSF) containing 50 \( \mu M \) 2-amino-5-phosphonovaleric acid (APV) did not affect the sEPSCs (APV). Inclusion of both of 10 \( \mu M \) 6-cyano-7-nitroquinolinyl-2,3-dione (CNQX) and 50 \( \mu M \) APV in the perfusate completely blocked the sEPSCs (APV+CNQX). B: miniature inhibitory postsynaptic currents (mIPSCs) recorded in a layer V pyramidal neuron from an undercut slice in the presence of 1 \( \mu M \) TTX (control). \( V_h = 0 \) mV. Addition of 10 \( \mu M \) bicuculline to the perfusate completely blocked the mIPSCs (Bicu). Note different current calibrations in A and B.

**FIG. 2. Abnormal evoked responses recorded from a layer V pyramidal neuron within the isolated area of an undercut slice.** A: at \( V_h = +5 \) mV, a 1.5 times threshold, 70-\( \mu s \) stimulus in layer V, \( \sim 80 \mu m \) lateral to the recorded cell evokes a short-latency brief outward current that is often followed by a larger, more prolonged, all-or-none outward current with multiple peaks. B: at \( V_h = -55 \) mV, currents evoked by the same extracellular stimulus are inward. A brief initial current is followed by an all-or-none, longer latency, more prolonged inward current. •, time of stimul. Calibrations in B for A and B.
SYNAPTIC ACTIVITY IN INJURED, EPILEPTOGENIC NEOCORTEX

FIG. 3. Spontaneous and miniature EPSCs and IPSCs (s- and mEPSCs and s- and mIPSCs) in layer V pyramidal neurons. A: sEPSCs (I) and sIPSCs (2) recorded from a control cell. B: sEPSCs (I) and sIPSCs (2) recorded from a neuron in an undercut slice. V_h’s for sEPSCs and sIPSCs in A and B were −55 and +5 mV, respectively. C: mEPSCs (I) and mIPSCs (2) recorded from a control cell. D: mEPSCs (I) and mIPSCs (2) recorded in TTX (1 μM) from a neuron in an undercut slice. V_h’s as indicated in A and B. The 3 traces in each panel of A–D are continuous. E: graph showing the mean ratio of the frequency of sEPSCs to sIPSCs (sEPSCs/sIPSCs) for layer V pyramidal cells in control (n = 26) and lesioned cortex (n = 23). F: graph showing the mean ratio of the frequency of mEPSCs to mIPSCs (mEPSCs/mIPSCs) for layer V pyramidal cells in control (n = 14) and lesioned cortex (n = 15).

undercut versus control cells (+21 and +23%, respectively), but these differences did not reach significance with the two-tailed t-test (but see RESULTS for whole population in Table 1). However, cumulative probability plots of interevent intervals for s- and mEPSCs in the groups of control and undercut neurons of Table 2 showed a significant shortening of intervals (KS test P < 0.0001). The ratio of frequencies of m- to sEPSCs did not change significantly in undercut versus control groups (Table 2). The increased sEPSC frequency in undercut versus control tissue was thus due to proportionally similar increases in both mEPSCs and impulse-dependent release. To rule out the possibility that the changes in mEPSC frequency were due to a relocation of excitatory synapses to electrotonically closer sites where they would be more readily recorded, we compared the 10–90% rise time of mEPSCs of these two groups (Fig. 5C), and no significant difference was detected (1.2 ± 0.06 vs. 1.2 ± 0.05 ms, for control and undercut, respectively; P > 0.5; K-S test, P > 0.001).

Decreased IPSC frequency in chronically injured cortex

sIPSCs recorded at V_h = 0 to +5 mV (Fig. 3, A2 and B2) had a significantly lower frequency in slices from the chronically injured brain area (18.8 ± 1.4 vs. 26.1 ± 1.4 Hz for lesioned and control cortex, respectively; P < 0.001; ~28% decrease; Table 1). This was confirmed in cumulative probability plots where the interevent intervals of sIPSCs were significantly longer in neurons from undercut slices (Fig. 6A, K-S test, P < 0.0001). The frequency of mIPSCs recorded in the presence of 1 μM TTX was also lower (~32% decrease) in undercuts (Fig. 7A; 14.0 ± 1.7 vs. 20.7 ± 1.2 Hz in neurons of injured and control cortex, respectively; t-test, P < 0.01; K-S

TABLE 1. Frequency and amplitude of spontaneous PSCs in partially isolated cortex

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Frequency, Hz</th>
<th>Amplitude, pA</th>
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<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Undercut</td>
</tr>
<tr>
<td>sEPSCs</td>
<td>7.4 ± 0.6 (33)</td>
<td>9.3 ± 0.7 (33)*</td>
</tr>
<tr>
<td>mEPSCs</td>
<td>5.6 ± 0.7 (19)</td>
<td>8.0 ± 0.9 (18)*</td>
</tr>
<tr>
<td>mEPSCs/sEPSCs</td>
<td>0.76</td>
<td>0.86</td>
</tr>
<tr>
<td>sIPSCs</td>
<td>26.1 ± 1.4 (27)</td>
<td>18.8 ± 1.4 (25)**</td>
</tr>
<tr>
<td>mIPSCs</td>
<td>20.7 ± 1.2 (19)</td>
<td>14.0 ± 1.7 (16)**</td>
</tr>
<tr>
<td>mIPSCs/sIPSCs</td>
<td>0.79</td>
<td>0.74</td>
</tr>
<tr>
<td>sEPSCs/sIPSCs</td>
<td>0.33 ± 0.06 (26)</td>
<td>0.60 ± 0.08 (23)**</td>
</tr>
<tr>
<td>Ratio of mE:mI</td>
<td>0.37 ± 0.06 (14)</td>
<td>0.81 ± 0.11 (15)**</td>
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All data are expressed as means ± SE. n values are in parentheses. sEPSC and mEPSC, spontaneous and miniature excitatory postsynaptic current; sIPSC and mIPSC, spontaneous and miniature inhibitory postsynaptic currents; mE/mL, miniature excitatory/inhibitory. * P < 0.05; ** P < 0.01; *** P < 0.005.
test, $P < 0.0001$). As in the case of spontaneous excitatory events, when frequencies of s- and mIPSCs were analyzed in the same neurons, mIPSCs made up a large and similar proportion of sIPSCs in both control (~78%) and undercut cells (77%, Table 2). There was an ~37% decrease in both s- and mIPSC frequency in undercut versus control neurons (Table 2), suggesting that decreases in both mIPSCs and impulse-related release contributed to the decrease in sIPSC frequency. Data obtained from the whole population shown in Table 1 were similar.

There was no significant difference in the rise times of mIPSCs in control versus injured neurons (Fig. 7C; 1.4 ± 0.1 vs. 1.4 ± 0.1 ms in control and undercut slices, respectively;

| TABLE 2. Contribution of action-potential-driven events to spontaneous EPSCs and IPSCs |
|---------------------------------|----------------|

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Undercut</th>
<th>Percent Δ</th>
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<tbody>
<tr>
<td>sEPSCs</td>
<td>7.8 ± 0.9</td>
<td>9.4 ± 1.1</td>
<td>+21</td>
</tr>
<tr>
<td>nEPSCs</td>
<td>6.5 ± 0.9</td>
<td>8.0 ± 1.1</td>
<td>+23</td>
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<tr>
<td>mEPSCs/nEPSCs</td>
<td>0.83</td>
<td>0.85</td>
<td>+2</td>
</tr>
<tr>
<td>sIPSCs</td>
<td>27.7 ± 2.4</td>
<td>17.6 ± 2.4</td>
<td>-37</td>
</tr>
<tr>
<td>mIPSCs</td>
<td>21.7 ± 12</td>
<td>13.6 ± 2.8</td>
<td>-37</td>
</tr>
<tr>
<td>mIPSCs/sIPSCs</td>
<td>0.78</td>
<td>0.77</td>
<td>-1</td>
</tr>
<tr>
<td>AP-dependent EPSCs</td>
<td>1.3</td>
<td>1.4</td>
<td>+8</td>
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<table>
<thead>
<tr>
<th>Amplitude, pA</th>
<th>Control</th>
<th>Undercut</th>
<th>Percent Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>sEPSCs</td>
<td>11.1 ± 0.6</td>
<td>12.8 ± 0.5</td>
<td>+15</td>
</tr>
<tr>
<td>nEPSCs</td>
<td>10.1 ± 0.8</td>
<td>11.6 ± 0.9</td>
<td>+15</td>
</tr>
<tr>
<td>mEPSCs/nEPSCs</td>
<td>0.89</td>
<td>0.91</td>
<td>0</td>
</tr>
<tr>
<td>sIPSCs</td>
<td>22.4 ± 1.9</td>
<td>23.0 ± 1.5</td>
<td>+3</td>
</tr>
<tr>
<td>mIPSCs</td>
<td>16.7 ± 1.1</td>
<td>20.1 ± 1.5</td>
<td>+20</td>
</tr>
<tr>
<td>mIPSCs/sIPSCs</td>
<td>0.75</td>
<td>0.87</td>
<td>+16</td>
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<tr>
<td>AP-dependent EPSCs</td>
<td>16.0</td>
<td>19.6</td>
<td>+20</td>
</tr>
</tbody>
</table>

All data are expressed as means ± SE. $n = 13$ and 12 for control and undercut, respectively, for both frequency and amplitude. All current parameters were obtained from each neuron. AP, action potential. *, ** $P < 0.05, 0.01$ between control and undercut; $\dagger, \ddagger P < 0.05, 0.01$ between spontaneous and miniature currents in control or undercut.
Amplitudes of sEPSCs and sIPSCs in chronically injured cortex

The mean amplitudes of sIPSCs and mIPSCs for control and undercut groups, calculated from the average value for each neuron, were not significantly different in cells of control versus injured cortex (Figs. 6E and 7E; Table 1). However, analysis of cumulative probability plots for large numbers of single events from groups of neurons showed small but significant shifts toward higher amplitudes in the undercut cortex (Figs. 6B and 7B). There was a small, but significant increase in mean amplitude of sEPSCs (Fig. 4, B and E; ~17%; \( P < 0.01 \)) and mEPSCs (Fig. 5, B and E; ~21%; \( P < 0.05 \)) in the chronically injured brain area (Table 1). Of note was the finding that the mean amplitude of sEPSCs in the whole population was not significantly different from that of mEPSCs in neurons of either control or undercut cortex (Table 1). This may have been due to the large proportion of mEPSCs in the sEPSC sample (Table 1). In the group of neurons of Table 2, the mean amplitudes of action-potential-dependent EPSCs (see METHODS) were greater than amplitudes of mEPSCs (19.6 vs. 16.0 pA).

The average decay time constant of mEPSCs from cells of isolated cortex was faster than that for neurons from control cortex (5.2 ± 0.26 vs. 6.7 ± 0.46 ms, \( P < 0.02 \); ~21% decrease). The possibility that this difference was related to
changes in electrotic structure or a more proximal location of excitatory synapses (e.g., Magee and Cook 2000) appeared unlikely, as the relationship between mean rise time and peak amplitude for both mEPSCs and mIPSCs was less correlated in neurons from undercut than control cortex (mEPSCs: \( R = -0.49, P < 0.05 \) for control; \( R = -0.31, P = 0.21 \) for undercut, Fig. 5D; mIPSCs: \( R = -0.72, P < 0.001 \) for control; \( R = -0.37, P = 0.07 \) for undercut, Fig. 7D). The average charge transfer for mEPSCs, measured during 1-min periods, was significantly greater in the undercut than control group of neurons (10.6 ± 2 nA/ms for controls and 19.7 ± 4 nA/ms for undercuts, \( P < 0.05 \)). The average charge transfer for mIPSCs measured in the same neurons was significantly smaller in the undercut group (99.4 ± 5 nA/ms and 74.2 ± 11 nA/ms for control and undercut groups, respectively, \( P < 0.05 \)).

Enhancement of evoked EPSCs in chronically injured cortex

EPSCs were evoked in layer V pyramidal neurons in the presence of 10 \( \mu \)M BMI (see METHODS) and input-output (I-O) curves obtained by varying the stimulus duration between 30 and 150 \( \mu \)s while keeping the stimulus intensity constant. To reveal NMDA receptor-mediated components, the membrane potential was depolarized to \( -30 \) mV. Only data from cells with stable access resistance between 7 and 14 M\( \Omega \) were analyzed (mean access resistance: 11.1 ± 1.1 vs. 10.9 ± 1.0 M\( \Omega \), in control and undercut neurons, respectively, \( P = 0.77 \)). The peak amplitude of non-NMDA components at threshold stimulus intensity (T) and \( V_h = -55 \) mV was similar in the two groups (39.1 ± 3.3 pA in control, \( n = 14 \), vs. 34.0 ± 3.8 pA in undercuts, \( n = 14 \); \( P = 0.26 \); Fig. 8, A and C), as was the stimulus intensity (duration) required to evoke the threshold responses (Fig. 8C). A cumulative probability plot of all responses showed no significant differences in evoked EPSC (eEPSC) amplitudes evoked by 1T stimuli in the undercut and control cells of Fig. 8B (not shown). In contrast, the amplitude of eEPSCs elicited by 2T stimuli from undercut slices was significantly larger than in control slices (215.2 ± 22.7 in undercuts vs. 145.9 ± 16.2 pA in controls, \( n = 14 \); \( P < 0.03 \); 47% increase; Fig. 8A, 2T; Fig. 8B).

The I-O slope, expressed as pA/normalized stimulus unit, was steeper for neurons from slices of isolated cortex than controls (158.3 ± 18.7 in undercuts, \( n = 14 \), vs. 103.3 ± 17.9 in controls, \( n = 14 \); \( P < 0.05 \); 33% increase; Fig. 8B). Further analysis showed that, at a stimulus intensity of 2T and \( V_h = -55 \) mV, there was no significant difference in decay time constant (9.3 ± 0.8 vs. 9.2 ± 0.9 ms, \( P = 0.92 \)), or half-width (8.5 ± 0.6 vs. 7.4 ± 0.8 ms; \( P = 0.24 \)), for EPSCs in neurons of control versus lesioned cortex.

To assess NMDA versus non-NMDA response components, amplitudes of EPSCs evoked in 10 \( \mu \)M bicuculline by 2T stimuli were measured at different membrane potentials and latencies (Fig. 9) (Stern et al. 1992). The NMDA components of eEPSCs, measured at \( V_h = -55 \) mV and a latency of 25 ms, were very small in amplitude (Fig. 9A1) but were prominent at \( V_h = -30 \) mV (Fig. 9B1). In some cells, stimuli evoked long-latency polysynaptic discharges so that the NMDA component could not be measured. Data from these neurons were not included in the analysis. The peak amplitude of EPSCs evoked at \( V_h = -30 \) mV by 2T stimuli at latencies of 5–7 ms (non-NMDA component) was larger in undercut neurons than

**FIG. 8. Increase in the input-output (I-O) slope of evoked EPSCs (eEPSCs) in lesioned cortex.** A: representative evoked EPSCs in layer V pyramidal neurons from a control (control) and undercut slice (undercut). Superimposed traces show responses in each cell to stimuli that are threshold for evoking an EPSC (1T), and stimuli 2 and 3 times threshold (2T, 3T). Each trace is an average of 5 responses. \( V_h = -55 \) mV; 10 \( \mu \)M BMI in perfusate. B: plot of response peak amplitude vs. normalized stimulus intensity (1–3 times threshold), derived from data similar to those in A, for 14 neurons. The I-O slope is significantly steeper in undercut slices. C: bar graphs of stimulus duration (intensity) at threshold (left) and the amplitude of eEPSCs at threshold (right) for neurons from control and undercut cortex. t-test, \( P > 0.05 \) for both stimulus intensity and EPSC amplitude in control vs. undercut groups.

in controls (Fig. 9B2; 168.3 ± 20.7 pA in undercuts, \( n = 9 \), vs. 107.1 ± 13.4 pA in controls, \( n = 13 \); 57% increase; \( P < 0.05 \)). However, the late components in these two groups were similar (44 ± 12.7 vs. 39.3 ± 8 pA for undercut and control groups; Fig. 9B2; \( P = 0.76 \); Table 3). The ratio of the amplitude of NMDA receptor-mediated responses to the sum of amplitudes of the AMPA plus NMDA components was not significantly different for undercut versus control neurons (0.20 ± 0.03 vs. 0.25 ± 0.04, for undercut and control, respectively; \( P = 0.36 \); Table 3). We also assessed the effects of pharmacological blockade of NMDA receptors with APV (50 \( \mu \)M in ACSF perfusate) on the amplitudes of non-AMPA and NMDA components of eEPSCs. At \( V_h = -30 \) mV, APV decreased the amplitude of the short (5–7 ms)-latency component by 20%, and the amplitude of the long (25 ms) component by 80% (not shown). No significant difference in APV effects on eEPSCs was found between control and undercut groups (20 ± 10%, \( n = 6 \), vs. 16 ± 5% reduction, \( n = 7 \) at short latency, \( P = 0.73 \); 81 ± 6 vs. 82 ± 4% reduction in amplitude at long latency for control and undercut, respectively; \( P = 0.84 \)). Results suggest that the increase in eEPSCs in chronically injured cortex is mediated predominantly via non-NMDA receptors.

**DISCUSSION**

We measured synaptic activities in layer V pyramidal neurons of partially isolated cortex weeks after the initial lesion to test the hypothesis that the hyperexcitability, known to develop after such chronic injury, is due at least in part to plastic
ALTERATIONS IN EXCITATORY CURRENTS

Changes in the efficacy of excitatory and inhibitory neurotransmission. Major results include an increase in the frequency and amplitude of sEPSCs and mEPSCs; a decrease in the frequency of sIPSCs and mIPSCs; and an increased I-O slope of eEPSCs due to an enhanced non-NMDA receptor-mediated component.

TABLE 3. Properties of evoked EPSCs in partially isolated cortex

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Undercut</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slope (pA/Δ normalized stim. threshold)</td>
<td>103.3 ± 17.9 (14)</td>
<td>158.3 ± 18.7 (14)*</td>
</tr>
<tr>
<td>AMPA component at −55 mV, pA</td>
<td>145.9 ± 16.2 (14)</td>
<td>215.2 ± 22.7 (14)*</td>
</tr>
<tr>
<td>AMPA component at −30 mV, pA</td>
<td>107.1 ± 13.4 (13)</td>
<td>168.3 ± 20.7 (9)*</td>
</tr>
<tr>
<td>NMDA component at −30 mV, pA</td>
<td>39.3 ± 8.0 (13)</td>
<td>44.0 ± 12.7 (9)</td>
</tr>
<tr>
<td>NMDA/(AMPA + NMDA)</td>
<td>0.25 ± 0.05</td>
<td>0.2 ± 0.04</td>
</tr>
</tbody>
</table>

All data are expressed as mean ± SE. n values are in parentheses. * P < 0.05.

In addition to these probable alterations in intracortical connectivity, recent immunocytochemical results indicate that there are complex changes in the expression and distribution of glutamate receptor subunits in neurons within the partial isolation, including a loss of immunoreactivity for the GluR2 subunit in populations of layer V neurons (Kharazia and Prince 2001) that might enhance AMPA receptor-mediated excitation (Gu et al. 1996). The small, but significant increases in the amplitude of s- and mEPSCs (Figs. 4, B and E, and 5, B and E; Tables 1 and 2) are consistent with alterations in the numbers or properties of postsynaptic receptors, and the faster decay time constant for mEPSCs also suggests a postsynaptic change.

Although the increase in mEPSC frequency in injured cortex (Table 1), in the absence of significant changes in mEPSC rise times, is consistent with a greater number of excitatory synapses on individual pyramidal cells, there might also be a contribution due to an enhanced probability of release from synaptic terminals. Recent results indicate that paired-pulse depression of eEPSCs is significantly greater in neurons of partially isolated cortex (Li and Prince 2000), suggesting that there is an increased probability of release from excitatory terminals (Gil et al. 1999; Thomson et al. 1993). The finding that mEPSC frequency, as a percentage of sEPSC frequency, is similar in control and undercut neurons (Table 2), suggests that both mEPSCs and the sEPSC component ascribed to impulse-related release contribute to the increased sEPSC frequency in chronically injured slices under our experimental conditions.

Analysis of eEPSCs also supports the conclusion that onset (Hoffman et al. 1994; Prince and Tseng 1993). More detailed anatomical studies are required to determine whether these presumed boutons are presynaptic structures and whether their targets are predominantly other pyramidal neurons in the same lamina, as is the case for layer V pyramidal cells in normal cortex (Kisvarday et al. 1986). Pyramidal neurons and neuropil within the isolation also show long-lasting enhanced immunoreactivity for a 68-kDa neurofilament antibody (D. A. Prince and I. Parada, unpublished observations), further supporting the suggestion that significant axonal alterations are occurring (Shetty and Turner 1995; Yaghmai and Povlishock 1992; Yang et al. 1997).

The increased mEPSC frequency in layer V pyramidal neurons (43%) is smaller than the >100% increase in presumed boutons previously reported. This discrepancy may be due to the proportion of contacts made onto interneurons or distal pyramidal dendritic sites where mEPSCs would not be detectable, or from the presence of “silent” synapses (Isaac et al. 1997) or those with a low probability of release (Gasparini et al. 2000). Also, the anatomical studies were done on neurons located deeper in thicker slices where connectivity is likely to be greater than in the present experiments.

FIG. 9. Amplitude of non-N-methyl-D-aspartate (non-NMDA) but not NMDA receptor-mediated EPSC components are increased in the undercut slice. A1: eEPSC from control neuron recorded at Vh = −55 mV showing predominant short latency non-NMDA, presumed AMPA/kainate (KA) component. A2: graphs of response peak amplitude to 2 times threshold stimuli (2T Stim.; left) and decay time constant (decay tau; right) for short-latency AMPA/KA component at Vh = −55 mV in control neurons (n = 14) and those from undercut slices (n = 14). Decay time constants were 9.3 ± 0.8 ms and 9.2 ± 0.9 ms for control and undercut groups (P = 0.92). There was a significant (P < 0.05) increase in response peak amplitude in neurons of undercut cortex. B1: response of neuron of A1 to the same stimulus at Vh = −30 mV. B2: graphs of the amplitudes of AMPA/KA (left, non-NMDA) and NMDA components (right) for 13 control and 9 undercut neurons from the groups of A2 at Vh = −30 mV and stimulus intensity as in A1. The non-NMDA and NMDA receptor-mediated response components in B were measured at latencies of 5–7 and 25 ms after stimulation, respectively (vertical dashed lines a and b in A1 and B1). The peak amplitude of the AMPA component was significantly larger in neurons of undercut slices than in controls as in left graph of A2.
functional alterations in excitatory neurotransmission occur in the undercut cortex. Although the peak amplitude of EPSCs evoked at threshold in neurons of the lesioned area was not increased (Fig. 8C), EPSCs evoked by 2T and 3T stimuli had larger amplitudes than controls (Figs. 8B and 9, A2 and B2), and the I-O slope was significantly steeper in injured cortex (Fig. 8C; Table 3). These results are consistent with the increased amplitude of s- and mEPSCs and could reflect increases in the numbers of presynaptic elements activated by higher intensity stimuli and/or changes in the number or properties of glutamate receptors on neurons in the injured cortex. It is difficult to explain the absence of a change in amplitude of EPSCs evoked at threshold in the undercut group unless this is due to relatively small alterations at individual excitatory contacts that would require a larger sample to detect.

Previous studies have suggested that alterations in NMDA receptor function play an important role in triggering of paroxysmal discharges in animal models of epileptogenesis and in man (Chapman 1998; Isokawa 1997). Recent evidence indicates that non-NMDA receptors also have a key role in seizure generation and propagation (Dozzi et al. 1999; Friedman et al. 1999). Our results show that a major change in NMDA receptor-mediated responses is not a pathogenetic factor in the partially isolated cortex; rather, non-NMDA receptor responses are enhanced (Fig. 9). However, as is the case in this and other chronic models, NMDA receptor blockade by APV does decrease polysynaptic epileptiform activity (Hoffman et al. 1994; Jacobs et al. 1996; Quesada et al. 1996). Assuming that AMPA/KA and NMDA receptors are in close proximity in the postsynaptic membrane (Bekkers and Stevens 1989), enhancement of the AMPA/KA component, without effects on NMDA receptor-mediated currents, would support a selective alteration in postsynaptic AMPA/KA receptors.

**Alterations in inhibitory currents**

The values for sIPSC frequency and conductance in control cortex in the present experiments agree well with those we previously reported for layer V pyramidal cells recorded using the “blind” slice-patch technique (Salin and Prince 1996); however, there are significant differences in mIPSC amplitude and frequency from this previous study that may be related to technical differences between the two experiments. The most parsimonious explanation for the decreases in s- and mIPSC frequencies without concurrent decreased amplitudes in pyramidal neurons of the partially isolated cortex would be a loss of interneurons and/or a decrease in inhibitory synapses. One ultrastructural study suggested that there was a decrease in inhibitory synapses within the isolation (Ribak and Reiffenstein 1982); however, other results indicated an increase in symmetrical synapses on dendritic shafts (Rutledge 1978). We have found that immunoreactivity (IR) for parvalbumin and calbindin is more prominent in interneurons of the injured cortex than controls (Prince et al. 1997), and IR for other markers of GABAergic neurons such as glutamic acid decarboxylase and neuropeptide Y is not decreased in the partially isolated cortex (D. A. Prince and I. Parada, unpublished observations). Further, cell counts show that there is no reduction in parvalbumin-immunoreactive interneurons, even though there is a small decrease in pyramidal cell density (Graber et al. 1999). Experiments involving postembedding immunocytochemistry for GABA have revealed an increase rather than a decrease in the density of GABAergic boutons on somata of layer V pyramidal neurons in undercut cortex (I. Parada and D. A. Prince, unpublished observations).

One possible explanation for these differences in anatomical versus electrophysiological data would be “stripping” of axonal terminals from their postsynaptic targets, such as occurs after axotomy of motoneurons (Mendell 1984; Sumner and Sutherland 1973; Takata 1981) and has been proposed in the postkainate model of hippocampal epilepsy (Franck et al. 1988). This process should have an effect on s- and mIPSC frequencies similar to interneuronal loss with preservation of GABAergic cells and terminals. An expected consequence of inhibitory stripping would be a decrease in amplitudes of evoked IPSCs (Takata 1981), which were not examined in these experiments. Other potential explanations for our findings such as injury-induced alterations in GABA A receptors (Gibbs et al. 1997) or internalization of the receptors (Wan et al. 1997) are unlikely in light of the absence of significant alterations in m- or sIPSC amplitudes. A positive shift in E Cl due to injury (Van Den Pol et al. 1996) and downregulation of the chloride-potassium co-transporter KCC2 (Prince et al. 2000) might result in a decrease in frequency of detectable spontaneous events; however, again, a reduction in m- and sIPSC amplitudes would be expected. Additional data are required to rule out decreased excitation or increased inhibition of interneurons or alterations in interneuronal intrinsic properties that would make these cells less responsive and contribute to a reduction in impulse-related sIPSC (but not mIPSC) frequency. Also, our whole cell recordings likely reflect inhibitory events predominantly on somata and proximal dendrites; measurements obtained from more distal dendritic sites might show more marked abnormalities (Cossart et al. 2001).

We previously reported an increase in s- and mIPSC frequency in a small population of layer V pyramidal neurons in slices cut through partial cortical isolations (n = 5), using the “blind” slice-patch technique (Prince et al. 1997). In these previous experiments, slices were somewhat thicker (400–450 μm), [K+]o was 5 mM, and recordings were from neurons deeper within slices. These factors and the small sample size may account for differences in results with respect to the present study.

The changes in frequency of spontaneous excitatory and inhibitory currents are relatively modest in the undercut neurons compared with controls, the largest alteration being an ~43% increase in mIPSC frequency (Table 1). However, the balance between the frequencies of these events, as reflected by the ratio of sEPSCs:sIPSCs or mEPSCs: mIPSCs, is strongly shifted toward excitation. These ratios have values that are approximately twice those found in layer V pyramidal neurons of control slices (Table 1). Such changes, together with the enhanced AMPA/KA receptor components of eEPSCs (Figs. 8 and 9), would favor generation of epileptiform activity in the cortical network (e.g., Traub et al. 1987; Wong et al. 1984). The frequency of impulse-related spontaneous synaptic activity is significantly greater in vivo, where connectivity has not been
affected by preparation of cortical slices (Pare et al. 1997, 1998); however, it is hard to predict whether the frequencies of excitatory and inhibitory events would be similarly or differentially increased.

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