Partial Hippocampal Kindling Decreases Efficacy of Presynaptic GABA$_B$ Autoreceptors in CA1

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The effect of partial hippocampal kindling, a model of temporal lobe seizure, on monosynaptic inhibition mediated by GABA was studied. Kindled rats were given 15 nonconvulsive hippocampal afterdischarges, and control rats were given low frequency or no stimulations. At 1–2 d after kindling, paired-pulse depression (PPD) of the IPSCs recorded in CA1 neurons *in vitro* was significantly smaller in kindled as compared with control rats. The difference in PPD persisted for at least 21 d after kindling. The decrease in PPD of the IPSCs after partial hippocampal kindling was likely caused by a reduced GABA autoinhibition after downregulation of presynaptic GABA$_B$ receptors. The GABA$_B$ antagonist CGP35348 (1 mM) suppressed PPD of the IPSCs more strongly in control than in kindled rats.

Direct activation of the presynaptic GABA$_B$ receptors by baclofen suppressed the monosynaptic IPSCs significantly more in control than in kindled rats. The decay rate of a single-pulse IPSC was faster in kindled than in control rats on day 1 or day 21 after partial kindling. The difference in IPSC decay between kindled and control rats was found with or without a GABA$_B$ receptor antagonist. The low efficacy of the presynaptic GABA$_B$ receptors in kindled rats may provide compensatory stabilization of the postsynaptic membrane against further seizures or plasticity.

Key words: inhibitory postsynaptic current; presynaptic inhibition; GABA$_B$ receptors; paired-pulse depression; kindling; seizures; hippocampus

The modulation of inhibition mediated by GABA is important for normal brain functions (Krnjević, 1991). A local and transient loss of inhibition may mediate physiological plasticity and memory storage (Davies et al., 1991; Mott and Lewis, 1991; Wilson and McNaughton, 1993; Buzsaki et al., 1994). GABAergic interneurons are subjected to various local and extrinsic control (Freund and Buzsaki, 1996). In addition, GABA release is controlled by autoinhibition via presynaptic GABA$_B$ receptors (Deisz and Prince, 1989; Thompson and Gahwiler, 1989; Davies et al., 1990, 1991; Nathan and Lambert, 1991; Silvilotti and Nistri, 1991; Bowery, 1993; Lambert and Wilson, 1994; Olpe et al., 1994; Pitler and Alger, 1994; Misgeld et al., 1995; Kaupmann et al., 1997). However, it is not known whether the presynaptic GABA$_B$ receptors are themselves regulated by neuronal activity.

GABA neurotransmission has long been associated with seizure activity. GABA$_A$ antagonists induce seizures (Gale, 1992; Schwartzkroin, 1993), and drugs that enhance GABA neurotransmission are effective anticonvulsants (Macdonald and McLean, 1986). Acute seizures suppress GABA function (Stelzer et al., 1987; Kapur et al., 1989). However, a decrease of GABA function in chronic epilepsy is still controversial (Babb et al., 1989; Engel, 1995).

In the kindling model of epilepsy, repeated stimulations are delivered to the brain that progressively evoke longer afterdischarges (ADs) and more severe convulsions (Goddard et al., 1969; McNamara et al., 1993). Full kindling refers to stimulations that evoked generalized tonic–clonic convulsions, whereas partial kindling evoked no convulsions. Partial hippocampal kindling is a good model of human temporal lobe seizures, which may not result in convulsions. Furthermore, the AD evoked by partial hippocampal kindling was mainly restricted to structures closely connected to the hippocampus (Leung et al., 1997), and no cell loss was detected (Cavazos et al., 1994). After hippocampal or amygdala kindling, paired-pulse inhibition of population spikes was increased in the dentate gyrus (Tuff et al., 1983; Oliver and Miller, 1985a; Zhao and Leung, 1992) but decreased in CA1 (Kamphuis et al., 1988; Zhao and Leung, 1991). The different paired-pulse response in the dentate gyrus and CA1 after kindling is consistent with the change in GABA$_A$ receptor binding (Titulaer et al., 1994, 1995), but not with the change in GABA immunoreactivity (Kamphuis et al., 1989) or GABA release (Kamphuis et al., 1991). In addition, EPSPs showed an increase in paired-pulse facilitation after kindling (Zhao and Leung, 1991, 1993).

The purpose of this study was to characterize the paired-pulse monosynaptic inhibitory response. At the time we began our study, the effect of kindling on monosynaptic inhibition and GABA autoinhibition was not known. Recently, Buhl et al. (1996) found a decrease in paired-pulse depression (PPD) of the IPSCs in dentate gyrus granule cells after full kindling of the perforant path. This study documents a similar finding in CA1 neurons after partial hippocampal kindling. In addition, we show that the kindling-induced decrease in PPD of the IPSCs was specifically mediated by a downregulation of presynaptic GABA$_B$ autoreceptors and lasted 3 weeks after partial kindling.

A brief summary of the work has been published previously (Wu and Leung, 1996; Leung et al., 1997).

MATERIALS AND METHODS

The kindling procedures and the *in vitro* slice preparation were reported previously (Leung et al., 1994). Briefly, repeated electrical stimulations were delivered through chronic, indwelling electrodes implanted in stratum radiatum of hippocampal CA1 of male Long–Evans rats. Experimental rats were given the kindling stimulus (1 sec, 100 Hz, pulses of 0.1 msec). About half of the control rats were given low-frequency stimulations (LFSs), each consisting of 100 pulses at 0.167 Hz, and the others were implanted with electrodes but not given any stimulations. There was no significant difference between the responses of the two types of control rats, and their data were combined into a single control group. ADs/LFSs were given hourly, 5 per day, for a total of 15 ADs/LFSs in 3 d.

On day 1–2 (day 1 group) or day 21–23 (day 21 group) after the last kindling or control treatment, the rat was anesthetized with halothane and decapitated. Most kindled and control rats were paired, and the experiments were done on consecutive days, with the experimenter unaware of the stimulation history of each rat. The hippocampus on the stimulated side was dissected out and 400-μm-thick transverse slices were obtained from the midseptotemporal level (Leung et al., 1994). The slices were placed in an interface chamber and perfused with artificial cerebrospinal fluid at 32 $\pm$ 1°C of the following composition (in mM): NaCl 124, KCl 5, NaH$_2$PO$_4$.H$_2$O 1.25, MgSO$_4$.7 H$_2$O 2, CaCl$_2$.6 H$_2$O 2, NaHCO$_3$ 26, and glucose 10. A concentric bipolar stimulating electrode was placed in stratum radiatum in CA1 (on the CA3 side) ~0.3 mm from the recording site. Sharp micropipettes (3 M potassium acetate; impedance 60–100 MΩ) were used to impale CA1 neurons in the pyramidal cell layer. QX-314 (167 mM) was added to the potassium acetate in the micropipette to block fast Na$^+$ channels (Connors and Prince, 1982; Leung and Yim, 1991) and GABA$_B$ responses (Nathan et al., 1990; Andrade, 1991; Otis and Mody, 1992). To isolate the IPSC without excitation, an NMDA antagonist D-2-amino-5-phosphonopentanoic acid (D-AP5; 20 μM) and a non-NMDA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX; 20 μM) were added to the perfusate. Single-electrode voltage clamp was made by an Axoclamp 2A amplifier at 4.5–5.0 kHz switching frequency, and the voltage before the sample-and-hold device was monitored to ensure that the transients had decayed before being sampled. Because inhibitory synapses were predominantly located near the cell body, lack of space clamp (Rall and Segev, 1985) was not a significant problem. Paired-pulse stimulations were given at 0.12 Hz, 2 or 4 $\times$ threshold intensity (0.2 msec duration), and an interpulse interval (IPI) of 50–500 msec, with the cell clamped at a potential of −40 to −120 mV. The threshold intensity was determined as the lowest intensity that evoked a detectable field response in each slice before the perfusion of glutamate antagonists, and typically only one (occasionally two) neuron was recorded per slice. The potentials were amplified, digitized at 10 KHz, averaged ($n = 4$), and stored on line by a custom program. In some experiments, GABA$_B$ receptor antagonist CGP35348 (1 mM) or GABA$_B$ receptor agonist baclofen (1–10 μM) was added to the medium containing CNQX and D-AP5.

In voltage clamp, PPD was evaluated by the ratio of the peak of the IPSC evoked by the second pulse divided by that evoked by the first pulse. All IPSC measures were evaluated at −50 mV holding potential if not otherwise specified. In current clamp, PPD was evaluated by the ratio of the peak IPSP evoked by the second pulse to that evoked by the first pulse. The reversal potential of the IPSC was estimated by a linear interpolation of the two IPSCs (recorded every 10 mV of the holding potential) yielding positive and negative responses, respectively. The latter reversal potential was within a few millivolts of the estimate given by linear regression analysis of the IPSC versus the holding potential (range −50 to −100 mV).

Statistical differences between measures in different groups were tested by a nonparametric Wilcoxon test, using either cells or rats as the basic unit. For "per cell" statistics, all of the cells studied were included; however, to minimize bias from a single rat, no more than four cells were selected from the same rat. For the "per rat" statistics, the average measure for all of the cells recorded for one rat ($n = 1$–6 cells) was used, and the sample number was the number of rats. In experiments in which repeated time measures were made, e.g., after drug perfusion, a repeated-measure ANOVA was applied, followed by *post hoc* t tests.

The half-peak durations of the IPSC1 [$t_{0.5}(1)$] and IPSC2 [$t_{0.5}(2)$] was estimated as the time interval from the onset of an IPSC (~2.4 msec after a stimulus) to the time the IPSC decayed to half the peak value. The falling phase of the IPSC was fitted by a single exponential IPSC(t) $= I_o \, e^{-t/\tau}$, with initial amplitude I_o and time constant τ. The time constants of the first- and second-pulse-evoked IPSC were named $\tau1$ and

$\tau2$ respectively; the values reported here were derived from regression yielding $R^2 > 0.85$. In most neurons, the single exponential decay was able to fit the IPSC well for a duration of 80 msec after the IPSC peak, and reducing the fitted duration did not substantially change τ. In some neurons (~15%), the τ value was estimated using a IPSC duration of 50–80 msec, if the shorter duration improved the fit (as estimated by R^2).

A nonparametric technique was also used to compare IPSC durations between ensemble of neurons recorded in different conditions. The mean IPSC of each recorded neuron was normalized by the first IPSC peak, and then the normalized IPSCs from different neurons were averaged at fixed times (0.1 msec bins for 250 msec duration). The ensemble SEM at each bin was also calculated. Because the IPSCs from different neurons were triggered at a fixed time by a digital pulse generator (Master 8, A.M.P.I., Jerusalem), their stimulation pulses were precisely lined up in time. When the ensemble averages of two conditions were compared, the lack of overlap between the SEMs of the two ensembles (compare Fig. 2) suggests a statistical difference between the ensembles, which was more precisely evaluated by a repeated-measure ANOVA followed by a *post hoc* t test, using samples at every 5–10 msec intervals.

RESULTS

Kindling and CA1 cell characteristics

The duration of the hippocampal AD, recorded in the homotopic CA1 contralateral to the stimulated CA1, increased with the number of ADs delivered, similar to previous results (Leung et al., 1994, their Fig. 1). In the group of nine kindled rats used for day 1 recordings (data in Table 1), the first AD measured 31.5 $\pm$ 4.2 sec (mean $\pm$ SEM) and the fifteenth (last) was 93.7 $\pm$ 17.9 sec. The increase in AD duration from the first to the last AD was statistically significant. Similar results were obtained in other kindled rats. No behavioral convulsion was observed during the ADs.

The resting membrane potential from cells recorded at the CA1 cell layer was −58.3 $\pm$ 0.7 mV (mean $\pm$ SEM; $n = 138$); resting membrane potential was not different between kindled and control groups. The input resistance was 61.5 $\pm$ 5.5 MΩ ($n = 22$). With QX-314 in the recording micropipette, slow, presumed Ca^{2+} spikes of >10 msec duration could be evoked if a neuron was depolarized to approximately −45 mV (Leung and Yim, 1991).

PPD of the IPSCs 1 d after partial hippocampal kindling

Monosynaptic IPSCs/IPSPs were obtained by direct stimulation of the stratum radiatum in the presence of CNQX and D-AP5 (see Materials and Methods). The recorded IPSC was mediated by GABA$_A$ receptors, because it was abolished by GABA$_A$ antagonist bicuculline (not shown). No postsynaptic GABA$_B$ current was detected, and the amplitude of the single-pulse IPSCs was not decreased by GABA$_B$ antagonist CGP35348 (Fig. 1*A*). In neurons from control rats recorded after paired-pulse stimulation, the amplitude and duration of the IPSC after the second pulse (IPSC2) was smaller than the respective measure of the first-pulse IPSC (IPSC1) (Fig. 1 *A*), as reported previously (McCarren and Alger, 1985; Davies et al., 1990; Lambert and Wilson, 1994; Olpe et al., 1994). In control neurons, the PPD ratio (peak IPSC2/peak IPSC1) was 0.63 $\pm$ 0.04 ($n = 16$) at an IPI of 100 msec (holding potential of −50 mV and using 2 $\times$ threshold intensity stimuli, i.e., 50 $\pm$ 1 μA, 0.2 msec duration pulses). In neurons from kindled rats, IPSC2 was strikingly similar in size and duration to IPSC1 (Fig. 1*A*). PPD was significantly smaller ($p < 0.01$) in kindled than in control rats at an IPI of 50, 100, and 150 msec but not 500 msec (Fig. 1*B*; Table 1). There was no difference in the stimulus intensities used to evoke the IPSCs in the kindled and control groups. The latency to reach the IPSC1 or IPSC2 peak did not differ between control and kindled rats (12 $\pm$ 1 msec). Also, the peak amplitudes of the monosynaptic

Table 1. Measures of monosynaptic IPSCs on day 1 after partial kindling or control procedures

	2 × threshold		4 × threshold	
	Kindled (21)	Control (16)	Kindled (11)	Control (13)
Peak IPSC1 (nA)	0.8 ± 0.07	0.98 ± 0.18	0.94 ± 0.13	1.08 ± 0.21
Peak IPSC2 (nA)	0.68 ± 0.07	0.68 ± 0.16	0.81 ± 0.12	0.75 ± 0.18
Paired-pulse depression	0.85 ± 0.03***	0.63 ± 0.04	0.85 ± 0.03**	0.65 ± 0.05
Half-duration IPSC1 $t_{0.5}(1)$ (msec)	46.1 ± 1.7**	57.7 ± 3.2	31.8 ± 2.1**	45.5 ± 3.2
Half-duration IPSC2 $t_{0.5}(2)$ (msec)	42.6 ± 1.6	42.3 ± 2.0	27.7 ± 1.7	30.4 ± 2.2
Time constant IPSC1 $\tau1$ (msec)	35.2 ± 3.3*	49.9 ± 5.4 (15)		
Time constant IPSC2 $\tau2$ (msec)	39.9 ± 5.3	44.8 ± 5.9 (15)		

IPSCs were recorded at −50 mV holding potential, after paired-pulse stimulation (100 msec interpulse interval) of stratum radiatum in a medium with 20 μM CNQX and 20 μM D-AP5, at either 2 × or 4 × threshold stimulus intensity. Paired-pulse depression was estimated by the ratio (peak IPSC2/peak IPSC1).

*** $p < 0.001$; ** $p < 0.01$; * $p < 0.05$ kindled different from control group for the same stimulus intensity.

IPSCs after single-pulse radiatum stimulation were not significantly different ($p > 0.3$; Wilcoxon) between the two groups (Table 1).

If the stimulus intensity was doubled from 2 to 4 × threshold, IPSCs increased slightly, but the PPD ratio was not significantly changed (Table 1). The difference between the kindled and control groups was also significant ($p < 0.01$; Wilcoxon) at 4 × threshold intensity (Table 1).

The time course of the IPSCs appeared to be different in kindled as compared with control rats (Fig. 1A). The duration of the IPSC1 [$t_{0.5}(1)$] was significantly longer in control than in kindled rats (Table 1). Time constant $\tau1$ was estimated to be ~50 msec in the control group, a value similar to that estimated by Roepstorff and Lambert (1994) or the slower time constant of Pearce (1993), and significantly larger ($p < 0.05$) than that in the kindled group (Table 1). The ensemble averages (normalized by the peak IPSC1 of each neuron) show that the average IPSC1 in the kindled group decayed much more rapidly than that of the control group (Fig. 2A). The ensemble averages of IPSC1 were significantly different from each other at 30–100 msec after the pulse ($p < 0.05$; post hoc t test after significant group, time, and group × time effects with repeated-measure ANOVA). Other than the difference near the peak of the IPSC2 (10–30 msec after the second pulse), the late time course of the IPSC2 was not different between the ensemble averages of the kindled and control groups ($p > 0.05$; post hoc t test) (Fig. 2A).

The differences between kindled and control groups were robust and were shown using either per cell or per rat statistics (see Materials and Methods). Per rat statistics also revealed significant differences between kindled ($n = 9$) and control rats ($n = 9$) in the PPD, $t_{0.5}(1)$, and $\tau1$ measures.

Current-clamp recordings on day 1 after kindling–control procedures

Some neurons were also recorded in a discontinuous current-clamp mode, and the results were congruent with the voltage clamp data. Radiatum stimulation at a prepulse membrane potential of −50 mV (maintained by current injection) yielded a ratio of IPSP peaks that was significantly larger ($p < 0.01$) in the kindled than in the control group. Similar results were found at 2 and 4 × threshold stimulus intensity (Table 2).

IPSCs/IPSPs on day 21 after kindling

At 21 d after kindling, neurons from kindled rats also showed significantly smaller PPD than did neurons from control rats ($p < 0.01$; Fig. 2B). At 100 msec IPI, the PPD ratio was 0.77 ± 0.05 ($n = 18$) in neurons of kindled rats as compared with 0.55 ± 0.06 ($n = 11$) in neurons of control rats (Table 3). Interestingly, IPSC1 amplitudes were similar among the day 21 neurons, and mean IPSC2 amplitude was larger in the kindled than the control group (Table 3). However, at 500 msec IPI, no significant difference in PPD was found between kindled and control groups (Table 3).

The difference in IPSC decay between kindled and control rats also persisted 21 d after partial hippocampal kindling (Fig. 2B). The measures $t_{0.5}(1)$, $\tau1$, and $\tau2$ were significantly smaller in the kindled than the control group (Table 3). Ensemble averages of the IPSCs (Fig. 2B) revealed that the decay time course of IPSC1 or IPSC2 was faster in the kindled than the control group at >20 msec latency ($p < 0.01$; post hoc t test after repeated-measure ANOVA, which gave significant group, time, and group × time effects).

When statistics per rat were performed, the day 21 kindled rats ($n = 6$) also showed significantly smaller $t_{0.5}(1)$, $\tau1$ ($p < 0.05$; Wilcoxon) than a pooled control group consisting of five day 21 control rats and nine day 1 controls. The control rats were pooled because there was no difference in any measure between the day 21 and day 1 control rats. PPD was also significantly different ($p < 0.05$) in day 21 kindled rats than in the pooled control rats.

IPSC reversal potentials and holding potentials

The reversal potentials of IPSC1 (RP1) were not significantly different between cells from day 1 kindled ($n = 8$ cells) and control groups ($n = 13$ cells) or between day 21 kindled ($n = 15$) and control groups ($n = 12$). Similarly, the reversal potential of IPSC2 (RP2) was not different between kindled and control groups, on either day 1 or day 21. However, when RP2 was compared with RP1 in the same neuron (Fig. 3), RP2 was more positive than RP1 by 4.3 ± 1.1 mV ($n = 13$) in control day 1 neurons and by 2.2 ± 0.6 mV in kindled day 1 neurons ($n = 8$). In the day 1 groups, RP1 and RP2 were 82.3 ± 7.1 mV and 78.3 ± 6.8 mV ($n = 13$), respectively, in the control group, as compared with 84.0 ± 3.3 mV and 82.9 ± 3.3 mV ($n = 8$) in the kindled
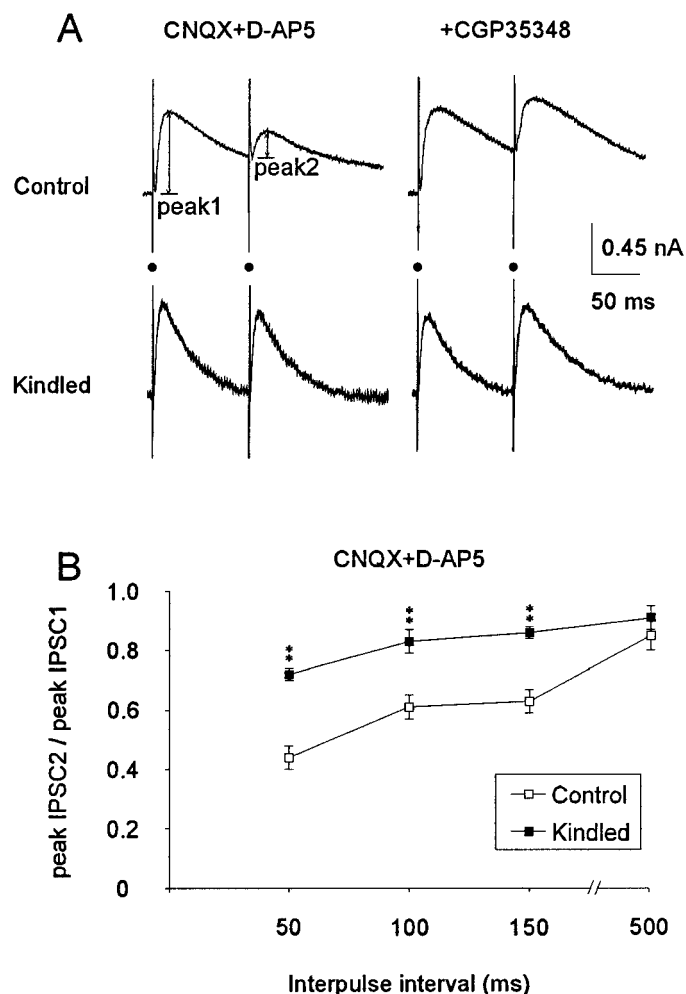
Figure 1. Kindled seizures reduced PPD of GABA$_A$ mediated IPSCs recorded at a holding potential of −50 mV. *A*, Examples of paired-pulse IPSCs in CA1 neurons from control and kindled rat, evoked by stratum radiatum stimuli of 2 × threshold intensity in the presence of CNQX and D-AP5, before and after perfusion with a CGP35348 (1 mM) medium. Peaks of IPSC1 and IPSC2 indicated by *peak1* and *peak2*, respectively. Resting membrane potential of control and "kindled" neuron was −59 and −60 mV, respectively. *Filled circles* indicate shock artifacts. *B*, Plot of the ratio of the peak IPSC evoked by the second pulse (*peak IPSC2*) to that evoked by the first pulse (*peak IPSC1*), in the absence of CGP35348, as a function of interpulse interval. Error bars are one SEM. Control group (C) = 8 neurons and kindled group (K) = 10 neurons, except for 500 msec interpulse interval data, where C = 6 and K = 4 neurons. ** (*p* < 0.01) indicates significant difference between control and kindled groups of neurons as assessed by the nonparametric Wilcoxon test.

group. The slight but significant (paired Wilcoxon; *p* < 0.01) depolarizing shift of RP2 with respect to RP1 was the main reason that PPD appeared to be weaker or absent at holding potentials of −80 to −110 mV (Fig. 3*A*). When the chord conductance of IPSC1 (*GI1*) and IPSC2 (*GI2*) was estimated at each holding potential [by peak IPSC/(holding minus reversal potential)], the paired conductance ratio (*GI2/GI1*) was similar across holding potentials for a particular group of neurons. The conductance ratio (*GI2/GI1*) was significantly different (*p* < 0.05) between day 1 kindled and control neurons at all holding potentials used (−50 to −120 mV). At −50 mV holding potential, the conductance ratio was ~15% higher than peak IPSC ratio, but the difference between day 1 kindled and control groups was
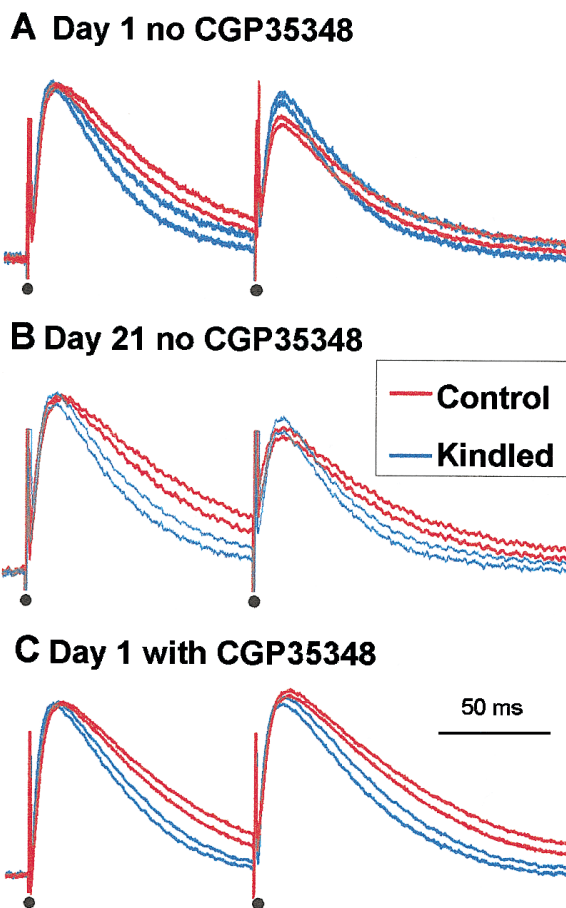

Figure 2. Ensemble averages of IPSCs from neurons recorded in CNQX and D-AP5 medium. For each pair of traces, the *top trace* is the ensemble mean + 1 SEM and the *bottom trace* is the ensemble mean − 1 SEM. The kindled group is *blue*, and the control group is *red*. Day 1 (*A*) and day 21 (*B*) averages of kindled and control groups, without GABA$_B$ antagonist CGP35348. *C*, Day 1 groups recorded with CGP35348. All IPSCs were recorded using 2 × threshold stimulus intensity, 100 msec interpulse interval, and −50 mV holding potential. For each neuron, the recorded trace was normalized by the first-pulse IPSC peak, and then all neurons in the same group were combined in the ensemble average (see Materials and Methods). Number of neurons in kindled (K) and control (C) groups: *A*, C = 14, K = 16; *B*, C = 11, K = 18; *C*, C = 17, K = 17. Note faster decay of the first-pulse IPSC in the kindled as compared with control group under all conditions.

similarly robust (*p* < 0.001) using either ratio of conductances or ratio of peak currents.

Effect of GABA$_B$ antagonist on paired IPSCs

To investigate the role of presynaptic GABA$_B$ receptors in the PPD of IPSCs, GABA$_B$ receptor antagonist CGP35348 (1 mM) was added to the medium with CNQX and D-AP5. Recordings were made 1 d after kindling and control procedures. In a medium with CGP35348, the mean PPD at 100 msec IPI was slightly smaller in the kindled as compared with the control group (Fig. 2*C*; Table 4), but the difference between groups was not statistically significant (*p* = 0.09).

The faster IPSC decay in the kindled than in the control group of neurons persisted in a medium with CGP35348. In the latter medium, $t_{0.5}(1)$ and $\tau 1$ were significantly different between kindled and control groups (Table 4), and in addition, a significant difference was found for $\tau 2$ (Table 4). Significant differences (*p* ≤

Table 2. Ratio of the IPSP peaks evoked by paired pulses at 100 msec interpulse interval, recorded in discontinuous current clamp mode at −50 mV membrane potential on day 1 or 21 after kindling/control procedures. Stimulation was at either 2 × or 4 × threshold stimulus intensity

	2 × threshold		4 × threshold	
	Kindled	Control	Kindled	Control
Day 1	0.84 ± 0.04 (16)*	0.67 ± 0.05 (16)	0.82 ± 0.03 (11)*	0.61 ± 0.05 (15)
Day 21	0.70 ± 0.05 (13)	0.53 ± 0.11 (5)		

* $p < 0.01$, difference between kindled and control groups.

Table 3. Measures of IPSCs recorded on day 21 after kindling/control procedures at −50 mV holding potential, in a medium with 20 μM CNQX and 20 μM D-AP5

	100 msec IPI		500 msec IPI	
	Kindled (18)	Control (11)	Kindled (14)	Control (6)
IPSC1 peak (nA)	0.72 ± 0.07	0.69 ± 0.07	0.65 ± 0.08	0.72 ± 0.10
IPSC2 peak (nA)	0.51 ± 0.04*	0.37 ± 0.05	0.57 ± 0.06	0.60 ± 0.09
Paired-pulse depression	0.77 ± 0.05**	0.55 ± 0.06	0.90 ± 0.02	0.85 ± 0.05
Half-duration IPSC1 $t_{0.5}(1)$ (msec)	43.8 ± 3.3**	62.0 ± 4.8	44.1 ± 3.2	66.2 ± 7.6
Half-duration IPSC2 $t_{0.5}(2)$ (msec)	32.9 ± 1.3	36.3 ± 1.1	37.8 ± 2.2	50.9 ± 3.1
Time constant IPSC1 $\tau 1$ (msec)	38.1 ± 4.6**	64.0 ± 7.2		
Time constant IPSC2 $\tau 2$ (msec)	37.2 ± 4.2*	60.2 ± 7.5		

Stimulation was at 2 × threshold stimulus intensity and either 100 or 500 msec interpulse interval (IPI).

** $p < 0.01$; * $p < 0.05$, kindled different from control groups for the same IPI.

0.05; Wilcoxon) in $t_{0.5}(1)$, $\tau 1$, and $\tau 2$ between kindled ($n = 6$ rats) and control ($n = 8$ rats) groups were also found if per rat statistics were used.

The ensemble averages (Fig. 2C) reveal the differences graphically. The IPSC1 ensemble averages of control and kindled groups were statistically different at 20–100 msec after the first pulse ($p < 0.01$; *post hoc t* test, after repeated-measure ANOVA revealed significant group, time, and group × time effects). In a medium with CGP35348, IPSC2 ensemble averages were also different between control and kindled groups at 20–140 msec after the second pulse ($p < 0.01$; *post hoc t* test after repeated-measure ANOVA yielding significant group, time, and group × time effects).

Single neurons were assessed before, during, and after washout of 1 mM CGP35348 (Fig. 1A). In the day 1 control group, CGP35348 decreased PPD and increased IPSC2 duration in all five neurons (Fig. 1A; $p < 0.05$; paired Wilcoxon). In the same group, CGP35348 had no consistent effect on IPSC amplitudes, but it increased $\tau 1$ or $t_{0.5}(1)$ in four of five neurons (Fig. 1A; $0.05 < p < 0.1$). In eight neurons from the day 1 kindled group, CGP35348 decreased PPD significantly ($p < 0.05$; Fig. 1A), although the PPD decrease was smaller than that in the control group. This suggests that the GABA$_B$ antagonist was still effective in blocking autoinhibition in the kindled group, but its effect was reduced as compared with the control group. CGP35348 increased $t_{0.5}(1)$ and $\tau 1$ significantly ($p < 0.05$) in the day 1 kindled group of neurons.

Effects of GABA$_B$ agonist baclofen on paired IPSCs

A decrease in GABA release and an increase in uptake may limit the activation of the presynaptic GABA$_B$ receptors (Isaacson et al., 1993; Roepstorff and Lambert, 1994). Thus, the GABA$_B$ agonist baclofen (10 μM in the bath) was used to directly activate the presynaptic GABA$_B$ receptors (Davies et al., 1990; Thompson and Gahwiler, 1992; Misgeld et al., 1995). Baclofen reduced IPSC1 in neurons from both control and kindled rats, but the reduction was significantly stronger in control than in kindled rats (Fig. 4A). The effect of baclofen was detected at 5 min after perfusion and was near maximal by 10 min (Fig. 4B). At 15 min after baclofen, the peak IPSC1 of the control group was 23 ± 3% of the baseline (seven neurons), whereas that of the kindled group was 44 ± 5% (nine neurons) (Fig. 4B). The effect of baclofen was predominantly on presynaptic inhibition because QX-314 in the micropipette already blocked the postsynaptic GABA$_B$ conductance (Nathan et al., 1990; Andrade, 1991).

Before the perfusion of baclofen, PPD was 0.61 ± 0.04 ($n = 7$) in control neurons and 0.83 ± 0.01 ($n = 9$) in "kindled" neurons. The difference in PPD between kindled and control neurons was significant ($p < 0.01$), confirming the result of the earlier experiment (Table 1). Baclofen decreased PPD in control neurons significantly to 0.92 ± 0.06 ($n = 7$; repeated-measure ANOVA; $p < 0.01$), consistent with the previous literature (Davies et al., 1990; Mott and Lewis, 1991). In contrast, baclofen did not significantly change PPD in the kindled rats (Fig. 4C).

DISCUSSION

PPD of IPSCs in CA1

The data on PPD in CA1 neurons in the control rats were in accordance with those reported previously (Davies et al., 1990; Davies and Collingridge, 1993). A notable exception is the finding that the reversal potential of IPSC2 (RP2) was significantly more
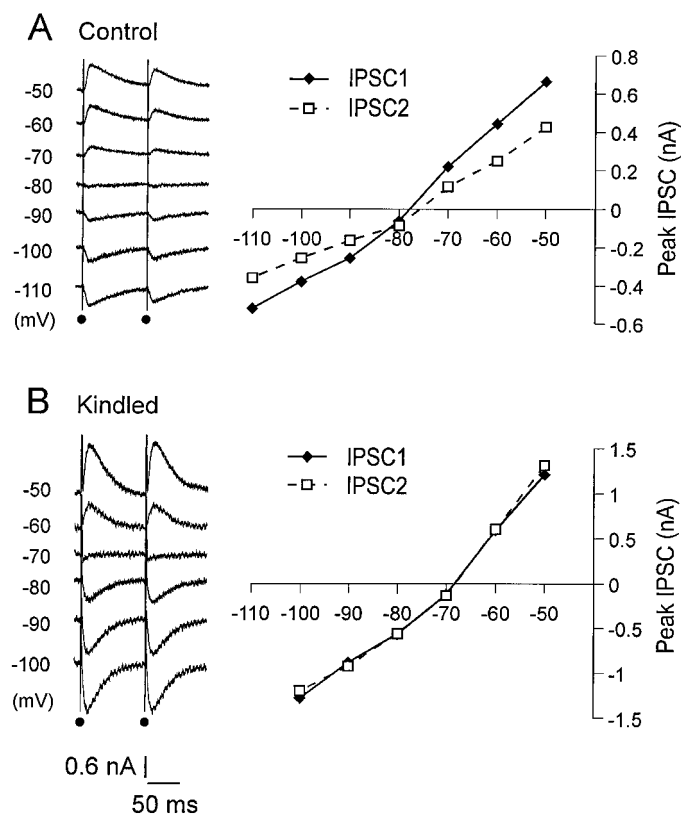
Figure 3. Average IPSC (*n* = 4 sweeps) versus voltage relation, in a medium with CNQX and D-AP5 but without CGP35348, at −50 to −110 mV holding potentials for a neuron from (*A*) day 1 control and (*B*) day 1 kindled rat. Outward rectification was seen in both neurons. A depolarizing shift of the reversal potential for IPSC2 as compared with IPSC1 is shown in *A*. At all holding potentials, PPD of the IPSCs was observed in *A* but not in *B*.

depolarized than that of IPSC1 (RP1) by ~4 mV, similar to the results of McCarren and Alger (1985). Davies et al. (1990) stated that PPD "was not associated with any change in reversal potential of the IPSC," but the resolution of their measures was unclear (see their Fig. 4). At −50 mV holding potential, the reversal potential shift contributed ~15% to the PPD, and this relatively

small contribution did not change the main results of this study or those of the previous studies (Davies et al., 1990; Davies and Collingridge, 1993). The shift in reversal potential may indicate a change in intracellular Cl^- concentration (McCarren and Alger, 1985; Thompson and Gahwiler, 1989).

Downregulation of presynaptic GABA$_B$ autoreceptors

As shown previously by Davies et al. (1990), PPD of the IPSC peak amplitudes is mainly a measure of presynaptic inhibition of GABA release. The larger ratio of paired-pulse IPSC amplitudes (and durations) in kindled as compared with control rats, at day 1 or day 21 after partial hippocampal kindling, suggests that GABA autoinhibition was weaker in kindled than in control rats. We infer that the weaker autoinhibition was mediated by a decreased efficacy or downregulation of the presynaptic GABA$_B$ receptors on GABA terminals. The PPD of IPSCs in control rats was strongly suppressed by GABA$_B$ antagonist CGP35348 or GABA$_B$ agonist baclofen (by occlusion), but the PPD of IPSCs in kindled rats was relatively insensitive to GABA$_B$ agonist or antagonist. Exogenously applied GABA$_B$ agonist baclofen suppressed the first-pulse IPSC in control rats much more than that in kindled rat, confirming that presynaptic GABA$_B$ receptor efficacy was reduced in kindled as compared with control rats.

The decrease in presynaptic GABA$_B$ receptor efficacy may be caused by a downregulation of the number of GABA$_B$ receptors or a decrease in the coupling of GABA$_B$ receptors to guanine-nucleotide-binding proteins (Pitler and Alger, 1994; Misgeld et al., 1995; Kaupmann et al., 1997) or to presynaptic Ca^{2+} currents (Pfrieger et al., 1994; Wu and Saggau, 1997). In the presence of a GABA$_B$ antagonist, no significant difference in PPD was detected between kindled and control groups, which does not suggest a role for non-GABA$_B$ mediated presynaptic inhibition (Lambert and Wilson, 1993; Wilcox and Dichter, 1994).

Effects of GABA$_B$ antagonist and kindling on the IPSC time course

The decay rate of IPSC1 was slowed by GABA$_B$ antagonist CGP35348, whereas the peak amplitude was not consistently changed. CGP35348 has little affinity for the GABA$_A$ receptor (Olpe et al., 1990), and suppression of residual postsynaptic GABA$_B$ currents does not account for the CGP35348 effect, because it should decrease rather than increase the late time

Table 4. IPSC measures recorded in a CGP35348 (1 mM) medium with CNQX and D-AP5, 1 day after kindling/control procedures, using 100 msec interpulse interval and −50 mV holding potential, at 2 × or 4 × threshold stimulus intensity

	2 × threshold		4 × threshold	
	Kindled (17)	Control (17)	Kindled (16)	Control (15)
Peak IPSC1 (nA)	0.99 ± 0.09	0.92 ± 0.10	1.00 ± 0.07	0.89 ± 0.10
Peak IPSC2 (nA)	0.94 ± 0.08	0.76 ± 0.08	0.93 ± 0.07	0.75 ± 0.09
Paired-pulse depression	0.96 ± 0.02	0.89 ± 0.04	0.92 ± 0.03	0.86 ± 0.03
Half-duration IPSC1 $t_{0.5}(1)$ (msec)	45.0 ± 3.3*	58.7 ± 3.5	49.7 ± 3.4	56.2 ± 2.6
Half-duration IPSC2 $t_{0.5}(2)$ (msec)	49.1 ± 3.4	55.6 ± 1.5	51.7 ± 2.2	54.7 ± 2.0
Time constant IPSC1 $\tau 1$ (msec)	30.0 ± 2.9 (16)**	53.1 ± 6.1		
Time constant IPSC2 $\tau 2$ (msec)	33.1 ± 2.6 (16)*	61.9 ± 6.4		

** $p < 0.01$; * $p < 0.05$ kindled different from control group for the same stimulus intensity.
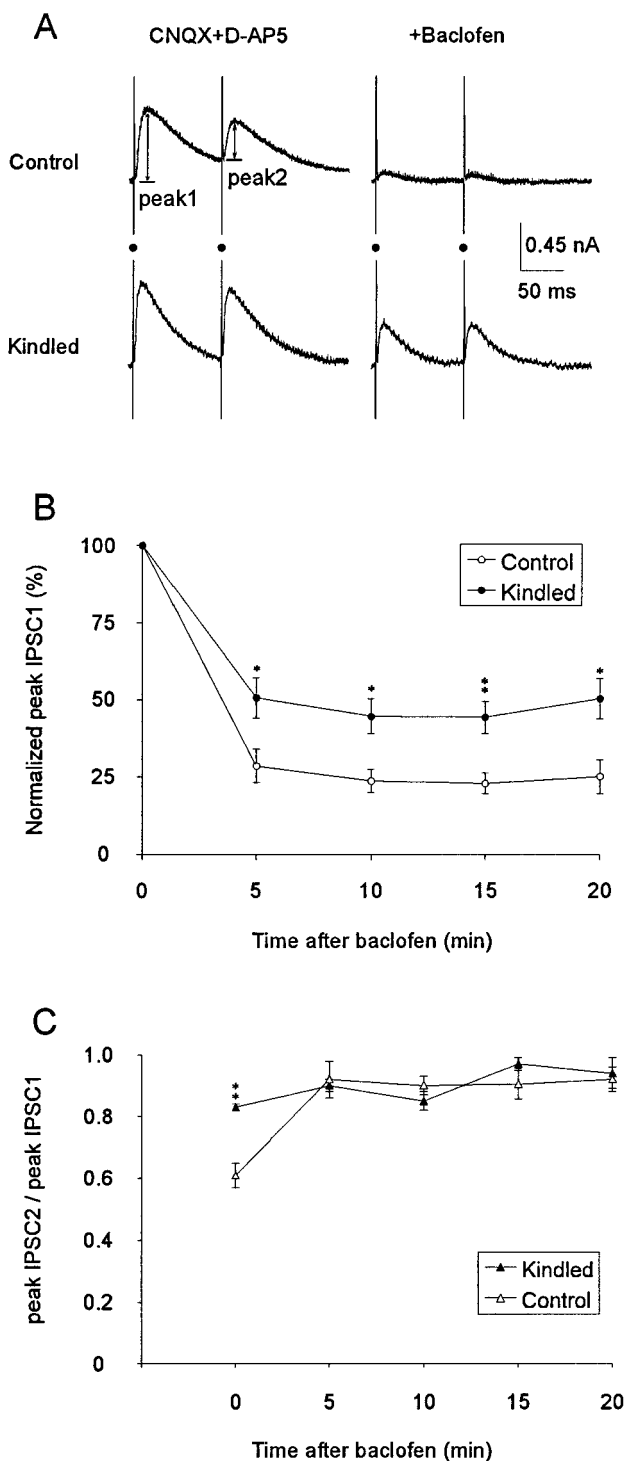
Figure 4. Partial hippocampal kindling reduced the effect of GABA$_B$ agonist baclofen in suppressing IPSCs. *A*, Examples of paired-pulse IPSCs in CA1 neurons from control and kindled rat, recorded at -50 mV holding potential, before and after the perfusion of 10 μM baclofen (added to the CNQX and D-AP5 medium). Resting membrane potential for control and "kindled" neuron was -60 and -63 mV, respectively. *Filled circles* indicate shock artifacts. *B*, Baclofen reduced the first IPSC in neurons from both kindled and control rats, but the effect was significantly larger in control as compared with kindled rats. *C*, The difference in the PPD (ratio of IPSC peaks) between kindled and control rats was abolished after 5 min of baclofen perfusion. * ($p < 0.05$), ** ($p < 0.01$) indicate significant difference between kindled and control groups (Wilcoxon) at a fixed time after baclofen.

course of IPSC1. The mechanism by which CGP35348 slowed the IPSCs is not known. It is possible that presynaptic GABA$_B$ inhibition after a single pulse may contribute to a shutdown of further GABA release (20 msec after the afferent stimulus). This is apparently the first time that a GABA$_B$ antagonist has been shown to affect the kinetics of a single evoked IPSC. It has not been reported before perhaps because of overlap with the postsynaptic GABA$_B$ currents (Davies et al., 1990; Wan et al., 1996) or a lack of quantitative analysis of the IPSCs.

The decay rate of the IPSC was faster in kindled as compared with control rats, best characterized by smaller $\tau 1$ values in the kindled than in the control group, and this effect persisted at least 21 d after partial hippocampal kindling. The effect was found in the presence of GABA$_B$ antagonist CGP35348, suggesting that it was independent of GABA$_B$ receptors. The mechanism underlying the difference in IPSC decay is not known. The release of GABA may be less sustained in kindled as compared with control rats. GABA uptake may be increased in the kindled rat, and a change in GABA uptake would affect the decay more than the peak of the IPSC (Isaacson et al., 1993; Roepstorff and Lambert, 1994). Changes in GABA$_A$ receptor subunits or phosphorylation (Stelzer, 1992; Macdonald and Olsen, 1994) may affect the IPSC kinetics, and GABA$_A$ receptor subunit changes have been reported in CA1 after hippocampal kindling, although the CA1 changes were small and did not last more than a few days (Clark et al., 1994; Kokaia et al., 1994; Kamphuis et al., 1995). Otis et al. (1994) found no change in the time course of miniature IPSCs in dentate granule cells after full kindling, but the decay time constant of miniature IPSCs (Collingridge et al., 1984; Ropert et al., 1990; Otis and Mody, 1992) is considerably smaller than that of evoked IPSCs (Pearce, 1993; Roepstorff and Lambert, 1994; this study).

Effect of kindling on neuronal excitation and inhibition

The loss of presynaptic GABA$_B$ receptor efficacy on GABA terminals after hippocampal kindling may be ubiquitous and pervasive, as suggested by the decrease in PPD of the IPSCs in the dentate gyrus after convulsive kindled seizures (Buhl et al., 1996). Because kindling induces the same change in PPD of the IPSCs but different changes of paired-pulse spike response in CA1 and the dentate gyrus (see introductory remarks), PPD of IPSCs cannot be the crucial factor involved in the paired-pulse population spike response. In CA1 neurons *in vitro*, the magnitude of IPSC2 was not a significant factor in the generation of an early latency spike after the second pulse (Leung and Fu, 1994). However, a faster decay of IPSC1 may account partly for the increased paired-pulse facilitation of the population spike in CA1 neurons, which persisted for approximately 6 weeks after partial hippocampal kindling (Leung et al., 1994).

We found no significant reduction of the peak of the monosynaptic IPSC1 in CA1 neurons after partial kindling. Similarly, Oliver and Miller (1985b) found no change in CA1 neuronal excitability during recurrent inhibition after kindling. Titulaer et al. (1994, 1995) reported a decrease in postsynaptic GABA$_A$ receptor binding of 10–25% in CA1, which may not be manifested in the IPSCs. The complexity of the inhibitory circuitry and its modulation cannot be determined by the monosynaptic IPSCs alone. Seizures have been suggested to selectively shut down afferent pathways to the GABAergic interneuron (Sloviter, 1991; Bekenstein and Lothman, 1993; Mangan et al., 1995). Preliminary studies (L. S. Leung and D. Zhao, unpublished observations) revealed that partial hippocampal kindling induced a sup-

pression of PPD of disynaptic inhibition but no apparent loss of single-pulse feedforward inhibition.

Significance of GABA$_B$ autoreceptor regulation

This is the first time that neural activity, in this case electrographic seizure, has been shown to regulate autoinhibition specifically through the presynaptic GABA$_B$ receptors. Buhl et al. (1996) showed a suppression of PPD of the IPSCs in dentate granule cells 1–2 d after full kindling (15 stage 5 seizures) of the perforant path but did not show the involvement of GABA$_B$ receptors. Downregulation of presynaptic GABA$_B$ receptors was proposed after kainic acid seizures, but not for the autoreceptors on GABAergic terminals (Haas et al., 1996). A decrease in efficacy of presynaptic GABA$_B$ receptors on glutamatergic terminals was shown in the basolateral amygdala after amygdala kindling (Asprodini et al., 1992) and in CA1 after partial hippocampal kindling (Wu and Leung, 1997).

Presynaptic GABA$_B$ receptors and autoinhibition may be of great but as yet unproven significance (Bowery, 1993; Misgeld et al., 1995). Release studies (Waldmeier et al., 1993) indicate that "basal release alone already substantially activated the autoreceptor," whereas no clear presynaptic GABA$_B$ effects have been demonstrated *in vivo* (Olpe et al., 1993; Misgeld et al., 1995). An important function of presynaptic GABA$_B$ receptors on GABA terminals may be to regulate the amount of postsynaptic inhibition and thus control the postsynaptic excitability that may lead to paroxysmal activity (Ben-Ari et al., 1979). Microinfusion of baclofen in the hippocampus of behaving rats induced seizure activity (Vaurio et al., 1996), which may be mediated by disinhibition through presynaptic GABA$_B$ receptors or postsynaptic GABA$_B$ receptors on inhibitory interneurons (Mott et al., 1989). The downregulation of GABA$_B$ receptors after seizures (Haas et al., 1996; this study) may thus serve to prevent more seizures and contribute to the refractory period after seizures (Engel, 1995). A possible trade-off is that normal physiological plasticity such as long-term potentiation may also be blocked (Hesse and Teyler, 1976; Wu and Leung, 1997).

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