Cortical Injury Affects Short-Term Plasticity of Evoked Excitatory Synaptic Currents

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Li, Huifang, Anita E. Bandrowski, and David A. Prince. Cortical injury affects short-term plasticity of evoked excitatory synaptic currents. J Neurophysiol 93: 146–156, 2005. First published September 1, 2004; doi:10.1152/jn.00665.2004. The hypothesis that plastic changes in the efficacy of excitatory neurotransmission occur in areas of chronic cortical injury was tested by assessing short-term plasticity of evoked excitatory synaptic currents (EPSCs) in neurons of partially isolated neocortical islands (undercut cortex). Whole cell recordings were obtained from layer V pyramidal neurons of sensorimotor cortical slices prepared from P36–P43 control and undercut rats. AMPA/kainate receptor-mediated EPSCs elicited by stimuli delivered at 40 to 66.7 Hz exhibited more paired-pulse depression (PPD) in undercut cortex than control, the time constant of depression evoked by trains of 20- to 66.7-Hz stimuli was faster, and the steady-state amplitude of EPSCs reached after five to seven EPSCs was lower. An antagonist of the glutamate autoreceptor, group II mGluR, increased the steady-state amplitude of EPSCs from undercut but not control cortex, suggesting that activation of presynaptic receptors by released glutamate is more prominent in undercut cortex. In contrast, the GABA<sub>δ</sub> receptor antagonist (2S)-3-[(1S)-1-(3,4-dichlorophenyl)ethyl]-amino-2-hydroxypropyl](phenylmethyl)phosphinic acid had no effect. Increasing [Ca<sup>2+</sup>]<sub>i</sub> from 2 to 4 mM increased PPD, with a smaller effect in neurons of the undercut. The F-V relationship of AMPA/kainate receptor-mediated EPSCs was close to linear in both control and undercut neurons, and spiking had no significant effect on the EPSCs, suggesting that decreases in postsynaptic glutamate receptors containing the GluR2 subunit were not involved in the alterations in short-term plasticity. Results are compatible with an increase in the probability of transmitter release at excitatory synapses in undercut cortex due to functional changes in presynaptic terminals.

INTRODUCTION

One important consequence of serious cortical injury, such as occurs with penetrating missile wounds, depressed skull fractures, and hemorrhagic stroke, is delayed development of partial seizures associated with epileptiform discharges arising from the lesioned area (Anneggeres et al. 1980; Hernandez and Naritoku 1997; Salazar et al. 1985). A number of mechanisms may contribute to development of hyperexcitability or epileptiform activity after such injuries, including enhanced excitatory synaptic activities, loss of GABA<sub>ergic</sub> inhibitory control of network activities, changes in membrane properties of individual neurons that increase their excitability, and other alterations in regulatory processes (Asprodini et al. 1992; Buhl et al. 1996; Esplin et al. 1994; Furtinger et al. 2001; McKinney et al. 1997; reviewed in Prince 1999). Partially isolated neocortex is a well-established chronic model of epileptogenesis following direct cortical trauma (Halpern 1972; Hoffmann et al. 1994; Jacobs et al. 2000; Prince and Tseng 1993). Neocortical islands with intact pial circulation, prepared by placing undercutting lesions and severing connections to surrounding cortex, become hyperexcitable after a latent period and generate epileptiform activity both in vivo (Sharpless and Halpern 1962) and when studied in vitro slice preparations (Hoffman et al. 1994; Prince and Tseng 1993; Salin et al. 1995). Interictal epileptiform discharges in these neocortical slices originate in layer V (Hoffman et al. 1994; Prince and Tseng 1993) where results from several experiments indicate that significant changes occur in excitatory synaptic processes affecting pyramidal neurons. Axonal sprouting in pyramidal cells of undercut cortex leads to development of extensive arbors, principally within layer V, which may be associated with new excitatory connectivity (Salin et al. 1995). An increase in the frequency of spontaneous and miniature excitatory postsynaptic currents (EPSCs) and in the slope of the output-input relation for AMPA/kainate receptor-mediated excitatory currents evoked in layer V pyramidal neurons suggest an increase in the number of excitatory synapses and/or the probability of release at terminals (Li and Prince 2002). Layer V pyramidal cells within the partially isolated area have also undergone changes in their intrinsic electrophysiological properties that render them more excitable, including an increased input resistance and membrane time constant, a reduction in spike frequency adaptation, and a steeper slope of the relationship between spike frequency and applied depolarization (I/I slope) (Prince and Tseng 1993). The latter changes in action potential generation are similar to those seen in axotomized corticospinal neurons and likely due in part to reductions in Ca<sup>2+</sup>-activated K<sup>+</sup> currents and slow spike afterhyperpolarizations (Tseng and Prince 1996).

These effects of injury on membrane properties have been detected with somatic recordings; it is not known whether they are also present in axons and their terminals where anatomical changes such as sprouting are known to take place and where alterations might result in significant changes in the efficacy of synaptic transmission (Ferhat et al. 2003; Nadler 2003; Salin et al. 1995; Scharfman et al. 2003; see Jacobs et al. 2000 for review). Axonal terminals in both acute (Gutnick and Prince 1972) and chronic epileptogenic cortical foci (Pinault and Pumain 1985) may themselves generate bursts of action potentials that propagate antidromically and presumably orthodromically to evoke transmitter release, further suggesting that presynaptic functional alterations are present (see Pinault 1995 for review).

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Direct electrophysiological assessment of the properties of presynaptic terminals has only been possible at a few restricted sites within the CNS (Sakaba et al. 2002; Zhang and Jackson 1993) and is not feasible in cerebral cortex with currently available techniques. However, studies of short-term synaptic plasticity can yield information about transmitter release from axonal terminals (Dobrunz and Stevens 1997; Gil et al. 1999) that might be affected by chronic neuronal injury. Electrophysiological results from several experiments in models of epileptogenesis suggest that alterations in short-term plasticity of transmitter release from presynaptic terminals are present (see Discussion and references therein). We therefore analyzed evoked EPSCs in layer V pyramidal neurons to determine whether responses to pairs and trains of stimuli differed in control versus partially isolated cortex.

METHODS

All experiments were carried out according to protocols approved by the Stanford Institutional Animal Care and Use Committee. A total of 28 Sprague-Dawley rats aged P36-43 (P0 = date of birth) were used for in vitro recordings. Nineteen 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 an ~3 × 5-mm bone window centered on the coronal suture was removed, leaving the dura intact and exposing a portion of the frontoparietal cortex unilaterally. Transcortical cuts in sensorimotor cortex were 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 parasagitally ~1-2 mm from the interhemispheric sulcus, advanced under direct vision tangentially through the dura and just beneath the pial vessels, 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 to make a second transcortical cut, and removed. An additional transcortical lesion was placed ~2 mm lateral and parallel to the initial 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 from the surgery and were reanesthetized for slice experiments 2 wk later.

Techniques for preparing and maintaining brain slices in vitro were as previously described (Li and Prince 2002). Neocortical slices (~300 μm) were transferred to a recording chamber where they were minimally submerged and maintained at 32 ± 1°C (mean ± SD). Slices were perfused at the rate of 1–2 ml/min with a solution [artificial cerebrospinal fluid (ACSF)] containing (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH2PO4, 2 CaCl2, 2 MgSO4, 26 NaHCO3, and 10 glucose; pH 7.4 when saturated with 95% O2-5% CO2. Patch electrophysiology experiments, typically 1–4 s as judged by alterations in EPSCs with some variation due to placement of the perfusion pipette.

RESULTS

Whole cell recordings were obtained from layer V pyramidal neurons and the properties of EPSCs evoked by focal stimulation in layer V were examined with membrane potential clamped at ~−60 or ~−62 mV. Pyramidal cells were identified as neurons with large somata and a single emerging apical dendrite extending toward the pial surface. Evoked EPSCs were completely blocked by bath application of 10 μM CNQX (n =

...continue
Injury decreases the PPR

Repetitive stimulation can elicit both paired-pulse facilitation (PPF) and paired-pulse depression (PPD). These phenomena can be described by the PPR that is regarded as an index of presynaptic efficacy (Markram and Tsodyks 1996; Thomson et al. 1993a) but see Discussion. We examined the PPR at different inter-stimulus intervals in control and undercut cortex to test the hypothesis that the synaptic efficacy in undercut cortex may undergo an alteration after injury. As expected from previous reports in other cortical regions (Hemipel et al. 2000), the PPR was closest to 1 at lower stimulus frequencies and smaller than 1 at higher stimulus frequencies, regardless of experimental condition (Table 1). However, there was a significant reduction in the PPR in undercut versus control cortex at 40, 50, and 66.7 Hz that was greater at higher frequencies (Figs. 1, B and C, and 2; Table 1). In control cortex, the PPR ranged from 1.01 at 10 Hz to 0.90 at 66.7 Hz, whereas in the undercut cortex, PPR was 0.92 at 10 Hz and 0.62 at 66.7 Hz.

As shown in Fig. 1C, control neurons and those from undercut cortex could show either PPF or PPD, although PPF was more frequent in the controls and PPD in the undercut group. In 14 cells that were stimulated only at 50 Hz, the amplitude of the initial EPSC in the train for PPD cells was 91.43 ± 14.38 pA (n = 9), which was not different from that of PPF cells (117.92 ± 25.01 pA, n = 5); input resistance was also not different (not shown). We noted no systematic difference in the placement of the stimulating electrode in PPF versus PPD cells.

We had previously shown that the peak amplitude of the AMPA/kainate receptor-mediated component of EPSCs is significantly larger in undercut versus control cortex (Li and Prince 2002). To exclude the possibility that the observed decrease in PPR in the undercut was due to a larger initial EPSC amplitude (e.g., Thomson et al. 1993a), we adjusted the stimulus intensity so that there was no significant difference in initial EPSC amplitude in the two groups of cells (~100 pA). In addition, we plotted the amplitude of the initial EPSC against the PPR and found that there was no correlation between these parameters (Table 1). These data suggest that the difference in PPR between control and partially isolated cortex cannot be accounted for by differences in EPSC amplitude.

To further evaluate the decrease in PPR in neurons of partially isolated cortex, we tested individual cells in control and in undercut cortex at 10, 20, 40, and 66.7 Hz. Control cells

### Table 1. PPR in control and undercut cells

<table>
<thead>
<tr>
<th>Frequency, Hz</th>
<th>Control</th>
<th>Undercut</th>
<th>ANOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPR</td>
<td>1st EPSC, pA</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>1.01 ± 0.05</td>
<td>116 ± 19 (19)</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>0.98 ± 0.03</td>
<td>112 ± 14 (22)</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>0.96 ± 0.04</td>
<td>103 ± 18 (18)</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>0.92 ± 0.05</td>
<td>97 ± 18 (17)</td>
<td></td>
</tr>
<tr>
<td>67</td>
<td>0.90 ± 0.05</td>
<td>110 ± 20 (12)</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.92 ± 0.05</td>
<td>97 ± 14 (15)</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>20</td>
<td>0.89 ± 0.06</td>
<td>103 ± 11 (20)</td>
<td>P &gt; 0.05</td>
</tr>
<tr>
<td>40</td>
<td>0.73 ± 0.04</td>
<td>93 ± 13 (14)</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td>50</td>
<td>0.67 ± 0.05</td>
<td>96 ± 11 (16)</td>
<td>P &lt; 0.01</td>
</tr>
<tr>
<td>67</td>
<td>0.62 ± 0.04</td>
<td>102 ± 14 (12)</td>
<td>P &lt; 0.05</td>
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All values are expressed as means ± SE. Paired-pulse ratio (PPR) is calculated as (2nd EPSC/1st EPSC) except for the number of cells tested is indicated in parenthesis.
showed PPR ranging between 1.03 (10 Hz) and 0.89 (66.7 Hz), whereas PPR values in undercut cells ranged between 0.92 (10 Hz) and 0.60 (66.7 Hz) (Table 2).

Effects of cortical injury on amplitude of successive EPSCs evoked by stimulus trains

In addition to the PPR, synaptic efficacy can be measured by the time constant of decay of amplitudes of successively evoked EPSCs, here termed the time constant of depression or $\tau$, and by the steady state amplitude of EPSCs during high-frequency stimulation. In this study, synaptic responses elicited by trains of 10 stimuli were measured in neurons from control and isolated cortex. At stimulus frequencies of 20, 40, and 66.7 Hz, the time constant of depression in neurons of injured cortex was significantly shorter than in control cortex (Table 3; Fig. 2, A1, A2, B1, and B2) (see also Doherty and Dingledine 2001). A similar trend was also present with 10 Hz stimulus trains; however, it did not reach statistical significance (Table 3; Fig. 2B3). An analysis of the average steady-state amplitude of the last three EPSCs evoked by stimulus trains revealed that, at frequencies of 20, 40, and 50 Hz, neurons of injured cortex had a lower steady-state EPSC amplitude than those in control cortex (Fig. 2, A2 and B1 and B2; $P < 0.01$ for 20, 40, and 50 Hz), but there was no difference with stimuli of 10 and 66.7 Hz (Fig. 2, B3 and A1).

Effect of extracellular calcium concentration ([Ca$^{2+}$]o) on synaptic depression

A large number of pre- and postsynaptic mechanisms may underlie the alterations in PPR, steady-state amplitude, and time constant of depression found in injured cortex, including those affecting presynaptic terminals and transmitter release (reviewed in Zucker and Regeh 2002). We tested several candidate mechanisms that might influence these properties. The effects of [Ca$^{2+}$]o on the PPR are well-established (Cummings et al. 1996; Zucker 1993). Increases in Ca$^{2+}$ entry into terminals during depolarization leads to an increased probability of release at a synapse and depletion of the releasable pool of vesicles such that subsequent evoked synaptic currents/potentials are smaller. To determine the degree to which PPD in neurons of control and undercut slices in the present experiments was Ca$^{2+}$ dependent, we recorded responses to pairs of stimuli in ACSF containing increased [Ca$^{2+}$] or in Ca$^{2+}$-free, Sr$^{2+}$-containing solution (Fig. 3, A and B). Increasing [Ca$^{2+}$]o from 2 to 4 mM significantly decreased the PPR at 10 Hz by 23 ± 4% in control ($n = 7$, $P < 0.01$) and by 12 ± 3% in cells of undercut ($n = 9$, $P < 0.01$). This change in PPR in neurons of injured cortex was significantly less than that in control cells (Fig. 3 B, left, and C). The effect of high calcium on mean peak amplitude of the initial EPSC in the train was, however, not significantly different between control and undercut (1st response in control was 129 ± 19 pA in 2 mM Ca$^{2+}$ and 135 ± 21 pA in 4 mM Ca$^{2+}$, ~5% increase; 1st response in undercut was 127 ± 18 pA in 2 mM Ca$^{2+}$ and 136 ± 29 pA in 4 mM Ca$^{2+}$, ~7% increase). This result may have been due to a large variability in effects of elevated [Ca$^{2+}$]o on peak EPSC amplitude from cell to cell; in both groups, some initial responses increased in high calcium while others were not affected. Thus changes in PPR appeared to be a more sensitive index of alterations in [Ca$^{2+}$]o than response amplitude. When extracellular Ca$^{2+}$ was replaced with equimolar Sr$^{2+}$, the PPR was increased by an average of 35 ± 9% ($n = 6$, $P < 0.01$) in control and 26 ± 4% in undercut neurons ($n = 4$, $P < 0.05$, Fig. 3B). The shifts in PPR in control versus undercut cortex with 4 mM Sr$^{2+}$ were not significantly different (Fig. 3B, right). In both control and undercut cortex, there was a strong correlation between the PPR in control solution and the change in PPR, defined as PPR in 2 mM [Ca$^{2+}$]o minus PPR in 4 mM [Ca$^{2+}$]o (Fig. 3D). Thus larger initial PPR values were associated with greater decreases in 4 mM [Ca$^{2+}$]o.

Potential role of presynaptic receptor activation

Another possibility for the decrease in PPR, steady-state EPSC amplitude and time constant of depression between undercut and control cells is a differential expression/activation of presynaptic receptors on excitatory terminals in the isolated cortex. For example, spillover of GABA onto presynaptic terminals has been shown to be associated with greater decreases in 4 mM [Ca$^{2+}$]o. A large number of pre- and postsynaptic mechanisms may underlie the alterations in PPR, steady-state amplitude, and time constant of depression found in injured cortex, including those affecting presynaptic terminals and transmitter release (reviewed in Zucker and Regeh 2002). We tested several candidate mechanisms that might influence these properties. The effects of [Ca$^{2+}$]o on the PPR are well-established (Cummings et al. 1996; Zucker 1993). Increases in Ca$^{2+}$ entry into terminals during depolarization leads to an increased probability of release at a synapse and depletion of the releasable pool of vesicles such that subsequent evoked synaptic currents/potentials are smaller. To determine the degree to which PPD in neurons of control and undercut slices in the present experiments was Ca$^{2+}$ dependent, we recorded responses to pairs of stimuli in ACSF containing increased [Ca$^{2+}$] or in Ca$^{2+}$-free, Sr$^{2+}$-containing solution (Fig. 3, A and B). Increasing [Ca$^{2+}$]o from 2 to 4 mM significantly decreased the PPR at 10 Hz by 23 ± 4% in control ($n = 7$, $P < 0.01$) and by 12 ± 3% in cells of undercut ($n = 9$, $P < 0.01$). This change in PPR in neurons of injured cortex was significantly less than that in control cells (Fig. 3 B, left, and C). The effect of high calcium on mean peak amplitude of the initial EPSC in the train was, however, not significantly different between control and undercut (1st response in control was 129 ± 19 pA in 2 mM Ca$^{2+}$ and 135 ± 21 pA in 4 mM Ca$^{2+}$, ~5% increase; 1st response in undercut was 127 ± 18 pA in 2 mM Ca$^{2+}$ and 136 ± 29 pA in 4 mM Ca$^{2+}$, ~7% increase). This result may have been due to a large variability in effects of elevated [Ca$^{2+}$]o on peak EPSC amplitude from cell to cell; in both groups, some initial responses increased in high calcium while others were not affected. Thus changes in PPR appeared to be a more sensitive index of alterations in [Ca$^{2+}$]o than response amplitude. When extracellular Ca$^{2+}$ was replaced with equimolar Sr$^{2+}$, the PPR was increased by an average of 35 ± 9% ($n = 6$, $P < 0.01$) in control and 26 ± 4% in undercut neurons ($n = 4$, $P < 0.05$, Fig. 3B). The shifts in PPR in control versus undercut cortex with 4 mM Sr$^{2+}$ were not significantly different (Fig. 3B, right). In both control and undercut cortex, there was a strong correlation between the PPR in control solution and the change in PPR, defined as PPR in 2 mM [Ca$^{2+}$]o minus PPR in 4 mM [Ca$^{2+}$]o (Fig. 3D). Thus larger initial PPR values were associated with greater decreases in 4 mM [Ca$^{2+}$]o.

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Another possibility for the decrease in PPR, steady-state EPSC amplitude and time constant of depression between undercut and control cells is a differential expression/activation of presynaptic receptors on excitatory terminals in the isolated cortex. For example, spillover of GABA onto presynaptic GABA_B receptors on glutamatergic terminals might produce such effects, particularly if GABA reuptake was reduced in the injured tissue (Isaacson et al. 1993). To test this possibility, we studied the effects of a GABA_B receptor antagonist on the amplitudes of EPSCs evoked by stimulus trains in neurons of control and undercut cortical slices (Fig. 4A). The PPR of control cells was 0.91 ± 0.10 and 0.92 ± 0.11 in standard ACSF and ACSF containing CGP55845 (0.2–1 μM), respectively ($n = 13$, NS). In undercut cells, the PPR was 0.54 ± 0.07 predrug and 0.52 ± 0.05 in CGP55845 ($n = 14$, NS). There was also no change in time constant of depression or steady-state EPSC amplitude after drug application in control or undercut cells (Fig. 4A). Thus in this population, activation of presynaptic GABA_B receptors did not contribute to alterations in EPSC amplitude during trains of stimuli.

Group II metabotropic glutamate receptors (mGluR) are known to mediate presynaptic inhibition of glutamate release in cortical neurons (Anwyl 1999; Stefani et al. 1996) and therefore might contribute to the PPD, the decrease in the
steady-state of EPSCs, or the time constant of depression in the undercut cortex. Perfusion of ACSF containing the group II mGluR antagonist LY341495 (2 μM) did not significantly affect the PPR in control cells (0.95 ± 0.07 in normal ACSF and 1.00 ± 0.09 after LY341495 application, n = 11, P > 0.05; Fig. 4Bi) or in undercut cells (0.69 ± 0.06 before drug and 0.73 ± 0.07 in LY341495, n = 12, P > 0.05; Fig. 4Bii). Although LY341495 did not affect the time constant of de-

FIG. 2. Decrease in decay time constant of EPSC amplitudes evoked by stimulus trains in epileptogenic cortex. A1–A2 and B1–B3: normalized EPSC amplitudes in layer V pyramidal cells plotted at times from onset of stimulus trains for different stimulus frequencies. □, controls, n = 10–13; ●, undercut, n = 10–14. Averaged data for each cell were normalized to the 1st response and then averaged across cells. ··· and —, single exponential fits. Error bars: SE in this and subsequent figs. A3: individual traces showing AMPA/kainate receptor-mediated EPSCs recorded from a control neuron (Co) and an undercut neuron (Un), evoked by 50-Hz stimulus trains. See Table 3 for statistical analysis.

FIG. 3. The effects of [Ca²⁺]₀ and [Sr²⁺]₀ on the PPR in layer V pyramidal neurons. A: single traces showing responses from 1 neuron evoked by pairs of stimuli (10 Hz) in ACSF containing 2 mM Ca²⁺, 4 mM Ca²⁺, and 0 mM Ca²⁺ and 4 mM Sr²⁺. B, left: percentage change in PPR in control (n = 7) and undercut neurons (n = 9) in control ACSF (2 mM Ca²⁺) vs. ACSF containing 4 mM Ca²⁺. Right: percentage change from control ACSF to perfusate containing 0 mM Ca²⁺ and 4 mM Sr²⁺ for control (n = 6) and undercut neurons (n = 4). *P < 0.05. C: plot of PPR in 2 mM [Ca²⁺]₀ vs. 4 mM [Ca²⁺]₀ for cells in control (left, n = 7) and undercut cortex (right, n = 9). Each — connects PPR values in 2 and 4 mM Ca²⁺ in single cell. D: plot of PPR in 2 mM [Ca²⁺]₀ vs. PPR in 2 mM [Sr²⁺]₀, minus PPR in 4 mM [Ca²⁺]₀. Each symbol represents 1 cell. ····· and —, undercut, r = 0.71; —, control, r = 0.89.
Injury-related alterations in postsynaptic glutamate receptors could play a role in the changes in short-term dynamics of evoked EPSC amplitude described in the preceding text. Anatomical data do suggest that there are chronic changes in AMPA receptor subunit composition in layer V pyramidal neurons of the partially isolated cortex, including a reduction in immunoreactivity for the GluR2 subunit (Kharaizia and Prince 2001). A downregulation of GluR2 has also been reported in the amygdaloid kindling model of epilepsy (Prince et al. 1995), and mice lacking GluR2 show a greater amount of long-term potentiation (LTP) and increased mortality (Jia et al. 1996). Because the presence of GluR2 in the AMPA receptor complex is linked to reduced Ca$^{2+}$ permeability (Hollmann et al. 1991), a decrease in GluR2 could result in increased Ca$^{2+}$ flux into neurons during glutamate receptor activation that might, in turn, influence the short-term plasticity of evoked EPSCs. As one test of such potential postsynaptic receptor alterations, we studied the effects of spermine, a biogenic polyamine that reduces the amplitude of EPSCs by specifically binding to those AMPA receptors lacking GluR2 (Donevan and Rogawski 1995; Kumar et al. 2002; Washburn and Dingledine 1996; Washburn et al. 1997). No significant effect of spermine (5–10 μM) on EPSC amplitude was detected in seven control or seven undercut cells during 10 min of bath perfusion (Fig. 5), although in five additional undercut cells exposed to spermine over 12 min, there was a slight decrease in EPSC amplitude that did not reach statistical significance (not shown), suggesting that reduction in the expression of postsynaptic GluR2 subunits is not a major factor underlying the change in PPD in undercut cells.

To further evaluate potential functional alterations in receptors, the I-V properties of AMPA receptor-mediated EPSCs were examined in control and undercut cells (Fig. 6). The short-latency, rapidly rising AMPA receptor-mediated component of evoked EPSCs (Fig. 6A, $V_h = -55$ mV) had a near linear I-V relationship in both control and undercut cell groups (Fig. 6, B and C) (see also Li and Prince 2002), suggesting that significant changes in rectification properties of postsynaptic AMPA receptors were not present (Kumar et al. 2002). The EPSC reversal potentials were 0.12 ± 2.8 mV ($n = 9$) and 2.97 ± 3.2 mV ($n = 7$) in the control and undercut groups, respectively ($P = 0.52$).

Changes in the strength of simultaneously activated neocortical excitatory and inhibitory synaptic events might contribute in differing degrees to the amplitudes of evoked PSCs during repetitive stimulation (Varela et al. 1999). To determine whether concurrent activation of GABA$_A$ receptor-mediated postsynaptic inhibition during stimulus trains might be shunting EPSCs (Tseng and Haberly 1988; Ulrich 2003) and accounting in part for changes in PPD or the time constant of depression, we examined the effects of perfusing slices with ACSF containing bicuculline (10 μM). Near-threshold focal stimulation was used, and APV (50 μM) included in the perfusate to avoid evoking epileptiform activity in slices after blockade of GABA$_A$ receptors with bicuculline (Fig. 7). Under these conditions, blockade of GABA$_A$ receptors had no significant effect on PPR, steady-state EPSC amplitude, or the time constant of depression at any frequency tested in control or undercut cortex (Fig. 7). As shown in the representative traces...
of Fig. 7, C and C1 and D and D1, bicuculline also had no significant effect on the amplitudes of EPSCs evoked by 20-Hz trains in either control (n = 4) or undercut cells (n = 5). Together with the lack of effects of GABA<sub>B</sub> receptor blockade (Fig. 4A), these data suggest that GABAergic mechanisms are not involved in the differences in short term plasticity between control and undercut cells.

**DISCUSSION**

A variety of evidence supports potential involvement of alterations in axonal terminals in the generation of epileptiform activity. Long-lasting changes in short-term plasticity at excitatory synapses, such as loss of PPF or increased PPD have been previously reported in the hippocampus after status epilepticus (Doherty and Dingledine 2001; Goussakov et al. 2000) and exposure to the convulsant drug, 4-aminopyridine in vivo and in vitro (Pena et al. 2002). Increased PPD of excitatory field potentials reported in epileptogenic human limbic structures (Wilson et al. 1998) might relate to changes in the functional properties of transmitter release, but this is difficult to evaluate due to potential concurrent activation of inhibitory circuits and other factors. Anatomical and biochemical alterations in axons and presynaptic terminals are a ubiquitous feature in models of both acute and chronic epileptogenesis (Arellano et al. 2004; Cavazos et al. 2003; Goussakov et al. 2000; Hinz et al. 2001; Hovorka et al. 1989; Marin-Padilla et al. 2003; Pierce and Milner 2001; Salin et al. 1995). In addition, alterations in excitability of axon terminals leading to spontaneous action potential generation and “backfiring” has been reported in both acute (Gutnick and Prince 1972; Noebels and Prince 1978; Rosen et al. 1973; Schwartzkroin et al. 1975a,b; Stasheff et al. 1993) and chronic models of epileptiform discharge (Pinault and Pumain 1985; see Pinault 1995 for review).

The present results show that, at stimulus frequencies >20 Hz, there is a significant decrease in the PPR (increase in PPD), as well as a more rapid time constant of decay of amplitudes of successively evoked EPSCs in presumably axotomized and otherwise damaged layer V pyramidal neurons of chronic partial cortical isolations (Fig. 2). The lesion producing the partial cortical isolation severs thalamocortical and other extrinsic inputs to cortex, and we assume that stimuli evoking EPSCs predominantly activate adjacent intracortical pyramidal cells and axons morphology and intrinsic properties of which have been altered by the chronic injury (Prince and Tseng 1993; Salin et al. 1995). Thalamocortical neurons and their axons projecting into the undercut cortex would be long gone 2–3 wk after the lesion (Barron et al. 1973). Assuming that layer V stimuli in controls activated both intracortical and
thalamocortical fibers and that intracortical stimuli generate PPF while thalamocortical inputs predominantly evoke PPD (Gil et al. 1999), a loss of the latter component in the lesioned cortex would tend to reduce rather than enhance PPD responses. Therefore the enhanced PPD in the undercut cells in our experiments (Fig. 1) is unlikely due to removal of the thalamic inputs by the lesion.

The decreases in PPR might involve a number of both pre- and postsynaptic mechanisms (reviewed in Zucker and Regehr 2002). There was a small but significant prolongation of the time constant for decreases in amplitudes of successively evoked EPSCs, as well as for steady-state EPSC amplitudes, and does not affect PPD. Preliminary experiments with group II metabotropic glutamate receptors were blocked by LY341495 (Fig. 4B), suggesting that these autoreceptors were activated by glutamate released during the later parts of the stimulus train. Such delayed effects might be expected from autoreceptor activation (Zucker and Regehr 2002). We found no significant differences in the amplitude of the first or second EPSC in control versus LY341495-containing perfusates, indicating that the same presynaptic receptors were not activated by ambient agonist levels. The small LY341495 effect suggests that activation of metabotropic glutamate autoreceptors makes only a minor contribution to the final amplitude of EPSCs in neurons from the injured cortex and does not affect PPD. Preliminary experiments with group I and III mGluR antagonists did not reveal any effect on the time constant or final steady-state amplitude (A. E. Bandrowski and D. A. Prince, unpublished observations), and therefore effects of other selective agonists of mGluRs were not tested. Presynaptic GABAB receptors did not appear to be involved in the PPD (Fig. 4A); however possible contributions by other mediators of presynaptic inhibition, such as adenosine (Phillis et al. 1979; Prince and Stevens 1992) and neuropeptide Y (Bacci et al. 2002; Colmers et al. 1988), were not explored.

Increases in \(\text{[Ca}^{2+}\text{]}_o\) decreased the PPR (increased PPD) in both control and undercut neurons (Fig. 3, B and C), while substitution of Sr\(^{2+}\) for \(\text{Ca}^{2+}\) had the opposite effects (Fig. 3). These results support a presynaptic mechanism contributing to the PPD in both cell groups. PPD reflects a decrease in the probability of release (\(P\)) at involved presynaptic terminals should be reflected as increases in PPD (Gil et al. 1999; Thomson et al. 1993a; Zucker and Regehr 2002). Thus increases in PPD seen in the neurons of partially isolated cortex suggest that \(P\) is increased in excitatory glutamatergic terminals. Such an increase in \(P\) could contribute, in part, to the increases in miniature EPSC frequency and larger peak amplitude of the AMPA/kainate receptor-mediated component of EPSCs as well as an increase in the slope of the input–output relation for evoked EPSCs in layer V pyramidal cells of partially isolated cortex found in previous experiments (Li and Prince 2002). However, other mechanisms, such as increased numbers of excitatory synapses (Salin et al. 1995), cannot be excluded.

Increases in \(\text{[Ca}^{2+}\text{]}_o\) caused a greater decrease of the PPR in control versus undercut neurons (Fig. 3B). One likely explanation for this finding would be a saturation or ceiling effect as a consequence of the presence of only a few (1–3) release sites for each synaptic terminal (Lisman and Harris 1993; Thomson et al. 2002; colmer et al. 1988), were not explored.

**FIG. 7.** EPSC paired pulse ratio is not dependent on activation of GABAA receptors. A and B: plots of PPR at stimulus frequencies of 10, 20, 40, and 66.7 Hz for control (A) and undercut (B) neurons. Numbers of cells tested at each frequency shown below symbols. □, mean PPR from neurons in slices perfused with ACSF containing APV (50 \(\mu\)M). ●, mean PPR from same neurons at each frequency after perfusion with ACSF containing APV + bicuculline (10 \(\mu\)M). PPR = peak amplitude of response 2/peak amplitude of response 1. ••••, PPR = 1. Bicuculline does not affect PPR at any frequency in control or undercut groups. C and D: representative single traces showing trains of EPSCs from a control (C and C1) and an undercut cell (D and D1), evoked by 20 Hz stimuli before (C and D) and after (C1 and D1) addition of bicuculline (10 \(\mu\)M) to the APV-containing perfusate. Calibrations in D for all traces.
Changes in short-term plasticity have been observed during maturation of neocortical circuits. For example, PPD is a prominent feature of monosynaptic connections in immature cortex (Kriegstein et al. 1987; Ramoa and Sur 1996; Thomson and West 1993); however, in adults, both PPF and PPD are reported (Thomson et al. 1993a,b; for review see Reyes and Sakmann 1999; Thomson and Deuchars 1994). If, as has been suggested (Cramer and Chopp 2000; Wood et al. 1990), injury to mature CNS can recapitulate some developmental processes, changes in the PPD and the decay time constant of successive EPSC amplitudes might be expected in the injured cortex.

The functional consequences of enhanced PPD in chronically injured epileptogenic cortex are hard to predict. Because the emergence of sustained epileptiform (ictal) activity is closely associated with high-frequency neuronal discharges (Matsumoto and Ajmone-Marsan 1964), enhanced PPD and increased falloff in EPSC amplitude at high frequencies might be regarded as mechanisms that serve as brakes on generation of such activity. This effect would be counterbalanced by the increased P, implied from the increased PPD, and increased excitatory connectivity within the cortical network (Salin et al. 1995) that would enhance the frequency and amplitude of spontaneous excitatory events and facilitate emergence of epileptiform discharges. Like other forms of short-term plasticity, synaptic depression causes the response of a cortical neuron to depend on the previous history of afferent signals. As a result, neuronal responses reflect the current state of presynaptic activity within the context of previous activity. However, unlike the other mechanisms, such as postsynaptic somatic inhibition that can reduce responsiveness to all inputs, synaptic depression is input-specific, leading to a dramatic increase in the selectivity and sensitivity of a neuron to subtle changes in the firing patterns of its afferents. It has been recently reported that synaptic depression greatly increased the sensitivity of a model cell to sudden small changes in the frequency of presynaptic inputs (Abbott et al. 1997). In this connection, it is interesting that increased baseline synaptic activity frequently precedes generation of spontaneous epileptiform activity in models of partial epileptogenesis (e.g., Chamberlin et al. 1990).

r a role in determining the PPR because, in several cortical cell types, stimulus frequencies as low as 20 Hz can result in some desensitization of AMPA receptors (Rozov et al. 2001). At other CNS synapses in hippocampus (Hjelmstad et al. 1999) and cerebellum (Hashimoto and Kano 1998), desensitization does not contribute significantly to PPD. The hypothesis that acute injury results in enhanced AMPA receptor desensitization has been directly tested in cell cultures of neocortical neurons subjected to stretch injury (Goforth et al. 1999). In this model, the opposite effect was found, i.e., injured neurons showed decreased desensitization of AMPA currents. Further experiments are required to directly assess potential alterations in AMPA receptor desensitization in models of chronic cortical injury or induced epileptiform activity, where increases in short-term synaptic depression have been found.

The decrease GluR2 subunit immunoreactivity in pyramidal neurons of layer V in partially isolated cortex (Kharazia and Prince 2001) might have a significant effect on the functional properties of AMPA receptors (Washburn et al. 1997), including an increase in Ca2+ permeability and a resulting increase in PPD. However, the immunocytochemically demonstrated reduction in GluR2 subunit expression did not appear to influence our results as the I-V relations of evoked EPSCs in control and undercut were relatively linear (Fig. 6) and EPSCs were relatively insensitive to external polyamine (Fig. 5). This discrepancy may be related to the relative abundance of this subunit remaining in the layer V pyramidal neurons after injury (Washburn et al. 1997).


Washburn MS and Dingleidine R. Block of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors by polyamines and polyamine toxins. J Pharmacol Exp Ther 278: 660–678, 1996.


