

# Inhibitory Control of LTP and LTD: Stability of Synapse Strength

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**Steele, Philip M. and Michael D. Mauk.** Inhibitory control of LTP and LTD: stability of synapse strength. *J. Neurophysiol.* 81: 1559–1566, 1999. Although much is known about the induction of synaptic plasticity, the persistence of memories suggests the importance of understanding factors that maintain synaptic strength and prevent unwanted synaptic changes. Here we present evidence that recurrent inhibitory connections in the CA1 region of hippocampus may contribute to this task by modulating the relative ability to induce long-term potentiation and depression (LTP and LTD). Bath application of the  $\gamma$ -aminobutyric acid (GABA) type A agonist muscimol to hippocampal slices increased the range of frequencies that produce LTD, whereas in the presence of the GABA type A antagonist picrotoxin LTD was induced only at very low stimulation frequencies (0.25–0.5 Hz). Because one source of GABAergic input to CA1 pyramidal cells is via recurrent inhibition, we tested the prediction that elevated postsynaptic spike activity would increase feedback GABA inhibition and favor the induction of LTD. By using an induction stimulation of 8 Hz, which alone produced no net change in synaptic strength, we found that stimulation presented during antidromic activation of pyramidal cell spikes induced LTD. This effect was blocked by picrotoxin. The influence of recurrent inhibition on LTP and LTD displays properties that may decrease the potential for self-reinforcing, runaway changes in synapse strength. A mechanism of this sort may help maintain patterns of synaptic strengths despite the ongoing opportunities for plasticity produced by synapse activation.

## INTRODUCTION

Activity-dependent mechanisms of synaptic plasticity are generally viewed as a plausible neural basis for learning and memory (Hebb 1949; Kandel and Schwartz 1982). In this context, learning relates to the mechanisms for inducing plasticity, and the persistence of memory relates to the stable and long-lasting expression of plasticity. The phenomena of long-term potentiation (LTP) and depression (LTD), as commonly studied in the CA1 region of hippocampus, are important in part because of the potential relationship between their long-term expression and the persistence of memories (Bear and Malenka 1994; Bliss and Collingridge 1993; Eichenbaum 1996).

However, forms of plasticity that display cellular and molecular mechanisms for the long-term expression of synaptic changes, such as LTP and LTD, do not necessarily confer synapses with the ability to store long-term memories. Clearly, the ability of synapses to undergo activity-dependent plasticity may allow experience to produce patterns of synaptic strengths that permit networks to store memories. Yet learning involves interactions between mechanisms/rules of plasticity and the

activity of the networks in which the modifiable synapses reside. In the artificial circumstances of an *in vitro* brain slice, most synapses are relatively quiescent unless stimulated by the experimenter. Thus the long-term expression of synaptic plasticity in a slice may require only a molecular mechanism that persists. In contrast, modifiable synapses in the intact brain are probably active quite often, providing frequent opportunities to change the patterns of synaptic weights that might encode a memory. Observations that hippocampal LTP and LTD are mutually reversing (Dudek and Bear 1993; Mulkey and Malenka 1992) reveal that these patterns can be changed and memories can be erased, despite the underlying ability for LTP and LTD expression to be long lasting. For example, potentiation at a set of synapses might encode a memory, but the induction of LTD at some or all of these synapses could degrade or completely erase this memory. Thus any systematic tendency for strengths to drift would mean that the persistence of memory cannot be explained entirely by *in vitro* observations that LTP and LTD expression can be long lasting.

Understanding the processes that prevent unwanted synaptic changes and contribute to the stability of synaptic strengths is highlighted further by the apparent potential for self-reinforcing, runaway induction of LTP and LTD. Because of the way these forms of plasticity depend on the postsynaptic membrane potential (Artola et al. 1990; Larson and Lynch 1989; Malinow and Miller 1986), changes in one set of synapses may increase the likelihood for further changes in the same direction (see Barrionuevo and Brown 1988). For example, the induction of LTP at one set of synapses could lead to stronger postsynaptic depolarization and therefore increase the likelihood of subsequent induction of LTP at all synapses onto the same postsynaptic cell. In this case there would be a complete loss of the patterns of synaptic weights onto the cell. Furthermore, because LTP and LTD are activity dependent, the ongoing activity displayed throughout the nervous system provides abundant opportunities for unwanted plasticity. Thus with bidirectional forms of plasticity (such as LTD and LTP) that are activity dependent and mutually reversing it seems important to understand both the mechanisms that lead to the induction of plasticity as well as the mechanisms that prevent the induction of unwanted plasticity and the potential loss of memories.

Negative feedback, in which action potential activity of the postsynaptic cell regulates excitability or the induction of synaptic plasticity, represents one general class of mechanism that could prevent runaway changes in activity or runaway induction of synaptic plasticity (see Bienenstock et al. 1982). Previous studies demonstrated the existence of processes within neurons that couple changes in synaptic strength and ion chan-

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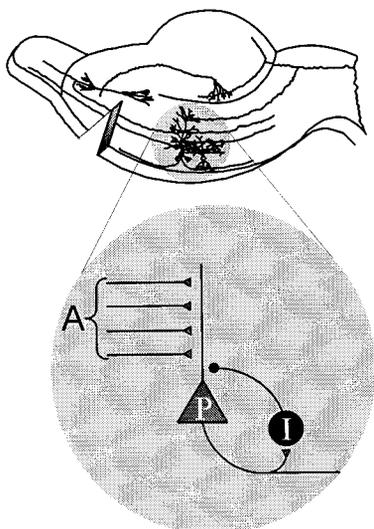


FIG. 1. A schematic representation of the hippocampal slice preparation and the recurrent inhibitory circuit relevant to the present experiments. Hippocampal pyramidal cells (P) receive excitatory input from Schaffer collateral afferents (A). These excitatory synapses undergo long-term potentiation (LTP) and depression (LTD). Pyramidal cells excite basket cells (B), which provide GABAergic recurrent inhibitory input back to the pyramidal cells.

nel densities with recent postsynaptic activity (Turrigiano et al. 1994). Moreover, computational studies suggest that negative feedback can maintain the activity of neurons within a useful dynamic range (LeMasson et al. 1993; Tsodyks et al. 1997). When applied to the induction of LTP and LTD, negative feedback could prevent runaway changes by making it easier to induce LTD (relative to LTP) when postsynaptic activity is high and by making it relatively easier to induce LTP when postsynaptic activity is low. Here we pursued the general hypothesis that  $\gamma$ -aminobutyric acid (GABA)-mediated recurrent inhibition may provide this sort of stabilizing negative feedback in the hippocampus (Fig. 1). We tested the specific hypothesis that spike activity of the postsynaptic pyramidal cell recruits GABA-mediated recurrent inhibition and influences the induction of plasticity by making it relatively easier to induce LTD when pyramidal cell spike activity is high and relatively easier to induce LTP when it is low.

## METHODS

### Slice preparation

Methods for preparing hippocampal slices were as previously described (Huber et al. 1995). Briefly, Harlan Sprague-Dawley rats (5–9 wk old) were deeply anesthetized with sodium pentobarbital (50 mg/kg) and were decapitated. The brain was rapidly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF) saturated with 95% O<sub>2</sub>-5% CO<sub>2</sub> and containing the following (in mM): 124 NaCl, 3 KCl, 1.3 NaH<sub>2</sub>PO<sub>4</sub>, 10 MgCl<sub>2</sub>, 0 CaCl<sub>2</sub>, 26 NaHCO<sub>3</sub>, 10 dextrose, and 10 *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (pH 7.35). One hippocampus was dissected free, and transverse slices (400- $\mu$ m thick) were prepared with a McIlwain tissue chopper. Immediately afterward, CaCl<sub>2</sub> (2 mM) was added to the ACSF, which was then warmed to 31°C over 20 min. Slices were then incubated for an additional 30 min in the standard ACSF (31°C), containing the same stock solution as described previously, except MgCl<sub>2</sub> (1.5) and CaCl<sub>2</sub> (2.5). Slices prepared from the same hippocampus were pseudorandomly assigned to experimental or control experiments. Imme-

diately before electrophysiological recordings, the CA1 region of all slices was surgically isolated from CA3.

### Preparation of compounds

The following compounds used in this study were purchased from Sigma and prepared as follows. A muscimol stock solution (100  $\mu$ M) was made fresh every other day, kept at 4°C, protected from light, and diluted in standard ACSF to 3 nM immediately before use. Picrotoxin (50  $\mu$ M) was prepared daily in standard ACSF with no added CaCl<sub>2</sub> and MgCl<sub>2</sub> (room temperature). After the picrotoxin completely dissolved, the solution was oxygenated, and CaCl<sub>2</sub> (2.5) and MgCl<sub>2</sub> (1.5) were added. D,L-2-amino-5 phosphonovalerate (APV) was prepared as a stock solution (50 mM) and diluted in standard ACSF to 50  $\mu$ M immediately before use.

### Recordings

All experiments were performed in a standard submersion chamber perfused with ACSF at a rate of 1.5 to 2 ml/min (31°C). Extracellular field potentials were recorded from the stratum radiatum in area CA1 of hippocampal slices with pipette electrodes (1 to 3 M $\Omega$ ) filled with ACSF with no MgCl<sub>2</sub> or CaCl<sub>2</sub> added. Intracellular recordings were obtained from the pyramidal cell layer with electrodes filled with 3 M potassium acetate (60–80 M $\Omega$ ). Schaffer collateral and commissural axons in stratum radiatum were stimulated with tungsten monopolar (20–50  $\mu$ m) electrodes (Frederick Haer, Brunswick, ME) for 30–60 min to obtain a stable baseline. Similar electrodes with larger exposed tips (1 mm) were placed against the alveus to stimulate pyramidal cell axons in antidromic studies. For each field potential experiment the stimulation intensity was set to produce synaptic responses that were ~50% of maximum, as measured by initial slope of the excitatory postsynaptic potential (EPSP). These responses were between 0.37 and 0.43 mV/ms with an amplitude of between 0.6 and 0.8 mV. The EPSPs recorded during intracellular experiments were set to 10–15% below threshold for an action potential. All experiments in which the input resistance of the pyramidal cell changed by >20% were excluded (3 were excluded). The baseline measurements were collected with single shocks every 15 or 30 s. Responses were digitized at 20 kHz and stored on computer for subsequent analyses.

The induction stimulation consisted of 600 pulses delivered at frequencies ranging from 0.25 to 50 Hz. The antidromic stimulation consisted of four pulses at 100 Hz given every 500 ms for the duration of the 8-Hz induction stimulation. In most analyses, changes in EPSP slope are expressed as the average EPSP slope over the last 5 min of the experiment (40–45 min postinduction) normalized to the last 5 min of baseline. Values reported in the text and figures are mean  $\pm$  SE. Two-tailed distributions with a critical *P*-value of 0.05 were used for all statistics.

## RESULTS

To test these hypotheses we began by examining the effects of a GABA type A receptor antagonist (picrotoxin) and agonist (muscimol) on the induction of LTD at the normally employed stimulation frequencies (600 pulses at 1 and 3 Hz; Fig. 2). Whereas either 1- or 3-Hz stimulation reliably induced LTD in control slices, the same stimulation protocol in the presence of picrotoxin produced only a transient decrease in EPSP slope that returned to baseline level within ~15 min (Fig. 2A). After washing out picrotoxin, the same stimulation reliably induced LTD. Application of picrotoxin after the induction of LTD had no noticeable effects (Fig. 2B), indicating that picrotoxin does not block the expression of LTD. In contrast, application of muscimol enhanced the induction of LTD compared with controls (Fig. 2C).

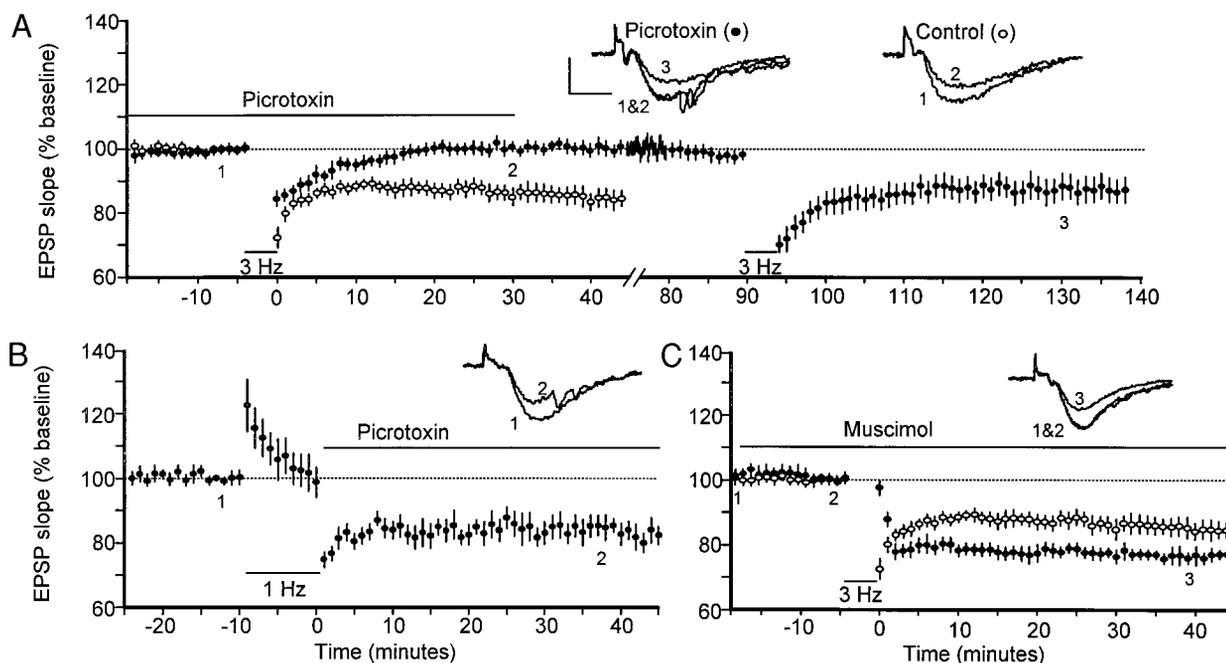


FIG. 2. Pharmacological manipulation of  $\gamma$ -aminobutyric acid (GABA) type A receptors influences the induction but not the expression of LTD. **A:** bath application of picrotoxin ( $50 \mu\text{M}$ ,  $\bullet$ ) blocked the induction of LTD with a 3-Hz, 600-pulse induction stimulus ( $100\% \pm 2$  at 45 min after induction stimulus,  $n = 8$ , compared with control slices  $\circ$ ),  $86.7\% \pm 2$ ,  $n = 8$ ,  $P < 0.001$ ). After washing out picrotoxin for 1 h, the same induction stimulus induced LTD ( $87.3\% \pm 4$ ,  $P < 0.001$  compared with picrotoxin), which was not significantly different from control slices. **B:** bath application of picrotoxin after the induction stimulation (1 Hz, 600 pulses) does not block the expression of LTD ( $83\% \pm 3$ ,  $n = 6$ ,  $P > 0.05$  compared to control). Excitatory postsynaptic potential (EPSP) traces are superimposed single sweeps taken from a representative experiment at the time intervals indicated. Calibration: 5 ms horizontal, 0.5 mV vertical. **C:** bath application of muscimol ( $3 \text{ nM}$ ,  $\bullet$ ) facilitated LTD induced with 3-Hz/600-pulse stimulation ( $77\% \pm 2$  at 45 min after induction stimulus,  $n = 10$ ,  $P < 0.01$  compared with control  $\circ$ ). This concentration of muscimol did not measurably change the initial EPSP slope compared with naive baseline stimulation (EPSP 1 and 2).

We then determined whether picrotoxin blocked LTD induction completely or simply changed the stimulation conditions required for inducing LTD relative to LTP. We first tested the ability of stimulation frequencies ranging from 0.25 to 50 Hz (600 pulses) to induce LTP and LTD. All experiments were performed as shown in Fig. 2A. Control experiments generally replicated previous findings (Dudek and Bear 1992, 1993; Mulkey and Malenka 1992) by inducing LTD at stimulation frequencies of 0.25 to 5 Hz and inducing LTP at higher frequencies (10 and 50 Hz; Fig. 3A). Bath application of picrotoxin had no measurable effect at 0.25 and 0.5 Hz, prevented the induction of LTD at 1 and 3 Hz, and enhanced the ability to induce LTP at 5 and 10 Hz (Fig. 3A). In contrast, bath application of muscimol enhanced the ability of stimulation to induce LTD relative to LTP (as seen at 10 Hz) and also significantly increased the magnitude of LTD induced by stimulation at 0.5 to 3 Hz (Fig. 3B).

The differences between each drug and control slices over the range of stimulation frequencies tested suggest two key properties regarding the role of GABA-mediated inhibition on the induction of LTP and LTD (Fig. 3C). First, as indicated by the relatively uniform effects of muscimol across all but the highest frequencies, inhibition of pyramidal cells makes it easier to induce LTD relative to LTP. Second, as suggested by the effects of picrotoxin, endogenous GABA released at stimulation frequencies of  $\geq 1$  Hz increases the ability to induce LTD relative to LTP. These data indicate that, at stimulation frequencies above a certain threshold (1 Hz in these experiments), endogenous release of GABA during repetitive stim-

ulation contributes to the final outcome of LTD, LTP, or no change in synaptic strengths. Although there is likely to be tonic release of GABA in slices, the discrete divergence of the picrotoxin-treated slices from control slices at 1 Hz suggests that the effects are due to inhibition of stimulation-induced GABA release.

It seemed important to address the possibility that picrotoxin may block the induction of *N*-methyl-D-aspartate (NMDA)-dependent LTD and that the synaptic depression seen at 0.25 and 0.5 Hz is mediated by mechanisms that differ from those that mediate the induction of LTD at 1 and 3 Hz. To address this we tested whether the LTD seen at 0.25 Hz (in picrotoxin) displays two key properties of LTD, pathway specificity and dependence on NMDA receptors (Dudek and Bear 1993). As shown in Fig. 4, two pathway experiments indicate that the synaptic depression seen at 0.25 Hz is pathway specific because no change was seen in the responses elicited by a separate stimulation pathway. Moreover, application of the NMDA receptor antagonist APV blocked the induction of LTD at 0.25 Hz in picrotoxin. Thus it appears that bath application of picrotoxin does not block LTD per se nor does it enhance a novel form of LTD seen only at low stimulation frequencies. Instead, bath application of picrotoxin and muscimol appears to systematically change the conditions required for the induction of the same NMDA-dependent, input-specific LTD commonly studied with 1- or 3-Hz stimulation.

In the CA1 region of hippocampus, recurrent inhibition represents one of the possible sources of GABAergic input that could be engaged during repetitive stimulation of Schaffer

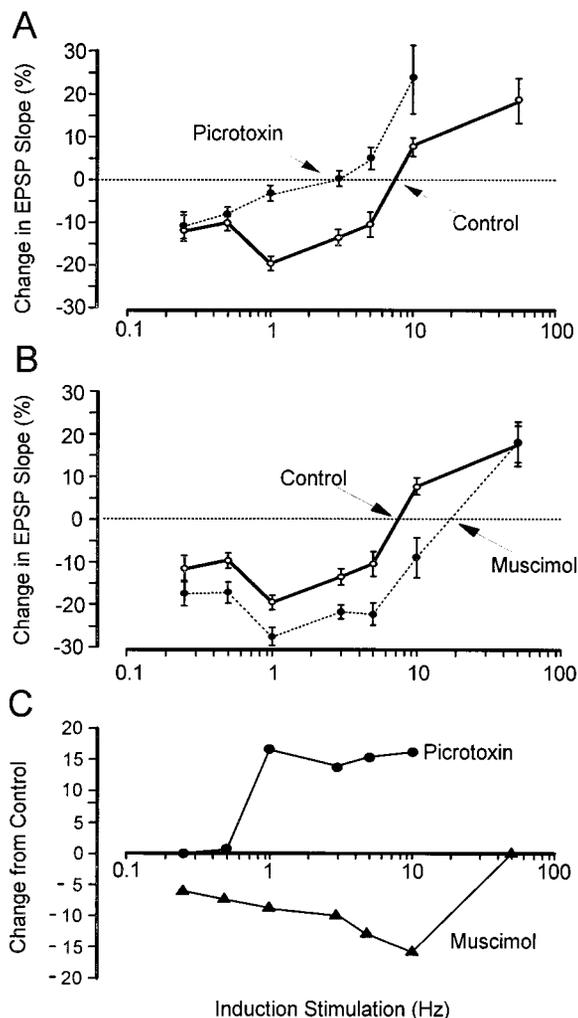


FIG. 3. The effects of picROTOXIN on LTD and LTP depend on the frequency of the induction stimulation. *A*: data from control slices (—, ●) shows LTD at frequencies from 0.25–5 Hz and LTP at frequencies of  $\geq 10$  Hz ( $n = 8$  per frequency). Note the large increase in LTD amplitude at 1 Hz compared with 0.5 Hz. Bath application of picROTOXIN (---, ●) had no effect at 0.25 and 0.5 Hz and favored the induction of LTP over LTD at frequencies  $> 1$  Hz ( $P < 0.05$ , except 0.25 and 0.5 Hz). *B*: application of muscimol (---, ●) enhances LTD relatively uniformly at all frequencies except 50 Hz ( $P < 0.05$  except 50 Hz); 10-Hz stimulation induced LTP in control slices and LTD in the presents of muscimol. Control data same as those shown in *A*. *C*: same data as *A* and *B* plotted as the difference between control and muscimol (▲) or picROTOXIN (●). Whereas the effects of muscimol were relatively uniform at  $\leq 10$  Hz, picROTOXIN had no effects at frequencies of  $\leq 0.5$  Hz. This suggests that endogenous GABA release may have been insignificant below 1 Hz under these conditions.

collateral afferents (Fig. 1). Because recurrent inhibition increases as pyramidal cell activity increases (Maccaferri and McBain 1995), the effects of picROTOXIN and of muscimol may reveal a role of negative feedback in controlling the induction of plasticity. Our results are consistent with the hypothesis that repetitive afferent stimulation above a threshold level engages recurrent inhibition, aiding in the induction of LTD. At midrange stimulation frequencies ( $\sim 8$ –10 Hz in our experiments) the effects of excitation and recurrent inhibition may combine to produce no net change in synapse strength. Higher stimulation frequencies may overwhelm this balance and lead to the induction of LTP. In this context, enhancing inhibition (as with muscimol) should favor the induction of LTD over LTP, whereas blocking inhibition (as with picROTOXIN) should

make the induction of LTD more difficult, as we have shown, and make the induction of LTP relatively easier, as is often observed.

These ideas predict that the induction of LTD would be favored over LTP when recurrent inhibition is enhanced through an artificial increase of pyramidal cell spike activity. To test this prediction we employed a frequency of induction stimulation (600 pulses at 8 Hz) that produced no net change in EPSP slope in control experiments (Fig. 3). To increase postsynaptic activity we used antidromic stimulation to elicit action potentials in pyramidal cells and intracellular recordings to confirm that the antidromic stimulation recruited recurrent inhibition (Fig. 5*E*). The antidromic stimulation (4 100-Hz pulses delivered twice per second) was similar to the firing characteristics of the inhibitory basket cells (Sik et al. 1995). Whereas 8-Hz stimulation alone did not produce a significant change in EPSP slope (Fig. 5*A*), pairing 8-Hz stimulation with antidromic activation of spikes in the pyramidal cells reliably induced LTD (Fig. 5*B*). This effect was blocked by bath application of picROTOXIN, where the combination of 8-Hz stimulation and antidromic stimulation produced, if anything, an increase in the EPSP slope (Fig. 5*C*). Each experiment included a second pathway to control for nonspecific effect of antidromic stimulation. The means for all three groups are shown in Fig. 5*D*.

The intracellular recordings collected during the induction stimulation illustrate the interactions among the 8-Hz stimulation, the antidromic stimulation, and the bath application of picROTOXIN (Fig. 5, *A–C*, top portions). During 8-Hz stimulation alone (which produced no significant change in EPSP slope), there was only a slight change in the underlying membrane potential (Fig. 5*A*). In contrast, adding antidromic stimulation (which induced LTD) produced a pronounced hyperpolarization during the 8-Hz induction stimulation (Fig. 5*B*). Application of picROTOXIN during this combined antidromic/8-Hz stimulation (which induced LTP) produced a small depolarization. These observations are consistent with the simple notion that recurrent inhibition affects the relative ability to induce LTD and LTP by influencing the membrane potential and the degree to which repetitive stimulation can activate NMDA receptor-gated channels.

To examine this notion further, we tested the ability of the 8-Hz stimulation/antidromic stimulation combination to induce LTD in the presence of APV (Fig. 6). Bath application of APV blocked the induction of LTD, whereas after washout of APV the same combined stimulation reliably induced LTD ( $n = 4$ ). These experiments also employed a second input pathway. Responses to this pathway did not change, showing that this LTD is also input specific. These observations further support the idea that increased inhibition facilitates the induction of LTD by controlling the activation of NMDA receptors.

## DISCUSSION

The main result we report is that the relative ability to induce LTP versus LTD is influenced by the degree of activation of recurrent inhibitory inputs. Application of the GABA type A receptor agonist muscimol or increasing endogenous recurrent GABAergic input both favor the induction of LTD relative to LTP. Thus when GABAergic activity is high LTD can be induced with stimulation protocols that normally either induce

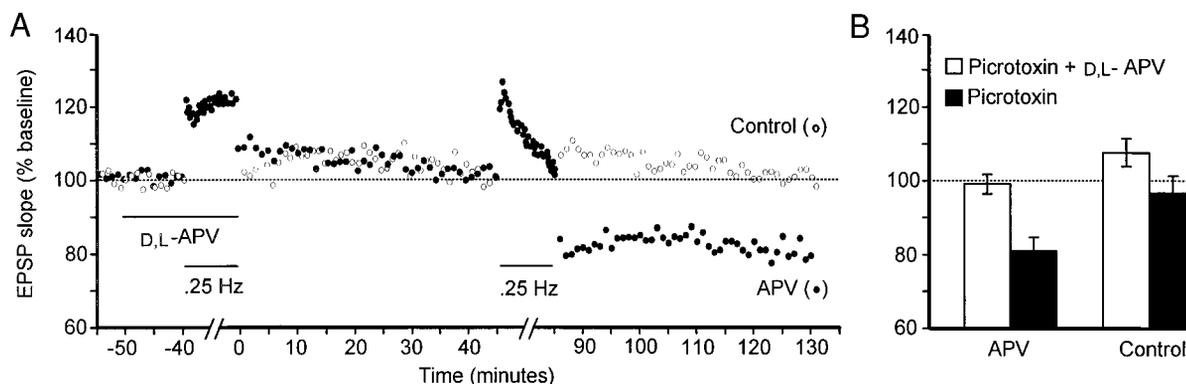


FIG. 4. LTD induced in picrotoxin with low-frequency (0.25 Hz) induction stimulation is input specific and *N*-methyl-D-aspartate (NMDA) dependent. *A*: representative 2-pathway experiment showing that the induction of LTD with 0.25-Hz stimulation in the presence of picrotoxin (present throughout the experiment) is blocked by a 10-min bath application of 50  $\mu$ M *D,L*-2-amino-5-phosphonovaleate (APV) [●]. LTD was induced by the same stimulation after a 45-min washout of the APV. *B*: summary data from 8 single-pathway and 4 dual-pathway experiments. APV consistently blocked the induction of LTD ( $98.3\% \pm 3$ ), whereas the 0.25 Hz reliably induced LTD after washing out APV ( $79.8\% \pm 4$ ). In the 4 two-pathway experiments there was no significant effect in the control pathway ( $108.2 \pm 4\%$ )

LTP or produce no change in EPSP slope. In contrast, picrotoxin increases the range of stimulation frequencies that induce LTP such that LTD induction is blocked at the stimulation frequencies employed in most LTD studies (1–3 Hz) and can be induced only at very low stimulation frequencies (0.25 and 0.5 Hz). The LTD induced at these low frequencies is pathway specific and sensitive to the NMDA antagonist (APV), suggesting that it is not a novel form of LTD. These data have a number of implications concerning both the induction of LTP and LTD in vitro and concerning the properties that LTD and LTP may display in hippocampal circuits.

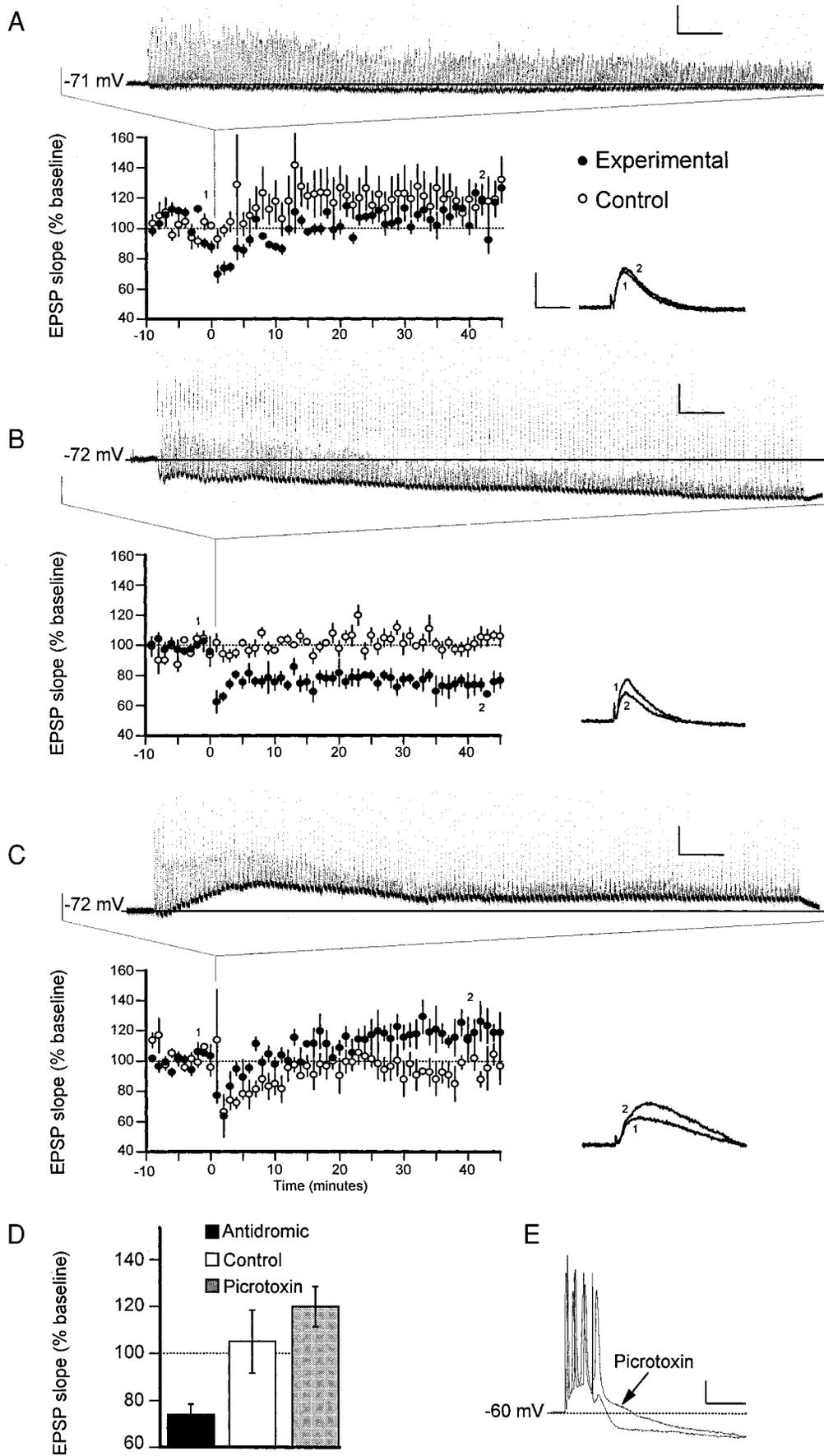
Our results indicate that the induction of LTD or LTP in vitro can involve interactions between direct excitatory input to the pyramidal cells and recurrent inhibition that is activated by spike activity in the pyramidal cells. Feed-forward inhibition is also likely to be activated by each orthodromic pulse. However, because its presence is a constant, it seems unlikely that feed-forward inhibition can explain the ability of antidromic activation to induce LTD with 8-Hz stimulation. Both the abolition of this effect by picrotoxin and the picrotoxin-sensitive hyperpolarization after antidromic stimulation suggest that recurrent inhibition may play an important role in controlling the direction of change in synaptic strength. Because both LTP and the LTD observed with 8-Hz/antidromic pairing are NMDA dependent, we suggest that under normal conditions the amount of feedback inhibition recruited by 8-Hz stimulation leads to calcium influx that falls between the levels required to induce LTD and LTP (Cummings et al. 1996). When inhibitory feedback is increased, as we have done with antidromic stimulation, the decreased amount of calcium influx favors the induction of LTD.

Our results imply that activating inhibitory synaptic transmission is required for the induction of LTD at the stimulation frequencies employed in most studies (1–3 Hz). Because picrotoxin had no effect at stimulation intensities of 0.5 and 0.25 Hz (Fig. 2A), we suggest that in our experiments the feedback inhibition is engaged at stimulation frequencies of 1 Hz and above. However, it seems likely that this threshold may vary, depending on circumstances such as the size and position of the

stimulation electrodes as well as the stimulation intensities used.

Variation in the activation of inhibitory synaptic transmission may therefore account for the variable ability to induce LTD in different laboratories and perhaps with different aged animals (Abraham et al. 1996; Bashir and Collingridge 1994; Dudek and Bear 1992, 1993; Thiels et al. 1994; Yang et al. 1994). For example, certain studies reported that it is difficult to induce LTD in brain slices from older animals (O'Dell and Kandel 1994; Wagner and Alger 1995). One explanation for this may be that LTD is saturated at these synapses, either because of the animal's experiences or because of events that occur during preparation of the slices (Bolshakov and Siegelbaum 1995). Our data also suggest that the induction of LTD might require different frequencies of stimulation in older animals as the balance between excitation and inhibition changes with age (Muller et al. 1989; Swann et al. 1989). Similarly, Thiels et al. (1994) have shown clearly that the induction of LTD in vivo requires recurrent inhibition. Stimulation protocols such as the ones that induce LTD in vitro had no effect, as were patterns of stimulation that paired synaptic inputs with recurrent inhibition-induced robust LTD. The induction of this LTD in vivo was blocked by bicuculline. Here again the role for recurrent inhibition may explain the inability to induce LTD in some in vivo preparations (e.g., Doyle et al. 1997).

Several previous studies addressed the potential role of GABAergic inhibition in the induction of LTD and LTP. Gustafsson and Wigström (1990) demonstrated that the induction of LTP is enhanced when inhibitory synaptic transmission is blocked. Conversely, Yang et al. (1994) demonstrated that the induction of LTD is facilitated in young rats by pairing synaptic activation with GABA. Our results and the hypothesis regarding the role of feedback inhibition are consistent with their findings. Wagner and Alger (1995) also reported and extensive analysis of the influence of both GABA<sub>A</sub> and GABA<sub>B</sub> receptors on the induction of LTD. These authors showed that, in apparent contrast to our findings, the GABA<sub>A</sub> antagonist bicuculline did not affect LTD in slices taken from young animals (16–22d) and enhanced the induction of LTD in



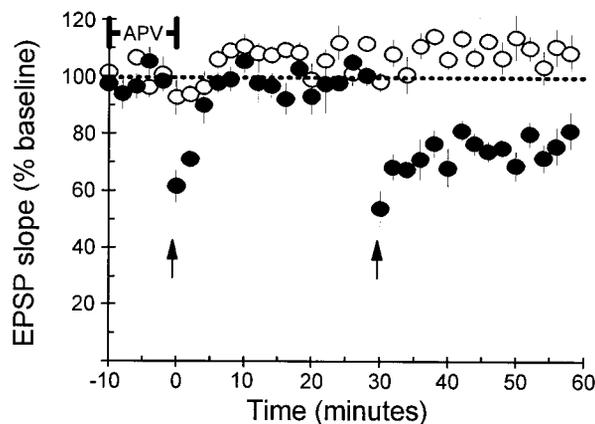


FIG. 6. LTD induced with combined 8-Hz/antidromic stimulation is input specific and NMDA dependent. An initial 8-Hz/antidromic stimulation (left-most arrow) was presented with 50  $\mu$ M APV present in the bath (●). This stimulation produced no measurable changes in EPSP slope ( $101\% \pm 7$ ,  $n = 4$ ). After washout of APV a second identical combined stimulation (second-up arrow) resulted in significant LTD induction ( $71.2\% \pm 6$ ). A second pathway did not change (○), demonstrating that LTD produced by the combined protocol is also input specific.

slices from mature animals. A GABA<sub>B</sub> antagonist significantly decreased LTD in slices from young animals but had no effect on depotentiation (inducing LTD after the induction of LTP). These results suggest that the effects of GABAergic transmission may be different before and after the induction of LTP, consistent with feedback control of LTP and LTD. These results also suggest, like our results, that the contributions of GABAergic inhibition—and thus the effects of GABA antagonists—vary depending on a number of factors. Thus both previous and present data indicate 1) the importance of testing the effects of GABA antagonists over a range of stimulation frequencies and thus 2) that caution is required in interpreting the implications of a pharmacological manipulation when tested at only one stimulation frequency.

Feedback inhibition may serve the useful role of preventing runaway induction of LTP or LTD. As mentioned previously, the induction of LTD and LTP requires relatively strong and weak pyramidal cell depolarization respectively, and each is potentially prone to positive feedback. Our results suggest that inhibitory control over the induction of LTD and LTP via recurrent pathways may help break this positive feedback. Such a mechanism appears potentially important for several reasons. First, a neuron whose inputs are all as strong or as weak as possible would have a diminished capacity for processing information. Its activity would depend mostly on the number of its inputs that are active and would depend much less on the pattern of the inputs. Second, with a typical number of active inputs, there would be an increased likelihood that the

spike rate of the cell would remain almost exclusively at its maximum or minimum level. The ability of such a uniformly active cell to pass along information to its follower cells would be greatly diminished. Third, an inherent tendency for all synaptic strengths to drift to their maximum or all to their minimum values would preclude the ability of training-induced patterns of synaptic strengths to encode memories (Sutton and Barto 1981).

The role for recurrent inhibition that our data suggest is similar to the hypothetical rule for bidirectional synaptic plasticity proposed by Bienstock et al. (BCM) (1982). In the BCM rule, the threshold activity separating the induction of potentiation and depression is a function of average recent activity of the postsynaptic cell. With increasing activity the threshold increases such that it is easier to induce depression, much like our observation that increased postsynaptic activity increases the range of stimulation frequencies that produce LTD by recruiting recurrent inhibition. Our hypothesis differs from the BCM rule in that it is implemented with a small network involving feedback inhibition rather than by mechanisms within the cell. It also differs in terms of the time period over which recent activity can influence the threshold.

Despite these differences, our hypothesis shares in common with the BCM rule the property that the threshold between increases (LTP) and decreases (LTD) in synaptic strengths varies as a function of recent postsynaptic activity. Given the conceptual appeal of the BCM rule, the existence of mechanisms that are similar or share certain important properties (such as the one we propose) should not come as a surprise. The important functional properties of a BCM-like rule may have led to the evolution of many variants and forms of implementation. For example, recent studies suggest a BCM-like negative feedback regulation of LTD and LTP that arises from the properties of calcium/calmodulin-dependent protein kinase II, an enzyme whose activity is known to be involved in the induction of LTP (Mayford et al. 1995).

Finally, our results suggest the importance of understanding not only the conditions under which synapses change in strength but also the mechanisms that are responsible for preventing unwanted changes. We suggest our results may illustrate one possible mechanism, namely, modulation of the induction of LTD and LTP via recurrent inhibition, that could help accomplish this apparently important task.

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FIG. 5. Recurrent GABAergic inhibition modulates the induction of LTD and LTP. The top 3 panels in A–C show different experiment protocols, each of which used intracellular recordings and 2 stimulation pathways. For 1 pathway the induction stimulus in each experiment was 600 pulses at 8 Hz (●), and the other pathway was used as a control (○). The membrane potential during the induction stimulation for a representative experiment is shown for each induction stimulus. EPSP traces are superimposed single sweeps taken from a representative experiment at the times indicated. A: 8-Hz stimulation alone induced no significant change in the EPSP ( $105\% \pm 13.3$ ,  $n = 5$ ). B: combining the 8-Hz stimulation with antidromic activation of the pyramidal cell axons (4 pulses at 100 Hz presented at 2 Hz) induced LTD ( $73.9\% \pm 4$ ,  $P < 0.05$ ,  $n = 4$ ). C: application of picrotoxin (50  $\mu$ M throughout the experiment) blocked the induction of LTD by using the combined 8-Hz/antidromic stimulation ( $120\% \pm 9$ ,  $n = 4$ ). D: summary data for the 3 groups measured 45 min after induction. E: sample intracellular traces showing a 4-pulse train of antidromic activation. Application of picrotoxin blocked a postburst hyperpolarization, indicating that the antidromic stimulation recruited recurrent inhibition. Similar results were obtained from all 4 picrotoxin experiments. Calibration for the induction sweeps is 20 ms horizontal and 10 mV vertical, the individual EPSPs are 5 ms horizontal and 0.5 mV vertical, and in E 50 ms horizontal and 10 mV vertical.

## REFERENCES

- ABRAHAM, W. C., MASON-PARKER, S. E., AND LOGAN, B. Low-frequency stimulation does not readily cause long-term depression or depotentiation in the dentate gyrus of awake rats. *Brain Res.* 722: 217–221, 1996.
- ARTOLA, A., BROCHER, S., AND SINGER, W. Different voltage-dependent thresholds for inducing long-term depression and long-term potentiation in slices of rat visual cortex. *Nature* 347: 69–72, 1990.
- BARRIONUEVO, G. AND BROWN, T. H. Associative long-term potentiation in hippocampal slice. *Proc. Natl. Acad. Sci. USA* 80: 7347–7351, 1988.
- BASHIR, Z. I. AND COLLINGRIDGE, G. L. An investigation of depotentiation of long-term potentiation in the CA1 region of the hippocampus. *Exp. Brain Res.* 100: 437–443, 1994.
- BEAR, M. F. AND MALENKA, R. C. Synaptic plasticity: LTP and LTD. *Curr. Opin. Neurobiol.* 4: 389–399, 1994.
- BIENENSTOCK, E. L., COOPER, L. N., AND MUNRO, P. W. Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. *J. Neurosci.* 2: 32–48, 1982.
- BLISS, T. V. P. AND COLLINGRIDGE, G. L. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361: 31–39, 1993.
- BOLSHAKOV, V. Y. AND SIEGELBAUM, S. A. Regulation of hippocampal transmitter release during development and long-term potentiation. *Science* 269: 1730–1734, 1995.
- CUMMINGS, J. A., MULKEY, R. M., NICOLL, R. A., AND MALENKA, R. C.  $Ca^{2+}$  signaling requirements for long-term depression in the hippocampus. *Neuron* 16: 825–833, 1996.
- DOYLEY, C. A., CULLEN, W. K., ROWAN, M. J., AND ANWYL, R. Low-frequency stimulation induces homosynaptic depotentiation but not long-term depression of synaptic transmission in the adult anaesthetized and awake rat hippocampus in vivo. *Neuroscience* 77: 75–85, 1997.
- DUDEK, S. M. AND BEAR, M. F. Homosynaptic long-term depression in area CA1 of hippocampus and the effects of NMDA receptor blockade. *Proc. Natl. Acad. Sci. USA* 89: 4363–4367, 1992.
- DUDEK, S. M. AND BEAR, M. F. Bidirectional long-term modification of synaptic effectiveness in the adult and immature hippocampus. *J. Neurosci.* 13: 2910–2918, 1993.
- EICHENBAUM, H. Learning from LTP: A comment on recent attempts to identify cellular and molecular mechanisms of memory. *Learning Memory* 3: 61–73, 1996.
- GUSTAFSSON, B. AND WIGSTROM, H. Long-term potentiation in the hippocampal CA1 region: its induction and early temporal development. *Prog. Brain Res.* 83: 223–232, 1990.
- HEBB, D. O. *The Organization of Behavior; A Neuropsychological Theory*. New York: Wiley, 1949.
- HUBER, K. M., MAUK, M. D., AND KELLY, P. T. Distinct LTP induction mechanisms: contribution of NMDA receptors and voltage-dependent calcium channels. *J. Neurophysiol.* 73: 270–279, 1995.
- KANDEL, E. R. AND SCHWARTZ, J. H. Molecular biology of learning: modulation of transmitter release. *Science* 218: 433–443, 1982.
- LARSON, J. AND LYNCH, G. Theta pattern stimulation and the induction of LTP: the sequence in which synapses are stimulated determines the degree to which they potentiate. *Brain Res.* 489: 49–58, 1989.
- LEMASSEON, G., MARDER, E., AND ABBOTT, L. F. Activity-dependent regulation of conductances in model neurons. *Science* 259: 1915–1917, 1993.
- MACCAFERRI, G. AND MCBAIN, C. J. Passive propagation of LTD to stratum oriens-alveus inhibitory neurons modulates the temporoammonic input to the hippocampal CA1 region. *Neuron* 15: 137–145, 1995.
- MALINOW, R. AND MILLER, J. P. Postsynaptic hyperpolarization during conditioning reversibly blocks induction of long-term potentiation. *Nature* 320: 529–530, 1986.
- MAYFORD, M., KANDEL, E. R., AND O'DELL, T. J. CaMKII regulates the frequency-response function of hippocampal synapses for the production of both LTD and LTP. *Cell* 81: 891–904, 1995.
- MULKEY, R. M. AND MALENKA, R. C. Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. *Neuron* 9: 967–975, 1992.
- MULLER, D., OLIVER, M., AND LYNCH, G. Developmental changes in synaptic properties in hippocampus of neonatal rats. *Brain Res. Dev. Brain Res.* 49: 105–114, 1989.
- O'DELL, T. J. AND KANDEL, E. R. Low-frequency stimulation erases LTP through an NMDA receptor-mediated activation of protein phosphatases. *Learning Memory* 1: 129–139, 1994.
- SIK, A., PENTTONEN, M., YLINEN, A., AND BUZSAKI, G. Hippocampal CA1 interneurons: an in vivo intracellular labeling study. *J. Neurosci.* 15: 6651–6665, 1995.
- SUTTON, R. S. AND BARTO, A. G. Toward a modern theory of adaptive networks: expectation and prediction. *Psychol. Rev.* 88: 135–170, 1981.
- SWANN, J. W., BRADY, R. J., AND MARTIN, D. L. Postnatal development of GABA-mediated synaptic inhibition in rat hippocampus. *Neuroscience* 28: 551–561, 1989.
- THIELS, E., BARRIONUEVO, G., AND BERGER, T. W. Excitatory stimulation during postsynaptic inhibition induces long-term depression in hippocampus in vivo. *J. Neurophysiol.* 72: 3009–3016, 1994.
- TSODYKS, M. V., SKAGGS, W. E., SEJNOWSKI, T. J., AND MCNAUGHTON, B. L. Paradoxical effects of external modulation of inhibitory interneurons. *J. Neurosci.* 17: 4382–4388, 1997.
- TURRIGIANO, G., ABBOTT, L. F., AND MARDER, E. Activity-dependent changes in the intrinsic properties of cultured neurons. *Science* 264: 974–977, 1994.
- WAGNER, J. J. AND ALGER, B. E. GABAergic and developmental influences on homosynaptic LTD and depotentiation in rat hippocampus. *J. Neurosci.* 15: 1577–1586, 1995.
- YANG, X.-D., CONNOR, J. A., AND FABER, D. S. Weak excitation and simultaneous inhibition induce long-term depression in hippocampal CA1 neurons. *J. Neurophysiol.* 71: 1586–1590, 1994.