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Failure to reverse long-term potentiation by coupling sustained presynaptic activity and *N*-methyl-D-aspartate receptor blockade

(hippocampus/memory/2-amino-5-phosphonovalerate/Hebbian synapse)

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ABSTRACT **The proposal that long-term potentiation (LTP) is a mechanism underlying memory in the mammalian brain rests on a number of properties of LTP that parallel characteristics of memory defined by behavior. A prominent feature of behaviorally defined memory is its reversibility. LTP is induced at synapses that correlate in their activity, and the signal for induction is calcium influx through *N*-methyl-D-aspartate (NMDA) receptor channels. By analogy to the reversibility of behaviorially defined memory, uncorrelated synaptic activity might be expected to reverse LTP, an anti-Hebbian effect called long-term depression, which has only recently been described in the hippocampus [Stanton, P. K. & Sejnowski, T. J. (1989) *Nature (London)* 339, 215–218]. Because the extent to which synaptic activity is correlated is represented by postsynaptic calcium concentrations, it seemed likely to us that long-term depression is related to the failure of calcium to pass through the NMDA channel. One way to block the calcium influx that signals correlated synaptic activity is with the NMDA receptor antagonist D-(−)-2-amino-5-phosphonovalerate. We performed a series of experiments in rat hippocampal slices designed to estimate the amount of synaptic depression per afferent test pulse under these conditions. Schaffer collateral–commissural afferents to field CA1 were repetitively stimulated in the presence of 2-amino-5-phosphonovalerate. No enduring synaptic depression nor reversal of LTP could be detected. We conclude that some other mechanism underlies long-term depression in the hippocampus.**

Long-term potentiation (LTP) is considered one of the best models for learning and memory in the human brain because many properties of LTP parallel those of behaviorally defined learning (1, 2). Memory is often distressingly reversible, but only recently has LTP been reported to have this particular property (3); just as correlated activity in synapses increases synaptic strength, so does uncorrelated activity weaken those same synapses, a phenomenon called long-term depression (LTD). This strengthening and weakening of synapses that depends on experience is thus reminiscent of learning and forgetting.

The triggering of LTP is now generally agreed to involve an increase in calcium levels in dendritic spines (4, 5). When activity correlates across synapses the neuron tends to depolarize, and this depolarization relieves the magnesium block of *N*-methyl-D-aspartate (NMDA) receptor channels (6, 7), permitting calcium to flow into the spine heads (8). The increased calcium levels that result from this influx are the first step in LTP induction. Calcium levels, then, represent the degree of correlated synaptic activity, and this information is used by the LTP-induction mechanisms. Because LTD results when a synapse is active, when its use is uncorrelated with activity in other synapses, we reasoned that the signal for lack of correlation might well be the lower calcium levels in dendritic spines. According to this view, synaptic activity with increased spine calcium levels would trigger increased synaptic strength (LTP), whereas the same activity with lower spine calcium levels would produce synaptic weakening (LTD).

The NMDA receptor antagonist D-(−)-2-amino-5-phosphonovalerate (APV) provides a simple tool for testing this idea. Because the antagonist blocks the calcium influx through NMDA receptor channels, any synaptic use in the presence of APV should appear to the neuron as uncorrelated synaptic activity. It has long been known that APV will block LTP induction (9, 10), but continued stimulation in the presence of the antagonist should, according to the above hypothesis, produce LTD and reverse preexisting LTP.

Brief episodes of tetanic stimulation in the presence of NMDA antagonists do not result in synaptic depression (9, 11), possibly because anti-Hebbian processes are less efficient (per pulse) than the Hebbian processes that result in LTP. We have therefore used multiple tetani and several stimulation patterns in an attempt to reverse LTP. Part of this work has been presented in abstract form (12).

METHODS

Transverse hippocampal slices were prepared from male Sprague–Dawley rats (150–220 g). Slices were preincubated in an interface chamber and individually transferred to the recording chamber, where they were kept fully submerged. The modified Krebs' solution consisted of 120 mM NaCl/3 mM KCl/1.2 mM NaH_2PO_4/2.5 mM $CaCl_2$/1.2 mM $MgCl_2$/23 mM $NaHCO_3$/11 mM D-glucose saturated with 95% O_2/5% CO_2 to maintain pH 7.4; temperature was held at 31–33°C. An extracellular recording electrode filled with Krebs' solution or a 9% NaCl solution (1–4 MΩ) was placed in the apical dendritic layer of field CA1. A monopolar platinum-iridium filament (35 μm, o.d.) delivered square, monophasic pulses (100 μs) to the Schaffer collateral–commissural fibers. One or two stimulating electrodes were placed in stratum radiatum to activate independent sets of inputs (for two electrodes). Pathways were considered independent when the response evoked by the simultaneous activation of both afferents was 85–100% of the algebraic sum of the field excitatory postsynaptic potential (EPSP) slope produced by stimulation of either pathway alone. LTP was induced by the application of 100 shocks at 100 Hz, delivered either in 5 bursts of 20 pulses, 1/6 s, or in 20 bursts of 5 pulses,

Abbreviations: LTP, long-term potentiation; LTD, long-term depression; NMDA, *N*-methyl-D-aspartate; APV, D-(−)-2-amino-5-phosphonovalerate; EPSP, excitatory postsynaptic potential; SHFS, sustained high-frequency stimulation; CNQX, 6-cyano-7-nitroquinoxaline-2,3-dione.

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1/200 ms. Data were sampled and digitized at 10 kHz, filtered at 2–5 kHz, and stored onto computer hard disk for subsequent analysis.

RESULTS

Dendritic field potentials were recorded in control medium, and a brief tetanus was delivered to induce LTP. The EPSP slope recorded 30 min after tetanic stimulation was enhanced by 56 ± 4% (mean of 10–20 successive responses ± SEM; n = 27) compared with the responses evoked 5 min before tetanus. APV was then added in the experimental slices, and sustained high-frequency stimulation (SHFS) was delivered. Fig. 1 illustrates the failure of 4000 pulses applied in the presence of 50 μM APV to reverse the LTP.

Several hypotheses can be proposed to account for our failure to reverse LTP: (*i*) A different number of afferent shocks or a stimulation pattern other than that used above may be more efficacious in reversing LTP. (*ii*) Nonspecific slow changes (increase) in synaptic efficacy may be occurring in the slice preparation that obscure the LTP reversal. (*iii*) The time after LTP induction when reversal was attempted was not optimal. (*iv*) LTD may be expressed in field CA1 only in the absence of LTP induced by a high-frequency train—i.e., in a nontetanized group of afferents. (*v*) The concentration of APV used (50 μM) may not be adequate to block the NMDA component of synaptic transmission in our preparation. (*vi*) The postsynaptic events mediated by the activation of the non-NMDA receptors may preclude LTP reversal.

(*i*) To address the first question, we tested the effect of several stimulation patterns (1000–5000 stimuli; burst applied at different frequencies: see Table 1 and Fig. 2) for their ability to reverse LTP. We found that prolonged stimulation in 50 μM APV produced a modest decrease in the EPSP slope directly related to the number of stimuli. For 1000 stimuli the decrease was not significant; for all experiments the average decrease was 17% (see Table 1). Because the LTP was produced by 100 stimuli, the average reversal of LTP per

Table 1. Effect of SHFS on EPSP slope evoked after LTP induction

SHFS, no. of pulses	EPSP slope, %					
	50 μM APV			Control medium		
	n	Pre-SHFS	Post-SHFS	n	Pre-SHFS	Post-SHFS
2000	7	139 ± 7	135 ± 9	4	161 ± 16	155 ± 20
4000	6	140 ± 9	120 ± 13	2	151	131
5000	4	127 ± 5	88 ± 11	4	140 ± 6	117 ± 13
Total	17	137 ± 4	119 ± 8	10	151 ± 7	135 ± 11

SHFS of Schaffer collateral–commissural fibers in the presence of APV does not affect potentiated synaptic responses recorded from CA1. Slices were previously tetanized in control medium to induce LTP, then APV was introduced and prolonged tetani were delivered. The EPSP slope evoked 0–5 min before and 30–45 min after SHFS is expressed as percentage of the response recorded 0–5 min before LTP induction. Several stimulation patterns were tested: (*i*) 400 trains of 5 shocks at 100 Hz; 1 train per 200 ms; 6-s interval between groups of 10 trains (2000 pulses); (*ii*) 800 trains of 5 shocks at 100 Hz; 1 train per 100 ms (n = 4) or 1 train per 200 ms (n = 3) in three sets of 400, 200, 200, delivered about once per 8 min (4000 pulses); (*iii*) 250 trains of 20 shocks at 50 Hz; 1 train per 6 s; three sets of 150, 100, 100 trains separated by ≈8 min each (5000 pulses). Data are presented as mean ± SEM and include the results obtained in experiments with one (n = 14) and two (n = 13) stimulating electrodes.

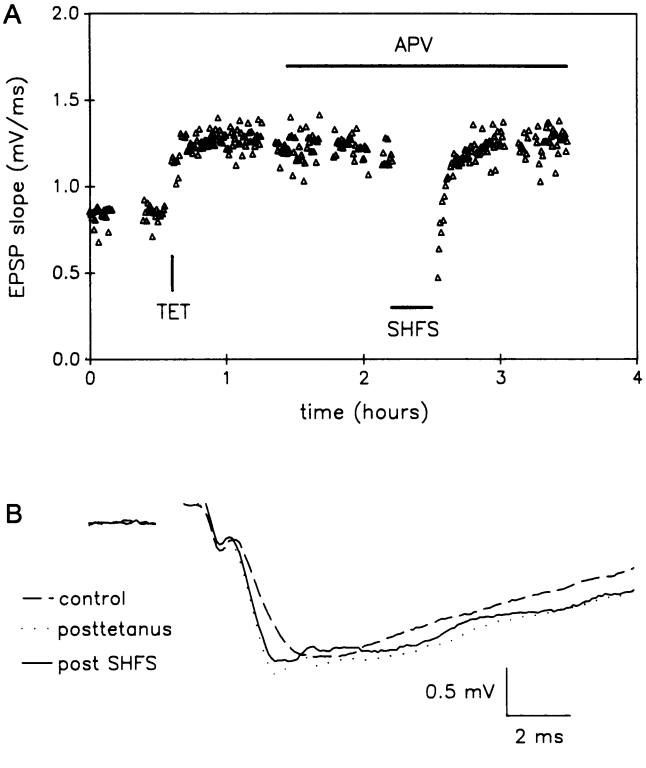

FIG. 1. Failure of 4000 pulses delivered in the presence of 50 μM APV to reverse LTP. (*A*) Tetanus (TET) of 100 pulses induced LTP of the EPSP slope recorded in the apical dendritic layer of area CA1. SHFS (800 trains of five shocks at 100 Hz, 1 train per 200 ms) in the presence of APV produced a transient depression that fully recovered. (*B*) Representative field potentials (average of 10 successive responses) sampled 5 min before and 30 min after tetanization and then 45 min after SHFS. No significant decrease of evoked synaptic potentials was seen after sustained stimulation in the presence of the NMDA antagonist.

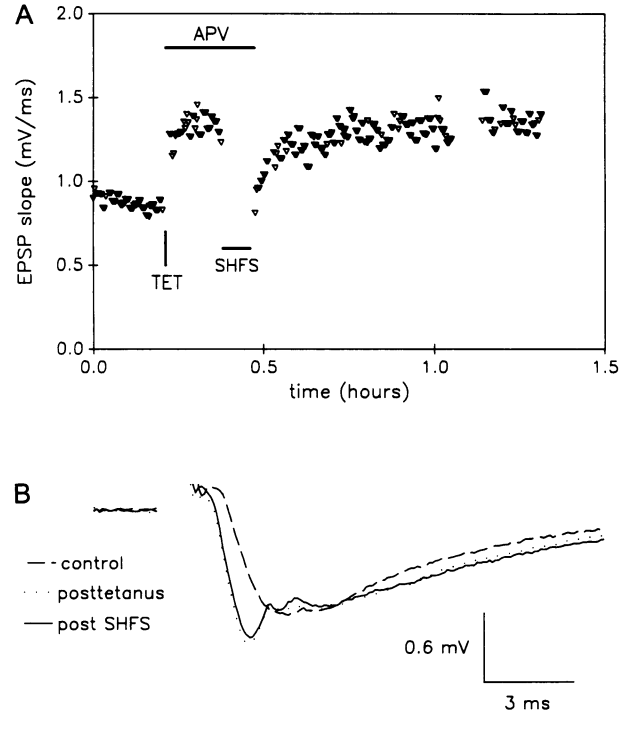

FIG. 2. Effect of SHFS delivered early after LTP induction. Immediately after an initial tetanus (TET), 50 μM APV was added. The high flow rate ensured that APV reached equilibrium rapidly. Ten minutes after tetanization 1000 pulses were applied, followed by APV washout. (*A*) Initial tetanus increased EPSP slope. After sustained stimulation there was an initial depression of the EPSP slope, which leveled off over 7 min to 59% above control. (*B*) Representative records (average of 10 successive responses) recorded before tetanus, 7–10 min posttetanus (just before SHFS), and 30 min poststimulation.

stimulus pulse in APV is <0.4% of the amount of LTP caused per stimulus pulse during induction.

Although massive stimulation in APV did decrease EPSP slope (Table 1), the preceding experiments cannot differentiate between LTP reversal and some "rundown-like" decrease in synaptic efficacy that attends heavy use. To distinguish between these two alternatives we examined the effects of the same heavy synaptic use in slices treated the same way except that APV was absent during stimulation. We anticipated that LTP would saturate in these slices, so that any "rundown" produced would be revealed in circumstances where LTD should not occur. Table 1 shows that the EPSP slope decreased about as much in these slices (16% overall) as it did in the slices stimulated in APV (18%). Although some slight reversal of LTP may have occurred, stimuli in APV are much less than 1% as effective in reversing LTP as are the stimuli that induce it.

(*ii*) To test the second hypothesis, a second stimulating electrode was used to monitor synaptic efficacy in an independent afferent pathway (9 of 17 experiments were done in APV). Brief tetanic stimulation resulted in LTP of the stimulated pathway alone, and no significant change occurred in the nontetanized pathway. LTP was produced alternatively in both pathways. Only one set of fibers received sustained stimulation in the presence of APV; the other pathway served as control. No difference was seen between stimulated ($-17 \pm 6\%$) and control pathway ($-11 \pm 5\%$, $P > 0.1$, Wilcoxon test; responses measured 30–45 min after sustained stimulation). No slow increase in the EPSP slope was recorded in the independent pathway that may have obscured the reversal of LTP.

(*iii*) Evidence favoring two temporal components of LTP has been reported (13, 14). In the experiments described above the prolonged stimulation in APV was given at least 1 hr after LTP induction and hence would test for reversal during the second stage. Perhaps the synapse can integrate the covariance of pre- and postsynaptic activity only during the initial stages of LTP induction. We tested this idea by applying a prolonged tetanus in APV within 5–10 min after giving a brief tetanus in control solution (Fig. 2). We believe that APV acted within a few minutes because in a separate group of experiments we have observed that with a rapid perfusion rate (5–10 ml/min), addition of 50 μM APV to a slice bathed in 0 mM Mg^{2+} and the quisqualate receptor antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (20 μM; ref. 15) produced near-complete suppression of evoked responses within 2 min (data not shown). One thousand to two thousand pulses delivered in 50 μM APV 5–10 min posttetanus did not decrease LTP in four of five slices (mean LTP +57 ± 19%). In one of five slices in this series of experiments LTP could not be produced.

(*iv*) LTD has been reported in CA1 synapses that have not been previously potentiated (3). The fourth hypothesis to explain our failure to produce synaptic depression was addressed by testing the effect of 2000 pulses delivered in the presence of 50 μM APV in slices not previously tetanized. No depression of evoked responses was recorded (+27 ± 8%, $n = 4$). Fig. 3*A* demonstrates the failure of 2000 pulses in either 50 or 200 μM APV to produce synaptic depression. Upon APV washout, a further 2000 pulses produced a long-lasting (33%) increase in EPSP slope.

(*v*) To estimate the blockade of the NMDA component of synaptic transmission by 50 μM APV, we recorded the amplitude of an APV-sensitive, long-lasting postsynaptic depolarization occurring during a high-frequency train of stimuli, which has been attributed to current flow through the NMDA receptor channel (16). Fig. 3 *B* and *C* demonstrate the response to the first burst of five pulses during SHFS. As expected, 50 μM APV significantly reduced the cumulative depolarization induced by the tetanus (Fig. 3*B*). However no appreciable difference was found between the response in 50 and 200 μM APV (Fig. 3*C*), indicating that 50 μM was as effective in blocking the NMDA channel as was the higher dose.

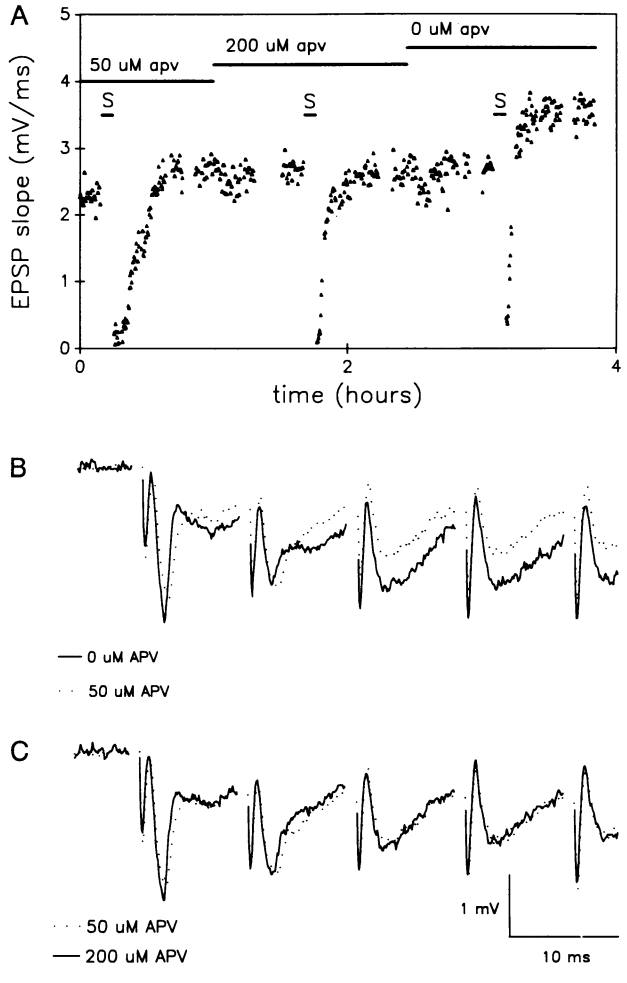

FIG. 3. Effect of SHFS in the presence of various concentrations of APV in a slice not previously tetanized. (*A*) Two thousand pulses given in the presence of 50 μM APV produced a small increase in EPSP slope. In 200 μM APV there was no further change in EPSP after the same stimulation. A long-lasting increase in EPSP is noted upon an additional 2000 pulses applied after APV washout. (*B*) The first burst of 5 pulses at 100 Hz during SHFS. Fifty micromolar APV inhibits cumulative postsynaptic depolarization recorded in the dendritic layer. (*C*) No additional effect on the slow synaptic component was produced by 200 μM APV.

In another series of experiments, we studied the effect of APV on the synaptic response evoked in 0 mM Mg^{2+}/20 μM CNQX/10 μM picrotoxin. This component, which represents the NMDA contribution to synaptic transmission (15), was reduced in a dose-dependent manner by APV (Fig. 4). Fifty micromolar APV blocked 97% of the EPSP recorded under these conditions. Calcium entry through the remaining unblocked NMDA receptor channels could conceivably be sufficient to prevent the detection of a negative covariance by the activated synapses. However, results from other experiments using different APV dosages did not show any significant difference from those obtained with 50 μM APV (see above).

(*vi*) Perhaps activation of non-NMDA receptors prevents LTP reversal. We tested this hypothesis by applying sustained stimulation (2000 pulses) in the presence of the broad-spectrum glutamate antagonist kynurenate (1 mM) or CNQX (2 μM) with APV (50 μM). This did not cause any decrement

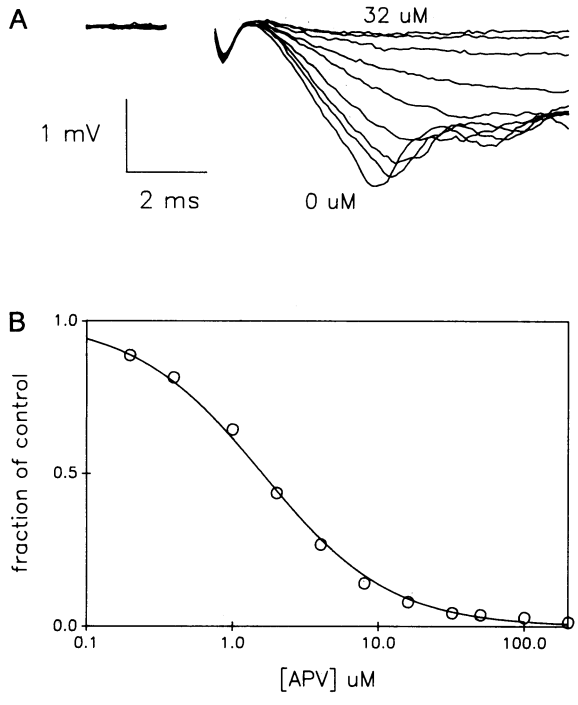

FIG. 4. Dose-dependent suppression by APV of the NMDA component of synaptic transmission. Slices were bathed in 0 mM Mg^{2+}/20 μM CNQX (one slice) and 0 mM Mg^{2+}/20 μM CNQX/10 μM picrotoxin (two slices). Addition of cumulative doses of APV progressively reduced EPSP. (*A*) Superimposed field EPSPs (average of 10 successive responses) evoked in the presence of 0–32 μM APV in a single slice. (*B*) Dose–response curve for APV effect on the EPSP amplitude measured at a fixed latency after test stimulus onset (4.4–5.0 ms). Data are expressed as a fraction of the response evoked without APV. ○, Experimental data (average of three slices); curve was fitted assuming a single binding site and K_d of 1.6 μM.

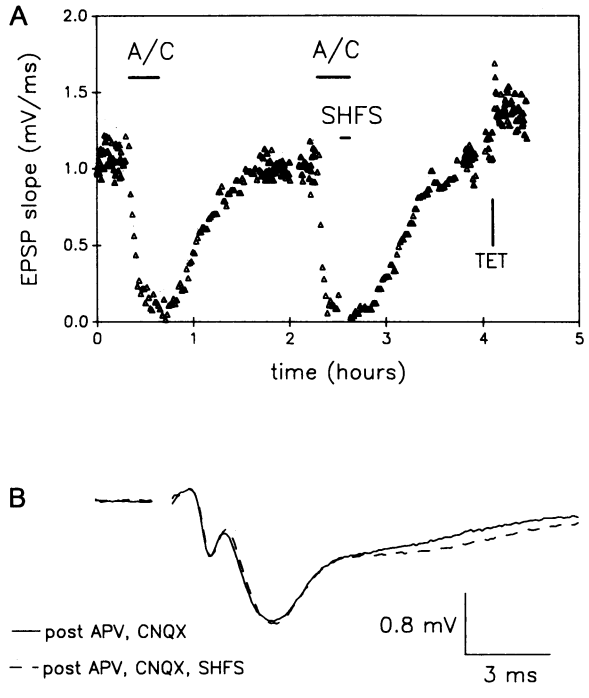

FIG. 5. Effect of SHFS in APV/CNQX (A/C) in a slice not previously tetanized. (*A*) Addition of APV/CNQX abolishes the evoked response, which returns to baseline upon washout. Applying SHFS in APV/CNQX does not produce a long-lasting change upon drug washout. Tetanization (100 pulses) after drug washout produced a long-lasting increase. (*B*) Representative records of the average of 10 successive responses recorded between 75 and 90 min after washout of APV/CNQX, demonstrating no effect of SHFS in the presence of the drugs.

of the EPSP slope either (+2 ± 3%, n = 4; response taken after washout of drugs). Fig. 5 illustrates an example of the failure of prolonged stimulation given in the presence of APV/CNQX to depress the evoked response.

DISCUSSION

Two different protocols of stimulation have been shown to induce a long-lasting depression of synaptic responses in the hippocampus. In the dentate, a depression of potentiated responses has been reported to be produced by high-frequency stimulation of a separate afferent pathway (heterosynaptic depression, refs. 17 and 18). However, heterosynaptic depression has not been observed in field CA1 (19, 20). We have found that even prolonged stimulation (2000 pulses) produced only transient (<15 min) heterosynaptic depression (data not shown).

Recently, a different protocol than the one we used has been shown to produce LTD, a synapse-specific reversal of LTP in field CA1 (3). Given the existence of endogenous mechanisms for NMDA receptor/channel modulation (21, 22), the key role played by NMDA receptors in LTP induction (9), and evidence that NMDA antagonists disrupt other types of synaptic plasticity (23, 24), one would anticipate that LTD associated with hyperpolarization is due to the blockade of the NMDA channel. Our main finding in this study is that presynaptic activity uncoupled from postsynaptic NMDA receptor activation does not reduce synaptic strength. We calculate that any synaptic decrease associated with presynaptic activity and inhibition of the NMDA channel is well under 1% of the increase-per-pulse associated with LTP. APV has been shown not to block LTD (3, 25), so we should have observed this effect if presynaptic activity in the absence of increased spine calcium concentration were the signal for lack of correlation. We conclude, therefore, that some unanticipated mechanism underlies LTD in the hippocampus.

Another alternative is that LTD is a transient, rather than an enduring, effect. As our protocol involved prolonged tetanization, from which the slice took some time to recover, we would not have detected synaptic depression of a brief duration. As the duration of LTD in hippocampus has not yet been established, this alternative remains a possible explanation for our observations.

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