Acute Injury to Superficial Cortex Leads to a Decrease in Synaptic Inhibition and Increase in Excitation in Neocortical Layer V Pyramidal Cells

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Yang, Lie, Larry S. Benardo, Helen Valsamis, and Douglas S. F. Ling. Acute injury to superficial cortex leads to a decrease in synaptic inhibition and increase in excitation in neocortical layer V pyramidal cells. J Neurophysiol 97: 178–187, 2007. First published September 20, 2006; doi:10.1152/jn.01374.2005. Injury to the superficial layers of cerebral cortex produces alterations in the synaptic responses of local circuits that promote the development of seizures. To further delineate the specific changes in synaptic strength that are induced by this type of cortical injury, whole cell voltage-clamp recordings were used to examine evoked and spontaneous synaptic events from layer V pyramidal cells in coronal slices prepared from surgically traumatized rat neocortices in which the superficial third of the cortex (layers I, II, and part of III) was removed. Slices from intact neocortices were used as controls. Examinations of fast inhibitory postsynaptic currents (IPSCs) indicated that traumatized slices were disinhibited, exhibiting evoked IPSCs (eIPSCs) with lower peak amplitudes. Measurements of spontaneous IPSCs (sIPSCs) revealed no difference in the mean amplitudes of sIPSCs recorded in traumatized versus control slices. However, the mean sIPSC frequency was lower in traumatized slices, indicative of a decrease in GABA release at these inhibitory synapses. Traumatized slices also displayed an increase in synaptic excitation, exhibiting spontaneous EPSCs (sEPSCs) with larger peak amplitudes and higher frequencies. Peak-scaled nonstationary fluctuation analysis of sEPSCs and sIPSCs was used to obtain estimates of the unit conductance and number of functional receptor channels. EPSC and IPSC channel numbers and IPSC unit conductance did not differ between traumatized and intact slices. However, the mean IPSC unit conductance of EPSCs was higher (+25%) in traumatized slices. These findings suggest that acute injury to the superficial neocortical layers results in a disinhibition of cortical circuits that stems from a decline in GABA release likely due to the loss of superficial inhibitory interneurons and an enhancement of synaptic excitation consequent to an increase in the AMPA receptor unit conductance.

Brain injury is a well-known cause of epileptogenesis, leading to increased network excitability and seizures (Lowenstein 1996). Although the underlying mechanisms of injury-induced epilepsy are complex and poorly understood, damage to the superficial cerebral cortex represents a common component of many forms of traumatic brain injury (TBI) that may contribute to the development of epilepsy. Epidemiological studies of TBI have shown that superficial cortical damage is a feature shared by several independent risk factors of epilepsy, such as subdural hematoma, epidural hematoma, and depressed skull fracture (Annegers et al. 1980, 1998). In addition, superficial cortical damage occurs with extra-axial lesions that result from other diseases that carry high rates of epilepsy, such as meningiomas (Ramamurthi et al. 1980).

Studies of mechanistically diverse animal models of epilepsy, such as status epilepticus and kindling (Loscher 2002; Lowenstein 1996; Morimoto et al. 2004), suggest that acute seizure activity may be one factor involved in postinjury epileptogenesis. In these studies, the presence of acute seizures independently predicts seizure recurrence. Given the rapidity with which injury-induced alterations to physiological and biochemical processes occur in neural circuits (D’Ambrosio and Perucca 2004; Miller et al. 1990; Morrison et al. 2000; Topolink et al. 2003a,b), the plastic changes in cortical function that support epileptogenesis could develop quickly after brain injury. However, in human epidemiologic studies, the role of early seizures is less clear. Although early seizures after brain trauma predict an increased risk of posttraumatic epilepsy (Angeleri et al. 1999; Annegers et al. 1980, 1998; Asikainen et al. 1999), it remains controversial whether acute seizures are independent risk factors (Asikainen et al. 1999) or markers of injury severity (Annegers et al. 1998). For example, early administration of anticonvulsant medications has been shown to decrease the percentage of brain injured patients who clinically exhibit early posttraumatic seizures but not the percentage who develop posttraumatic epilepsy (Temkin et al. 1990, 1999). However, it is worth noting that these treatments do not suppress electrographic seizures in a high proportion of patients (Vespa et al. 1999). Thus despite the absence of early clinical seizures, subclinical seizure-like activity could be present that contributes to the subsequent development of postinjury epilepsy. Given the multiple processes at work, studies using controlled models of acute brain injury are needed to complement chronic injury models as a means of gaining insights into the specific processes affecting network excitability (Herman 2002).

To evaluate the acute effects of superficial cortical damage on the local neuronal networks, our laboratory has developed a traumatized neocortical slice preparation (Yang and Benardo 1997). As a reductionist model, this in vitro preparation allows examination of the immediate changes in the intrinsic physiological properties of the local neuronal circuitry caused by the removal of the superficial cortical layers (layers I, II, and part of III), without the additional factors associated with injury (Willmore 1990). Using this model, we previously demonstrated that this type of purely mechanical insult induces an
increase in circuit excitability, manifested as epileptiform activity, in a majority of neocortical slices (Yang and Benardo 1997, 1998, 2000). The hyperexcitability appears to be a consequence of disinhibition that arises from two distinct sources: the physical removal of the inhibition-rich superficial cortical laminae and glutamate-triggered increases in intracellular calcium (Yang and Benardo 1997). Maneuvers designed to either enhance the strength of synaptic inhibitory transmission or inhibit rises in intracellular calcium prevented the development of epileptiform activity when applied within 20 min after the injury (Yang and Benardo 1997, 2000). However, one key issue that remains is the elucidation of the specific changes in GABA- and glutamatergic transmission that give rise to the injury-induced hyperexcitability. The present study examines the specific alterations in synaptic transmission induced by acute cortical injury to the superficial cortex using whole cell voltage clamp recordings of layer V pyramidal cells. Comparisons of evoked and spontaneous excitatory and inhibitory postsynaptic currents (EPSCs and IPSCs) recorded from traumatized and control slices revealed that injured slices exhibited both decreased inhibition and increased excitation. The decrease in synaptic inhibition appears to stem from diminished activity in presynaptic inhibitory circuits, whereas the enhancement of excitation derives from an increase in α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPAR) unit conductance. Thus acute trauma to the superficial neocortical layers rapidly produces distinct modifications in synaptic inhibition and excitation that combine to promote epileptogenicity in cortical circuits. Some of the results of this study have been presented in abstract form (Yang and Benardo 2004).

**Methods**

**Preparation and maintenance of slices**

All the experiments were performed according to the protocols approved by the Animal Care and Use Committee of SUNY Downstate Medical Center. In vitro neocortical slices were prepared and maintained using procedures previously described (Edwards et al. 1989; Ling and Benardo 1999, 2005; Moyer and Brown 1998). Briefly, Sprague-Dawley rats (postnatal day 17–28) were deeply anesthetized by either intra-peritoneal injection of ketamine (87 mg/kg) and xylazine (13 mg/kg) or inhalation of halothane and then decapitated. The brain was rapidly removed and placed in ice-chilled (0–4°C) low-Ca2+, high-Mg2+ saline (see following text). The somatosensory cortical tissue was then blocked off and isolated. Tissue blocks were then secured to a specimen mounting unit with cyanoacrylate glue and transferred to the chamber of a Vibratome tissue sectioner (St. Louis, MO), where they were submerged in ice-chilled, high-Mg2+ saline (see following text). The so-called superfused saline was made by adding 130Cs-methanesulfonate, 0.15CaCl2, 2.0MgCl2, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), picrotoxin (PTX, 50 μM), and 10 lidocaine N-ethyl bromide (QX-314), pH 7.2–7.3 (adjusted with CsOH). In some recordings, neurobiotin (4 mg/ml) was included in the intracellular solution and slices were recovered for histological processing using the avidin-biotin-peroxidase complex procedure (ABC, Vector Laboratories, Burlingame, CA) as previously described (Benardo 1997). Currents were recorded under voltage clamp with a Warner PC-501A patch-clamp amplifier (Warner Instrument, Hamden, CT). Cells accepted for study had a mean input resistance of 283.9 ± 34.4 MΩ (mean ± SE, n = 38, range: 108–969 MΩ) and mean access resistance of 16.8 ± 0.67 MΩ (n = 38, range: 8.4–25.6 MΩ). If the access resistance increased by >20% during experiments, the recording was terminated and the data discarded. Signals were digitized at 47 kHz via a 14-bit PCM interface (VR-10B Digital Data Recorder, Instrotech, Elmont, NY) and stored on VHS videotape for post hoc analysis or recorded directly to computer hard disk using pCLAMP 9.0 software (Axon Instruments, Foster City, CA). Data were filtered at 2 kHz (4 pole, –3 dB Bessel filter) and digitally sampled at 20 kHz.

Synaptic events were evoked by extracellular electrical stimuli (2–20 V, 0.2 ms, 0.05 Hz) delivered through a coated, tungsten, bipolar electrode (5 MΩ resistance) placed laterally to the recording electrode (~200 μm). EPSCs were recorded by holding the membrane at the empirically determined IPSC reversal (approximately −75 mV), whereas IPSCs were recorded at the EPSC reversal (0–5 mV). Once whole cell access was achieved, the membrane potential was held initially at −75 mV to monitor spontaneous EPSCs. Data collection was carried out only if the responses were stable for >5 min. In 70% of the cells recorded, both spontaneous and evoked EPSCs and IPSCs could be obtained from the same cell.

Electrophysiological recording

Whole cell recordings were obtained from layer V pyramidal cells visually identified by shape of cell soma and apical dendrites. Patch pipettes were pulled from borosilicate glass capillaries (1.5 mm OD, World Precision Instrument, Sarasota, FL) and had a tip resistance of 3–5 MΩ when filled with an intracellular solution composed of (in mM) 130 Cs-methanesulfonate, 0.15 CaCl2, 2.0 MgCl2, 0.1 EGTA, 10 HEPES, 2 Na2-ATP, 0.25 Na3GTP, and 10 lidocaine-N-ethyl bromide (QX-314), pH 7.2–7.3 (adjusted with CsOH). In some recordings, neurobiotin (4 mg/ml) was included in the intracellular solution and slices were recovered for histological processing using the avidin-biotin-peroxidase complex procedure (ABC, Vector Laboratories, Burlingame, CA) as previously described (Benardo 1997). Currents were recorded under voltage clamp with a Warner PC-501A patch-clamp amplifier (Warner Instrument, Hamden, CT). Cells accepted for study had a mean input resistance of 283.9 ± 34.4 MΩ (mean ± SE, n = 38, range: 108–969 MΩ) and mean access resistance of 16.8 ± 0.67 MΩ (n = 38, range: 8.4–25.6 MΩ). If the access resistance increased by >20% during experiments, the recording was terminated and the data discarded. Signals were digitized at 47 kHz via a 14-bit PCM interface (VR-10B Digital Data Recorder, Instrotech, Elmont, NY) and stored on VHS videotape for post hoc analysis or recorded directly to computer hard disk using pCLAMP 9.0 software (Axon Instruments, Foster City, CA). Data were filtered at 2 kHz (4 pole, –3 dB Bessel filter) and digitally sampled at 20 kHz.
Data analysis

Spontaneous synaptic events were detected and analyzed using Mini Analysis software (Synaptosoft, Decatur, GA), which identifies spontaneous events on the basis of several criteria, including threshold amplitude and the area under each event. As a routine check, all events detected were visually inspected and those that did not exhibit the general shape expected for synaptic currents were rejected for analysis. To select for events generated more proximally and thus minimize potential contributions from space-clamp error and dendritic filtering, only events with 10–90% rise times <1.5 ms were included in our analyses (Ling and Benardo 1999). Due to the variability in threshold stimulus values among neurons, input-output relationships were constructed using relative stimulus intensities normalized to the threshold stimulus value (T) of each cell (Li and Prince 2002), defined as the intensity that recruits the smallest discernable evoked synaptic current. In all cells the currents evoked by T stimuli were significantly larger than average spontaneous events recorded in the same cell (cf. Li and Prince 2002). Analysis of evoked and spontaneous events was performed using Mini Analysis or Clampfit software (pCLAMP 9.0). Data were evaluated using a two-tailed t-test (2 data groups) or ANOVA (>2 data groups) at the P < 0.05 significant level. Distributions of spontaneous events were compared using the Kolmogorov-Smirnov (K-S) test at the P < 0.001 significant level.

Total charge flux, defined as the integral of the current trace, was calculated from the point of current onset to its decay back to baseline using piecewise approximations of EPSC and IPSC integrals (Ling and Benardo 1994; Yang and Benardo 1997).

Estimates of unit conductance (γ) and the number of active channels (N, defined as number of physical channels, if their open probability) were obtained using peak-scaled nonstationary fluctuation analysis (NSFA) of eEPSCs and eIPSCs (DeKoninck and Mody 1994; Traynelis et al. 1993). Individual synaptic currents (50–100 events) were aligned on the rising phase of the half-maximal amplitude and averaged. The averaged trace was scaled to the peak of the individual traces (i.e., “peak-scaled”) and its decay phase divided into 60 bins (from the peak to the end of the decay). The variance of the individual synaptic currents around the averaged trace was calculated within each bin and plotted against the mean decay amplitude (i.e., bin value). Plots of current variance versus amplitude were fitted with the following parabolic function using a least-squares algorithm to obtain estimates of the unit current (i) and number of active channels at the peak amplitude (N): σ² = π²/4 - 2πiN + b, where σ² is the variance, I is the mean current, and b is the baseline variance (Fig. 7). The unit conductance was calculated as γ = i(Vn - Vrev), where Vn is the membrane holding potential and Vrev the reversal potential of the synaptic event.

All data throughout this report are expressed as means ± SE unless indicated otherwise.

RESULTS

Decrease in evoked IPSC amplitude in traumatized slices

Evoked EPSCs (eEPSCs) and IPSCs (eIPSCs) were recorded from 46 layer V pyramidal cells in slices prepared from 40 animals; 25 neurons were from control slices and 21 from traumatized slices. For all cells, the peak amplitudes of eEPSCs and eIPSCs increased as the stimulus intensity was increased (Fig. 1, A and B). Threshold stimulus intensities for eEPSCs and eIPSCs for traumatized slices were similar to those of control, varying between 2 and 4 V. The shape and amplitude of eEPSCs and eIPSCs evoked with T stimuli varied between slices, some of which exhibited multiple peaks (not shown). Application of T strength stimuli-evoked EPSCs with peak amplitude values that ranged from 35.4 to 350.0 pA, and IPSCs that ranged from 37.6 to 601.0 pA. Averaged eEPSCs and eIPSCs from control (13 cells) and traumatized (11 cells) slices are shown in Fig. 1, A and B. At all stimulus intensities applied, there was no significant difference in the peak amplitude of evoked events evoked in intact slices (n = 25 cells (slices)) vs. traumatized slices (n = 18 cells (slices); P > 0.05; t-test; D). Input-output relationships of eIPSCs revealed that inhibitory events evoked with 2 T to 4 T stimuli were significantly smaller in traumatized slices (n = 18) vs. control (n = 25, *P < 0.05, **P < 0.01, applies to this and all subsequent figures). E: decline in evoked inhibition was reflected in the eEPSC/eIPSC ratio of peak amplitude, which was significantly higher in traumatized slices at all stimulus strengths applied (n = 18 cells (slices) for both intact and traumatized slices).
However, in both traumatized and control slices, the mean decay time (90–10%) of eEPSCs was prolonged as stimulus intensity was increased (Fig. 2, A1, B1, and C1), reaching a maximal value at stimulus strengths between 2 T and 4 T (Fig. 2E). The mean decay times of eEPSCs recruited with 2 T and 4 T stimuli in traumatized slices were significantly longer than those in control slices (Fig. 2E). However, despite this prolongation of eEPSC decay time, there was no significant difference in the total charge carried by eEPSCs recorded in traumatized slices versus those of control (Fig. 3A).

In traumatized slices sEPSC amplitude and frequency are increased, whereas sIPSC frequency is decreased

Spontaneous synaptic events were examined to further delineate the effects of acute cortical injury on synaptic function. sEPSCs recorded at −75 mV holding were not measurably affected by bath application of the N-methyl-D-aspartate (NMDA) receptor antagonist CPP but were completely and reversibly blocked by CNQX (n = 5 slices, Fig. 4A), confirming that these events were mediated by AMPA/kainate (KA) receptors. The average (Fig. 4D, •••) of 120 sEPSCs recorded from a control slice (Fig. 4C; randomly chosen from 618 consecutive events) had a peak amplitude of 12.4 ± 4.6 pA (mean ± SD, range: 3.4–70.0 pA). The mean decay phase was best fitted by a single-exponential function (r = 0.98, Fig. 4D, —), which yielded a time constant of 4.2 ms, confirming the absence of NMDA components at this holding potential (Edmonds et al. 1995). Isolated sIPSCs were recorded in the same cell by shifting the membrane holding potential to 0 mV and were reversibly blocked by bath-applied picrotoxin (PTX, 50 μM), confirming these events were GABAAergic (n = 4 slices, Fig. 4B). The average trace (Fig. 4F, •••) of 120 sIPSCs (Fig. 4E; randomly sampled from 772 consecutive events) had a mean amplitude of 19.9 ± 11.0 pA (mean ± SD, range: 3.5–90.0 pA). The mean decay phase was best fitted by a single-exponential function (r = 0.99, Fig. 4F, —) that provided a time constant of 9.9 ms. The fast rise and brief decay times confirmed that the sIPSCs were GABAergic (Ling and Benardo 1999).

To determine whether acute injury to the superficial cortex altered spontaneous synaptic responses, sEPSCs recorded from traumatized slices (n = 23 slices) were compared with control (n = 25 slices). sIPSCs recorded from traumatized (n = 19) and control (n = 22) slices were likewise compared. The number of cells sampled for sIPSCs was lower than that for sEPSCs due to deterioration of whole cell access in these slices before sIPSC recordings could be completed. Representative sEPSCs and sIPSCs from intact and traumatized slices are shown in Fig. 5, A and B (sEPSCs: A1 and B1; sIPSCs: A2 and B2). Both the mean peak amplitude and frequency of sEPSCs recorded in traumatized slices were higher than those in control slices (Fig. 5, C and D; average of events analyzed over 2- to 3-min epochs from each cell). The mean peak amplitude of
sEPSCs recorded in traumatized slices (13.0 ± 0.6 pA) was 17.1% higher than that of control (11.1 ± 0.9 pA; P < 0.05), whereas the mean frequency was 29.1% greater in traumatized slices vs. control (2.9 ± 0.7 Hz) versus control (6.9 ± 0.6 Hz; P < 0.05). In contrast, the mean peak amplitude of sIPSCs (Fig. 5C) recorded in traumatized slices (17.5 ± 1.1 pA) was not significantly different from control (17.3 ± 1.1 pA). However, the mean sIPSC frequency (Fig. 5D) in traumatized slices (5.9 ± 0.57 Hz) was 24.5% lower than in control (7.8 ± 0.7 Hz; P < 0.05). These differences in spontaneous synaptic activity were reflected in the sEPSC/sIPSC ratios of peak amplitude and frequency (Fig. 5E). The sEPSC/sIPSC ratio of peak amplitude was 29% higher in traumatized slices (0.8 ± 0.1) versus control (0.6 ± 0.1, P < 0.05), whereas the ratio of event frequency was increased 81.0% in traumatized slices (1.8 ± 0.2) relative to control (1.0 ± 0.1, P < 0.01). Thus acute injury to the superficial layers of neocortex produces an overall bias in spontaneous synaptic transmission toward excitation.

The alterations in the magnitude and frequency of spontaneous synaptic activity were confirmed by examinations of the cumulative distributions of sEPSC and sIPSC peak amplitude and interevent interval values (Fig. 6). Due to the slice-to-slice variability of event amplitude and frequency for both intact and traumatized slices, cumulative plots were constructed by sampling 150 consecutive sEPSCs and sIPSCs from each cell. Cumulative amplitude distribution plots for sEPSCs recorded in traumatized slices exhibited a rightward shift relative to control (Fig. 6A, P < 0.0001), indicating an increase in the proportion of large amplitude events. Amplitude distributions for sIPSCs in traumatized slices overlapped the distribution plots for events recorded in control (Fig. 6D, P < 0.05), confirming there was no significant difference in sIPSC am-
plitudes between traumatized and control slices. Examination of cumulative distribution plots for sEPSC and sIPSC total charge corroborated these findings, indicating an increase in the total charge carried by sEPSCs (Fig. 6B, P < 0.0001) but not by sIPSCs (Fig. 6E, P > 0.05) in traumatized slices. Distribution plots of interevent intervals showed that in traumatized slices, the sEPSC interevent interval distribution curve was shifted to the left (Fig. 6C, P < 0.0001), denoting an increase in sEPSC frequency, whereas the sIPSC inter-event interval curve was slightly shifted to the right (Fig. 6F, P < 0.001), indicating a decrease in sIPSC frequency.

Thus acute trauma to the superficial neocortical layers leads to a decrease in the frequency, but not amplitude, of spontaneous GABA_A-mediated IPSCs, which is consistent with a loss of inhibitory interneurons and, in turn, decline in synaptic GABA released. However, the increase in both sEPSC amplitude and frequency may reflect a dual enhancement of both presynaptic glutamate release and postsynaptic AMPAR-mediated responses.

Acute neocortical injury increases EPSC unit conductance but not the number of functional channels

To determine whether the potentiation of sEPSC magnitude was reflective of an increase in AMPAR unit conductance (\( \gamma \)) or number of active channels (\( N \), defined as the product of the number of physical channels per synapse and the channel open probability), peak-scaled NSFA of spontaneous synaptic events was used to obtain estimates of \( \gamma \) and \( N \) (De Koninck and Mody 1994; Traynelis et al. 1993). Peak-scaled NSFA, which examines the variance in fluctuations in the decay phase of synaptic events, is based on the assumption that the opening and closing of individual channels are independent events. Thus event-to-event fluctuations in synaptic currents will be smallest at the peak and end of the event’s trajectory (i.e., the time points when most channels are opened and closed, respectively) and largest in the intermediate decay phase, as determined by the single channel conductance and number of functional channels. Estimates of unit current and functional channel number can thus be derived from parabolic fits to plots of current amplitude versus variance (see METHODS) and used, in turn, to identify the specific receptor properties affected.

Estimates of \( \gamma \) and \( N \) were calculated from peak-scaled NSFA of sEPSCs recorded in both traumatized and control slices. Current versus variance plots were well fit by parabolic functions (Fig. 7A2, solid lines), yielding estimates for \( \gamma \) and \( N \) (summarized in Table 1). EPSC unit conductance was 23.6% higher in traumatized slices relative to control with a mean \( \gamma \) value of 26.7 ± 2.1 pS in traumatized slices versus 21.6 ± 1.4 pS for control (\( P < 0.05 \)). In contrast, there was no significant difference in \( N \) for sEPSCs recorded in traumatized slices (16.2 ± 2.2) versus control (13.2 ± 1.5, \( P > 0.05 \)). These findings suggest that acute injury to the superficial neocortex leads to an increase in AMPAR unit conductance within a relatively short time frame (i.e., hours). To check for possible contributions from dendritic filtering of events, plots of event rise time versus peak amplitude were routinely assessed and indicated no correlation between these two parameters (Fig. 7A3).
FIG. 7. Peak-scaled nonstationary fluctuation analysis (NSFA) of sEPSCs and sIPSCs. A1, top: representative traces of sEPSCs recorded from a control slice showing the average of 87 sEPSCs (thin line) peak-scaled to an individual sEPSC trace (thick line). Bottom: subtraction of individual traces from the average response was used to calculate the variance of individual traces about the mean sEPSC. A2: representative plot showing parabolic fit to the current vs. variance relationship for sEPSCs, which yielded estimates for the mean unit conductance (and the number of active channels N). A3: plots of sEPSC rise time (10–90%) vs. peak amplitude showed no correlation between these 2 parameters. B1: peak-scaled NSFA of sIPSCs recorded from a traumatized slice. B2: representative plot showing parabolic fit to the sIPSC current vs. variance relationship. B3: there was no correlation between sIPSC rise time and amplitude.

In contrast to excitatory events, IPSC unit conductance (Fig. 7B and Table 1) did not differ significantly between traumatized (γ: 13.2 ± 1.1 pS) and control slices (γ: 16.1 ± 2.1 pS; P > 0.05). Values for N were also statistically similar for sIPSCs recorded in injured (37.5 ± 3.8) and control slices (38.1 ± 4.5). These findings further support our proposal that the decrease in inhibition caused by acute injury to superficial cortex stems solely from a loss of inhibitory projections to target pyramidal cells with no changes to postsynaptic GABA_A receptor properties.

DISCUSSION

Previously, we have described the development of a novel in vitro model of cortical trauma to investigate the basic mechanisms of injury-induced excitotoxicity and epileptogenesis. This model serves to complement in vivo studies aimed at elucidating the basic mechanisms of cortical injury and epileptogenesis by allowing detailed examination of the pathophysiological changes to cortical circuit activity that are induced purely by mechanical injury to the superficial cortical laminae. Our previous studies have shown that superficial cortical injury quickly induces the development of epileptiform activity despite the absence of other complicating events, such as inflammation and hemorrhage. The resultant hyperexcitability is a consequence of disinhibition owing to a decrease in GABA_ergic inhibition and a glutamate-triggered increase in intracellular Ca^{2+}. The present study extends our previous findings by identifying the specific modifications to both excitatory and inhibitory synaptic transmission that arise acutely following cortical injury to support the development of posttraumatic seizures. We have found that injury to the superficial neocortex triggers distinct alterations in both evoked and spontaneous excitatory and inhibitory synaptic transmission, including: prolongation of eEPSC decay; decrease in eIPSC amplitude, decay time, and charge transfer; increase in sEPSC amplitude and frequency; decrease in sIPSC frequency; and increase in AMPAR unit conductance. Together these changes shift the excitation-inhibition balance toward a state favoring heightened excitation and the generation of epileptiform activity.

The decline in inhibition induced by acute cortical injury appears to derive primarily from the loss of inhibitory neurons located in the superficial layers (Chu et al. 2003; Salin and Prince 1996; Yang and Benardo 1997, 2002) as the decrease in evoked inhibition was most pronounced at high stimulus intensities with no differences observed in eIPSCs recruited with threshold stimuli relative to control slices. Low-intensity stimuli principally engage local networks proximal to the target pyramidal cells, whereas high-intensity stimuli also recruit inhibitory cells situated more distally, e.g., in the superficial laminae (Ling and Benardo 1999; Salin and Prince 1996). Thus the loss of the interneurons in the superficial layers would not significantly influence the magnitude of spontaneous and unitary inhibitory events in layer V pyramidal cells (Soltesz et al. 1995). When superficial and deeper laminae are separated, local inhibitory connections within the deep layers are unlikely to be affected. This was confirmed by Nissl staining of injured slices, which showed that neurons survived the lesioning procedure, including those proximal to the cut (Fig. 8, A and B). Damage to deep layer pyramidal neurons in lesioned slices was restricted primarily to the apical dendrites (Fig. 8, D–F) with pyramidal cell morphology otherwise identical to that in intact slices. However, alterations to the local circuits were likely substantial as illustrated by the circuit diagram summarizing the major neuronal connections within the neocortex (Fig. 8C) that include reciprocal excitation between pyramidal neurons both within and between cortical laminae and feed-forward and -back GABA_ergic inhibition to cells in all layers (Connors and Amitai 1993). The horizontal cut applied in upper layer III (Fig. 8C, dashed line) separates the neocortical circuit into superficial and deep segments. Compared with intact neocortex, the isolated deeper segment contains circuits in which the apical dendrites of most pyramidal neurons are truncated and the excitatory and inhibitory connections from the superficial layers severed. As such, these circuits presumably lose the inhibitory inputs from layer I and layer II/III GABA_ergic interneurons, e.g., Martinotti cells and double bouquet cells (Jones 1993; Thomson and Bannister 2003). In addition, layer IV/V intrinsic bursting neurons, which are believed to play a

| TABLE 1. Results of peak-scaled NSFA for spontaneous EPSCs and IPSCs |
|------------------|------------------|------------------|------------------|
|                  | Intact           | Traumatized      | Intact           | Traumatized      |
| **Unit γ, pS**   | 21.6 ± 1.4       | 26.7 ± 2.1*      | 16.1 ± 2.1       | 13.2 ± 1.1       |
| **Unit I, pA**   | 1.6 ± 0.2        | 2.0 ± 0.1*       | 1.2 ± 0.1        | 1.0 ± 0.1        |
| **Channel No.**  | 13.2 ± 1.5       | 16.2 ± 2.2       | 38.1 ± 4.5       | 37.5 ± 3.8       |
| **I_{max} (pA)** | 22.8 ± 1.9       | 27.1 ± 1.6       | 42.2 ± 4.6       | 33.7 ± 2.4       |
| **N**            | 16               | 11               | 14               | 13               |

Data were expressed as means ± SE; *P < 0.05, t-test compared with intact slices.
critical role in driving synchronized network activity (Chagnac-Amitai and Connors 1989), are also likely deprived of inputs from superficial circuits. Such an extensive loss of synaptic inhibition would undoubtedly lead to a decline in the recruitment of GABAergic drive in deep layer principal cells, leading to a state of hyperexcitability in these local networks. It is worth noting that in the 350-μm-thick slices used in this study, the basal dendrites of pyramidal cells were likely truncated to some degree, since these processes extend 500–600 μm (Salin and Prince 1996). However, this presumed, partial loss of the basal dendritic trees does not appear to destabilize the excitatory-inhibition balance as evidenced by the absence of hyperexcitability and epileptiform activity in control slices (Yang and Benardo 1997). In contrast, we previously showed that hyperexcitability develops in horizontal slice preparations of neocortex in which deep layer circuits (including basal dendritic trees) are well preserved but the superficial layers eliminated, further highlighting the importance of the superficial inhibitory circuits to the regulation of cortical activity (Yang and Benardo 1998).

However, direct modifications to postsynaptic GABA_\text{A} receptor function must also be considered. As shown in our previous studies, maneuvers aimed at increasing GABAergic responses or buffering intracellular Ca^{2+} prevented the development of epileptiform activity (Yang and Benardo 1997, 2000). The disinhibition that results from the loss of the superficial cortical layers could ultimately lead to an increase in intracellular Ca^{2+} in pyramidal cells that would, in turn, desensitize postsynaptic GABA_\text{A} receptors (Chen et al. 1990). However, GABA receptor desensitization would be expected to attenuate the magnitude of eEPSCs recruited with threshold stimulus intensities, which was not observed. Moreover, the decay times of eEPSCs recruited with lower stimulus intensities were not altered in traumatized slices, providing further evidence that GABA_\text{A} receptor desensitization was not affected.

The increase in sEPSC frequency and amplitude in traumatized slices may reflect a dual enhancement of both glutamate release and postsynaptic AMPAR function. Peak-scaled NSFA of sEPSCs, which yielded estimates of AMPAR unit conductance and channel number close to previously reported values (Benke et al. 1998; Otis et al. 1991; Trayanlis et al. 1993), indicated a significant increase in unit conductance in injured slices, but no change in the number of active AMPAR channels at individual synapses. Past studies have shown that AMPAR conductance can be rapidly modulated, such as by activity-induced long-term potentiation (LTP) of excitatory synaptic transmission (Benke et al. 1998) and the actions of protein kinases (Greengard et al. 1991; Ling et al. 2002; Wang et al. 1991). Acute brain injury is known to trigger increased levels of extracellular glutamate (Faden et al. 1989; Nilsson et al. 1994), which could, in turn, initiate plastic changes by engaging regulatory responses similar to those of LTP, such as the activation of NMDA receptors (Collingridge et al. 1983).

Mechanical injury to neocortical neurons may also increase AMPAR current by altering receptor desensitization (Goforth et al. 1994). Potentiation of AMPAR conductance could lead to the higher sEPSC frequencies measured in injured slices by raising the amplitude of events normally obscured in the baseline noise and thus increase the number of spontaneous events detected. The higher sEPSC frequencies observed in injured neocortex could also be a consequence of the disinhibition of cortical circuits releasing spontaneous excitatory activity. Although acute cortical injury enhanced the amplitude and frequency of sEPSCs, there was no difference in the magnitude of eEPSCs between injured slices and control. It is likely that excitatory afferents were also lost with the removal of the superficial cortical laminae, and such losses could offset injury-induced increases in AMPAR conductance.

Recent studies have shown that electrophysiological paroxysmal activity develops in vivo within a few hours after partial deafferentation of cortex via undercutting (Topolnik et al. 2003a,b), suggesting a rapid reorganization of local networks that could lead to seizure initiation. In another, well-established model of chronic brain injury involving neocortical undercutting (Hoffman et al. 1994; Li and Prince 2002; Prince and Tseng 1993; Salin et al. 1995), cortical slices exhibit both evoked and spontaneous epileptiform activity that may arise.
from alterations in synaptic transmission that include increased eEPSC amplitude, increased sEPSC frequency, and decreased sIPSC frequency (Li and Prince 2002). Thus the manifest changes in synaptic function observed with chronic cortical injury appear to parallel those that develop acutely. However, the underlying mechanisms may differ. The enhancement of synaptic excitation in chronic injury models is ascribed to increased glutamate release (Li et al. 2005) and axonal sprouting in pyramidal cells, leading to new excitatory AMPAR connections (McKinney et al. 1997; Salin et al. 1995). Alterations in intrinsic neuronal properties have also been identified that may additionally contribute to enhanced cortical excitability, including increased pyramidal cell input resistance, prolonged membrane time constant, and increased spike discharge frequency (Prince and Tseng 1993).

In contrast, our data indicated no change in glutamate release or AMPAR number with acute injury, but instead, an increase in AMPAR conductance. This difference may reflect distinct temporal phases of synaptic modification (i.e., acute vs. chronic) over the postinjury period, possibly similar to the early and late phases of activity-induced LTP (though on a significantly longer time scale). The early, induction phase of LTP is characterized by an increase in AMPAR unit conductance (Benke et al. 1998), whereas the later maintenance phase is supported by an increase in the number of active AMPARs (Ling et al. 2006). The increase in AMPAR conductance observed shortly after injury may represent a transient, early, induction phase of postinjury hyperexcitation that promotes subsequent increases in AMPAR number or glutamate release.

Morphological alterations, such as the axonal sprouting reported with chronic cortical injury, are not likely to be significant in the time span of our experiments (i.e., 2–8 h postlesion). However, the increase in synaptic excitation observed acutely could trigger trophic effects that modify cortical circuits over time by promoting the expression of neurotrophins, such as brain-derived neurotrophic factor and nerve growth factor (Gall 1992, 1993; Gall et al. 1991; Lauterborn et al. 2000). Although we did not observe any changes in the intrinsic properties of layer V pyramidal cells, past studies showed that cortical trauma increases the incidence of intrinsic bursting cells (Topolnik et al. 2003a), which are located in layer IV/V and would effectively increase synaptic excitation to local circuits, including those in layer V (Chagnac-Amitai and Connors 1989).

It is notable that hyperexcitability develops more rapidly in our acute injury model versus the chronic undercut model, and this may be due to physical differences in the type and nature of injury. Compared with undercutting the cortex, removal of the superficial layers may cause more extensive gray matter lesions, greater loss of apical dendrites, and, in turn, more severe excitotoxic responses (Choi 1992, 1995; Faden et al. 1989; Greengard et al. 1991; Lipton and Rosenberg 1994). It also represents a widespread removal of the interneuron-rich superficial layers (especially layer I) and, as such, would cause dramatic changes in the excitation-inhibition balance throughout the neocortical network.

Clinical correlates suggest a role for the anatomic reorganization after acute superficial cortical injury in setting the stage for later development of epilepsy. In the case of traumatic brain injury, it is noteworthy that in the largest epidemiologic study of factors predictive of posttraumatic epilepsy, injury severity markers of superficial cortical damage such as subdural hematoma, epidural hematoma, and depressed skull fracture independently predict increased rates of posttraumatic epilepsy, whereas those without clear superficial cortical injury, such as linear skull fracture and depressed Glasgow Coma Scale (<10), do not (Annegers et al. 1998). Although pathologic damage is more widespread and severe than radiologic lesions indicate (Polvoshock and Katz 2005) and injuries that cause more extensive damage, such as cortical contusions (Annegers et al. 1998) and penetrating head wounds (Salazar et al. 1985), are also epileptogenic, it is interesting to note that these lesions all possess a component of superficial cortical injury.

The findings of this study demonstrate that within a relatively short time frame, acute injury to the superficial neocortical layers produces specific alterations in both excitatory and inhibitory synaptic transmission that bias cortical circuits toward hyperexcitability. This could represent one component of the complex mechanisms involved in injury-induced epileptogenesis. Future work will be required to further characterize the precise nature, e.g., sequence and time course, of the acute changes in cortical function that arise from injury, which will aid in the development of rational and efficacious interventions in the clinical setting.

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