Long-Term Depression of Temporoammonic-CA1 Hippocampal Synaptic Transmission

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INTRODUCTION

The hippocampus is a brain structure that plays a critical role in learning and memory (Eichenbaum et al. 1992; Press et al. 1989; Squire and Zola-Morgan 1991; Zola-Morgan and Squire 1990). The fundamental information processing pathway in the hippocampus is usually considered to be the trisynaptic circuit, in which the entorhinal cortex sends a perforant path projection to the granule cells of the dentate gyrus, which send mossy fibers to the pyramidal cells of the CA3 region, which send Schaffer collaterals to the CA1 pyramidal cells, which then project back to the entorhinal cortex (Andersen et al. 1966; Brown and Zador 1990). These projections are all glutamatergic and excitatory (Andersen 1975; Misgeld 1988). Within each region there are local GABAergic interneurons that provide feedforward and feedback inhibition (e.g., see Freund and Buzsáki 1996; Lacaille et al. 1989; Ribak and Seress 1983; Woodson et al. 1989).

However, there is more to hippocampal processing than this simple trisynaptic loop. In particular, there is a direct projection from entorhinal cortex to area CA1, effectively bypassing the first two stages of the conventional circuit. This projection is referred to as the temporoammonic pathway (Maccaferri and McBain 1995; Ramón y Cajal 1911) because of its origins in the entorhinal cortex (in the temporal lobe) and its termination in CA1, part of the cornu ammonis of the hippocampus. Unlike the perforant path, which consists of axons from stellate excitatory neurons of layer II of the entorhinal cortex, the temporoammonic pathway consists of axons from pyramidal cells of layer III of the entorhinal cortex (Steward and Scoville 1976). These temporoammonic axons terminate preferentially in the area of the distal dendrites of the pyramidal cells, stratum lacunosum-moleculare (SLM). These axons make asymmetric (and hence probably excitatory) synapses, >90% of which are onto the spines of CA1 pyramidal cells (Desmond et al. 1994). Temporoammonic axons also synapse onto the inhibitory basket and chandelier cells of CA1 (Kiss et al. 1996) and are likely to innervate the interneurons of SLM (Lacaille and Schwartzkroin 1988; Vida et al. 1998).

There is some controversy as to whether the temporoammonic input to the hippocampus is primarily excitatory or primarily inhibitory (see Soltész and Jones 1995). Field recordings in vitro (Colbert and Levy 1992; Doller and Weight 1982) and in vivo (Leung et al. 1995; Yeckel and Berger 1990, 1995) reveal a population excitatory postsynaptic potential (EPSP) in SLM after stimulation of the temporoammonic pathway, supporting the ultrastructural evidence for an excitatory input onto the distal dendrites of CA1 neurons. However, intracellular recordings (Empson and Heinemann 1995) show a mixed response, including a nonsynaptic glutamatergic EPSP and a disynaptic inhibitory postsynaptic potential with both GABA_A and GABA_B components.

Various forms of short- and long-term plasticity have been extensively studied at all three synapses in the trisynaptic circuit of the hippocampus (Bliss and Collingridge 1993). As yet, however, relatively little is known about plasticity in the temporoammonic pathway, whose activity can modulate plasticity in the Schaffer collateral pathway to CA1 (Levy et al. 1998) and whose position in the hippocampal circuitry suggests a potent role in the modulation of hippocampal output from CA1. A previous study reported induction of long-term potentiation (LTP) in this pathway in vitro only when fast inhibition was blocked by addition of the GABA_A antagonist bicuculline (Colbert and Levy 1993); in vivo, LTP of the excitatory current sink in the distal dendrites of area CA1 evoked by stimulation of the perforant path (including tem-
Tissue preparation

Slices were prepared from 6- to 8-wk old male Sprague-Dawley rats. All use of animals was performed according to the guidelines of the Caltech Institutional Animal Care and Use Committee. Rats were decapitated after halothane anesthesia, and the brain was rapidly removed to ice-cold, oxygenated artificial cerebrospinal fluid (ACSF) containing (in mM) 119 NaCl, 2.5 KCl, 1.3 MgSO₄, 2.4 CaCl₂, 1.0 NaH₂PO₄, 26.2 NaHCO₃, and 11.0 glucose. The dorsal surface of the posterior half of each hemisphere was glued onto the stage of a cooled chamber at room temperature for 1 h before experiments were started. Further microdissection was performed either in ice-cold ACSF immediately after slice preparation or in the recording chamber before the start of the experiment. All electrophysiology was done with the slices submerged and constantly perfused with oxygenated ACSF at room temperature.

To clearly isolate the temporoammonic response, it was necessary to further dissect the slice (see Fig. 1). The entire dentate gyrus was removed to eliminate the possibility of activation of the trisynaptic pathway and to prevent contamination of a field response recorded in SLM by the much larger field elicited in dentate gyrus by concurrent activation of the perforant path. In most experiments, including all those in which bicuculline was used, CA3 was also removed to prevent induction of seizure-like activity as well as to eliminate the possibility of disynaptic activation via the perforant path projection to CA3. Also, a cut was made through stratum radiatum (SR) in distal CA1 (near the subiculum) perpendicular to the cell body layer to prevent antidromic activation of Schaffer collaterals by the stimulating electrode in SLM (see Fig. 1). Schaffer collaterals do not enter SLM to any appreciable extent (Amaral and Witter 1989), so this cut cleanly isolates temporoammonic axons.

Electrophysiology

Bipolar tungsten electrodes, either concentric or paired needles, were used for stimulation. One electrode was placed in SR to stimulate the Schaffer collaterals; the other was placed in SLM to stimulate the temporoammonic pathway (Fig. 1). Stimulus pulses were 100 μs long, monophasic, and ranged from 10 to 100 μA in the Schaffer collateral pathway and 100 to 200 μA in the temporoammonic pathway. Stimulus intensities were selected to produce submaximal responses with no population spike. Field recordings were made with low-resistance micropipettes filled with 3 M NaCl. The Schaffer collateral response was recorded in SR, and the temporoammonic response was recorded in SLM. Separation of the two pathways was further confirmed by the observation of a positive-going field potential in the other layer (Fig. 1) (Colbert and Levy 1992).

The following stimulation paradigms were used: high-frequency stimulation (HFS) = 100 Hz for 1 s, repeated four times at 20- or 30-s intervals; θ-burst stimulation (TBS) = four bursts of five pulses at 100 Hz, 200 ms between bursts, repeated four times at 20- or 30-s intervals; LFS = 1 Hz stimulation for 10 min. All stimulus pulses were of the same length and amplitude as test pulses. Test pulses were applied once every 20 or 30 s to each pathway. The initial slope of the field potential after the end of the fiber volley was measured.

Drugs were applied by dilution of concentrated stock solutions into the perfusion medium. Stock solutions were made up in water, with the exception of nifedipine, which was prepared in DMSO (×1,000) and stored protected from light. Experiments with nifedipine were performed in low light. CGP 55845A was a kind gift from Novartis (Basel, Switzerland); all other drugs were obtained from Sigma (St. Louis, MO).

Data analysis

Data were collected directly onto an IBM-compatible computer with in-house software. All numerical values are listed as means ± SE unless otherwise stated. Depression and potentiation were measured by taking an average of the initial slopes of the field EPSPs (fEPSPs) over 10-min periods immediately before and 20–30 or 50–60 min after the end of LFS, HFS, or TBS. Student’s paired t-test was used to determine statistical significance for

FIG. 1. A: microdissected slice recording configuration for field temporoammonic (TA) responses, recorded in stratum lacunsum-moleculare (SLM), and field Schaffer collateral (SC) responses, recorded in stratum radiatum (SR). The dentate gyrus and CA3 regions, shown in dotted lines, are dissected away before starting the experiment. A cut is made through stratum radiatum in distal CA1 to prevent antidromic SC stimulation from contaminating the TA response. B: representative field potentials showing negative-going fields in SR in response to SC stimulation and in SLM in response to TA stimulation; meanwhile, positive-going fields are seen in SR in response to TA stimulation and in SLM in response to SC stimulation. Scale bar: 0.2 mV/30 ms.
within-group comparisons; the unpaired \( t \)-test was used between groups. Results from each experimental manipulation were compared with the same control group. \( P \) values >0.05 are reported in the text as not significant (NS). Points in figures represent means ± SE across all experiments; each point is the average of data taken over 5 min. Representative traces, shown in insets, are averages of five consecutive sweeps from a representative experiment, taken 5 min before LFS, HFS, or TBS and 25 min after the end of LFS, HFS, or TBS.

**RESULTS**

**Temporoammonic field response is depressed by LFS**

When LFS (see METHODS) was applied to the temporoammonic pathway in normal ACSF, the response was significantly depressed (Fig. 2A) (mean percent of baseline at 30–40 min, 75.9 ± 3.4%, \( n = 26 \), \( P < 0.0001 \)). This synaptic depression persisted for >1 h (mean percent of baseline at 60–70 min, 73.7 ± 5.0%, \( n = 7 \), \( P < 0.01 \)) and was not accompanied by any significant changes in the size of the presynaptic fiber volley (Fig. 2A).

During the application of LFS to the temporoammonic pathway, the Schaffer collateral pathway was not stimulated; during the rest of the experiment, test pulses were applied to the Schaffer collateral pathway at the same frequency as to the temporoammonic pathway. Synaptic strength in the Schaffer collateral pathway was not affected, whereas the temporoammonic response was depressed by LFS (Fig. 2A) (mean percent of baseline at 30–40 min, 95.0 ± 4.7%, \( n = 9 \), NS).

LTD has also been observed after LFS of the Schaffer collateral pathway (Mulkey and Malenka 1992) but only in slices prepared from younger animals (Dudek and Bear 1993; Wagner and Alger 1995). When the LFS protocol was applied to the Schaffer collateral pathway in this study, little or no depression was seen. On average, a small but not statistically significant depression of the response was observed (Fig. 2B) (mean percent of baseline at 30–40 min, 87.3 ± 6.2%, \( n = 8 \), NS); this trend was due only to results from two slices that were depressed to 55 and 65% of baseline.

To determine whether LTD of the temporoammonic pathway can be saturated, the LFS protocol was applied repeatedly for 10 min every 30 min either four or five times. The response asymptotically approached a level of ~30–50% of the original baseline (Fig. 2C), reaching its maximally depressed level after three or four epochs of LFS.
Synaptic depression of the temporoammonic response could be induced in the absence of fast GABAergic inhibition. After the GABA_A antagonist bicuculline (20 μM) was added to the perfusion solution, a slight but not significant increase in the temporoammonic field response was generally observed (mean percent of original response 20–25 min after bicuculline application, 105 ± 6%, n = 4, NS). The response was allowed to reach a steady baseline for ≥20 min before application of LFS. LFS in the presence of bicuculline induced depression of the field response (Fig. 3) (mean percent of baseline at 30–40 min, 75.7 ± 2.6%, n = 20, P < 0.0001) that was not significantly different from that observed in control slices. This depression lasted ≥1 h (mean percent of baseline at 60–70 min, 79.9 ± 3.2%, n = 14, P < 0.001).

Depression of the temporoammonic response was also possible in the absence of slow, GABA_B-mediated inhibition. Addition of the GABA_B antagonist CGP 55845A (1 μM) had no significant effect on the baseline field potential elicited by temporoammonic stimulation (mean percent of original response 20–25 min after CGP 55845A application, 112 ± 7%, n = 5, NS). When LFS was applied in the presence of CGP 55845A, the field response was still significantly depressed (Fig. 3) (mean percent of baseline at 30–40 min, 72.7 ± 3.5%, n = 5, P < 0.01). This amount of depression was also not significantly different from that seen in control slices.

Blockade of NMDA receptor-mediated transmission reduces temporoammonic LTD

What are the early signaling events important for establishing temporoammonic LTD? Calcium ion is an important ini-
tiator of many short- and long-term plasticity processes in neurons (Bliss and Collingridge 1993; Delaney et al. 1989; Katz and Miledi 1968; Neveu and Zucker 1996). LTD induction in other hippocampal pathways has been shown to be NMDA receptor dependent (Cummings et al. 1996; Mulkey and Malenka 1992; Thiels et al. 1996). When LFS was applied to the temporoammonic pathway in the presence of the NMDA receptor antagonist, n,N'-2-amino-5-phosphonovaleric acid (AP5; 50 μM), LTD was significantly reduced relative to control (P < 0.05) (Fig. 4) (mean percent of baseline at 30–40 min, 90.1 ± 5.4%, n = 15, P < 0.05; 60–70 min, 91.2 ± 4.8%, n = 5, P < 0.05), although a small, but significant amount of residual depression was still observed. It is worth noting that there was variability between experiments; in half of the experiments AP5 treatment appeared to block LTD, whereas in the other half it had little effect. LTD was not blocked further when higher concentrations of AP5 were used; in the presence of 100 μM AP5, LTD was similarly reduced but not completely blocked (data not shown) (mean percent of baseline at 30–40 min, 88.3 ± 1.5%, n = 3, NS different from depression in 50 μM AP5).

In the hippocampus, some forms of heterosynaptic LTD are dependent on L-type calcium channel activation (Christie and Abraham 1994; Wickens and Abraham 1991). However, homosynaptic temporoammonic LTD was not blocked by the presence of the L-type calcium channel blocker nifedipine (20 μM) (data not shown; mean percent of baseline at 30–40 min, 82.6 ± 1.7%, n = 5, P < 0.05). The combination of nifedipine and AP5 produced slightly, although not significantly, greater inhibition of temporoammonic LTD than AP5 alone (Fig. 4) (mean percent of baseline at 30–40 min, 93.4 ± 4.3% of baseline, n = 7, NS different from AP5 alone, significantly different from baseline (P < 0.05)). The block of temporoammonic LTD by AP5 and nifedipine was reversible; when LFS was applied in the presence of AP5 and nifedipine, LTD was not blocked further when higher concentrations of AP5 were used; in the presence of 100 μM AP5, LTD was similarly reduced but not completely blocked (data not shown) (mean percent of baseline at 30–40 min, 88.3 ± 1.5%, n = 3, NS different from depression in 50 μM AP5).
was applied again to slices 30 min after washout of the drugs, significant depression was observed (data not shown; mean percent of baseline at 30–40 min, $70.7 \pm 6.4\%$, $n = 7$, $P < 0.0001$).

Blockade of muscarinic receptors does not affect temporoammonic LTD

In addition to the temporoammonic projection, SLM of CA1 also receives a substantial cholinergic input from the medial septum (Matthews et al. 1987). Activation of the muscarinic ACh receptor (mAChR) is implicated in other forms of hippocampal synaptic plasticity (Auerbach and Segal 1996). To determine whether mAChRs are involved in temporoammonic LTD, we applied LFS in the presence of 1 mM atropine.

Atropine itself had no significant effect on the temporoammonic field response. In the presence of atropine, the temporoammonic field response was depressed to the same extent as in control ACSF (Fig. 5) (mean percent of baseline at 30–40 min, $75.1 \pm 5.0\%$, $n = 4$, NS different from control).

Reversal of temporoammonic LTD

To test whether LTD in the temporoammonic pathway is reversible, HFS (see METHODS) was applied either 30 or 60 min after the end of LFS. In experiments conducted in normal ACSF, HFS induced a significant recovery of the depressed synaptic response (Fig. 6A, $\bullet$) (mean percent of original baseline at 20–30 min, $89.4 \pm 3.6\%$, $n = 22$, $P < 0.05$). The increase relative to the depressed baseline was $116.3 \pm 3.6\%$ of baseline ($n = 22$, $P < 0.0001$). The reversal of LTD was even greater when HFS was applied in the presence of 20 mM bicuculline (Fig. 6A, $\Delta$) (mean percent of original baseline at 20–30 min, $115.3 \pm 4.8\%$, $n = 18$, $P < 0.05$). The increase relative to the depressed baseline was $150.1 \pm 9.1\%$ of baseline ($n = 18$, $P < 0.0001$). This difference in response to HFS after LTD is similar to that seen when HFS was applied to naive slices.

Complete reversal of temporoammonic LTD was achieved by repeated application of HFS. In normal ACSF, three applications of HFS at 5-min intervals resulted in a full recovery to the original baseline response, which persisted for 1 h after the last HFS (Fig. 6B) $[\text{mean percent of original baseline at 30–40 min, } 106.8 \pm 9.0\%, n = 5, \text{ significantly different from depressed level of } 76.4 \pm 4.5\% (P < 0.01)]$, NS different from baseline; mean percent of original baseline at 60–70 min, $103.3 \pm 6.4\%$, $n = 5$, significantly different from depressed level ($P < 0.01$), NS different from baseline).

Long-term potentiation

In several hippocampal pathways, HFS or TBS can induce long-term potentiation (LTP) (Bliss and Collingridge 1993). When HFS was applied to the temporoammonic pathway in naive slices, little potentiation of the field response was observed (Fig. 7A, $\bullet$) (mean percent of baseline at 20–30 min,

A

control $\bullet$

bic $\triangle$

B

4X HFS

3X (4X HFS)

FIG. 6. Temporoammonic LTD can be partially or wholly reversed by high-frequency stimulation (HFS = 100 Hz for 1 s). $A$: in normal ACSF ($\bullet$), HFS applied to the depressed temporoammonic pathway resulted in a recovery above the depressed baseline but below the original baseline (---). In the presence of 20 mM bicuculline, HFS resulted in a potentiation significantly above the original baseline. $Top$: superimposed representative field potentials taken 5 min before and 35 min after the first HFS application. Scale bar: 0.2 mV/30 ms.

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observed when TBS was applied (Fig. 7B). This potentiation requires activation of NMDA receptors. A: HFS applied in the presence of 20 µM bicuculline (bic) results in significant potentiation of the temporoammonic response, while the same protocol applied in normal ACSF (●) results in little or no potentiation. In the presence of both bicuculline and 50 µM AP5 (bic + AP5), no potentiation is observed. Top: superimposed representative field potentials taken 5 min before and 25 min after HFS of temporoammonic pathway in normal ACSF (control), 20 µM bicuculline (bic), or 20 µM bicuculline plus 50 µM AP5 (bic + AP5). Scale bar: 0.2 mV/30 ms. B: θ-burst stimulation (TBS) potentiates the temporoammonic response only when applied in the presence of 20 µM bicuculline (bic); TBS in normal ACSF (●) has little effect. Top: superimposed representative field potentials taken 5 min before and 25 min after TBS of temporoammonic pathway in normal ACSF (control) or 20 µM bicuculline (bic). Scale bar: 0.2 mV/30 ms.

**DISCUSSION**

We examined the capacity for long-term synaptic modification of the temporoammonic CA1 synapse in the hippocampus. LTD of the commissural input to CA1 has been shown in adult animals in vivo, although only by means of application of a paired-pulse LFS protocol (Thiels et al. 1994).

Repeated application of LFS to the temporoammonic pathway resulted in saturation of depression at a maximal level of ~40–50% of the original baseline, similar to or lower than that seen in the Schaffer collateral pathway (Dudek and Bear 1993; Mulkey and Malenka 1992). However, after cessation of LFS, the response rebounded somewhat, suggesting that there may be a “floor” below which the temporoammonic response can be pushed only temporarily. This transient, larger depression may also be analogous to the short-term potentiation seen after tetanic stimulation, which then decays away to reveal LTP of lesser magnitude.

LTD in the temporoammonic pathway is independent of GABA_A or GABA_B receptor activation. This also contrasts with Schaffer collateral LTD, where LTD induced by LFS in slices from adult animals is enhanced in the presence of the GABA_A antagonist bicuculline, and LTD in young animals is inhibited by the GABA_B antagonist CGP 35348 (Wagner and Alger 1995). Although stimulation of the temporoammonic pathway does clearly activate interneurons, which in turn make both GABA_A^- and GABA_B^-mediated synapses onto CA1 pyramidal cells (Dvorak and Schuman 1997; Empson and Heinemann 1995), depression of the monosynaptic, excitatory component of this pathway is neither enhanced nor reduced by interneuron activity. The difference between Schaffer collateral and temporoammonic LTD in terms of the involvement of inhibition may be due to the fact that stimulation in SLM may activate GABAergic pathways only disinaptically, in a feed-forward fashion (Empson and Heinemann 1995), whereas stimulation in SR to activate the Schaffer collaterals can also directly activate axons of CA1 interneurons (e.g., see Arai et al. 1995; Lambert et al. 1991). The differences in temporal patterning of excitation and inhibition in the Schaffer collateral and temporoammonic pathways may play a role in the differential responses to LFS shown by these two pathways.

The induction of homosynaptic LTD in other pathways requires an increase in intracellular Ca^{2+} concentration, either by influx through NMDA receptors (Cummings et al. 1996;
fibers closer to the subiculum (Witter et al. 1989). It should CA1, with medial fibers terminating closer to CA2 and lateral projection, on the other hand, maps along the transverse axis of the gyrus; the LPP terminates in the outer third of the molecular layer. The two projections may also differ in their dependence on NMDA receptor dependence of LTP induction (Colino and Mulkey 1993), although in vitro studies show no difference in NMDA receptors are found in the distal dendrites of CA1 pyramidal cells, although not as densely in SLM as in SR (Jacobson and Cottrell 1993; Jarvis et al. 1987; Monaghan and Cotman 1985), and an NMDA receptor-mediated response to temporoammonic stimulation was observed physiologically (Colbert and Levy 1992; Empson and Heinemann 1995); furthermore, LTP of the temporoammonic pathway was fully blocked by 50 μM AP5 (Fig. 7). It therefore seems unlikely that the incomplete block of temporoammonic LTD by AP5 is due to an absence or paucity of NMDA receptors in SLM. Conversely, although calcium imaging studies show that some voltage-dependent calcium channels are clearly present in the distal dendrites of hippocampal pyramidal cells, L-type calcium channels are most abundant close to the soma, with much lower densities in the more distal dendrites (see Johnston et al. 1996). This may account for the relatively small effect of nifedipine on temporoammonic LTD. The mechanism of the induction of the residual, non-NMDA component of temporoammonic LTD remains to be elucidated. Other studies found a contribution of calcium release from intracellular stores (Reyes and Stanton 1996; Monaghan and Cotman 1985), and metabotropic glutamate receptor activation (Oliet et al. 1997; O’Mara et al. 1995) to homosynaptic LTD at other hippocampal synapses; these calcium sources may also contribute to temporoammonic LTD.

One possible explanation for the variability between experiments in the sensitivity of temporoammonic LTD to blockade of NMDA receptors may be a difference between temporoammonic fibers arising in the lateral and medial entorhinal cortex. There are distinct physiological differences between the lateral and medial perforant path (LPP and MPP) projections to dentate gyrus (McNaughton 1980; McNaughton and Barnes 1977). The two projections may also differ in their dependence on NMDA receptor activation for LTP, at least in vivo (Bramham et al. 1991), although in vitro studies show no difference in NMDA receptor dependence of LTP induction (Colino and Malenka 1993). The LPP and MPP can be activated and recorded independently in vivo or in vitro by virtue of the laminar segregation of their axon terminals along the proximo-distal axis of the dendrites of the granule cells of the dentate gyrus; the LPP terminates in the outer third of the molecular layer, whereas the MPP terminates in the middle third of the molecular layer (Witter et al. 1989). The temporoammonic projection, on the other hand, maps along the transverse axis of CA1, with medial fibers terminating closer to CA2 and lateral fibers closer to the subiculum (Witter et al. 1989). It should therefore in theory be possible to selectively record responses to either medial or lateral inputs by varying the position of the electrode along the transverse axis of the hippocampal slice. We did not systematically monitor this, but the bulk of our experiments was performed with the recording electrode somewhere in the middle of the CA1 area, where it would likely record inputs from both pathways. We did perform a small number of experiments with two recording electrodes in SLM at either end of CA1 but observed no consistent differences in LTD expression or AP5 sensitivity (Dvorak-Carbone and Schuman, unpublished data). However, this question would likely best be resolved by in vivo experiments where the lateral and medial temporoammonic fibers could more unambiguously be stimulated independently.

SLM, the terminal field of the temporoammonic pathway, receives a strong cholinergic input from the septum (Matthews et al. 1987), and mAChR activation has been implicated in some forms of hippocampal LTD (Auerbach and Segal 1996). Because stimulation directly in SLM could have activated septal axons remaining in the slice, it was important to show that the LTD observed was not due to release of ACh from septal afferents. Temporoammonic LTD was unaffected by application of the mAChR antagonist atropine (Fig. 5), showing that the observed depression was not due to the activation of cholinergic inputs.

When studying synaptic depression, it is important to show that the effect of LFS on the synaptic response does not simply reflect damage to the synapses or general degradation of the slice. To monitor the health of the slice, we applied test stimuli to the Schaffer collateral pathway alternately with the test stimuli applied to the temporoammonic pathway and observed no change in the Schaffer collateral response while the temporoammonic response was depressed (Fig. 2A). The ability to reverse synaptic depression with HFS can also counterindicate synaptic rundown or poor slice health (Dudek and Bear 1993; Mulkey and Malenka 1992). When we applied HFS to a depressed temporoammonic pathway, we observed a partial recovery of the response to a level between the depressed level and the original baseline; repeated application of HFS brought the response back up to the original baseline, suggesting that the depressed temporoammonic pathway had not suffered some nonspecific damage (Fig. 6). Furthermore, HFS applied to the depressed pathway in the presence of bicuculline resulted in complete recovery and potentiation of the response above the original baseline level (Fig. 6).

The requirement for multiple applications of HFS to bring the depressed temporoammonic response back to baseline after LTD is consistent with the lack of LTP seen in naive slices. In agreement with a previous report (Colbert and Levy 1993), we were able to potentiate the temporoammonic response in naive slices by HFS or TBS only in the presence of bicuculline. Compared with the Schaffer collateral response, the temporoammonic field response is fairly small to begin with and might therefore require disinhibition before it can be potentiated; indeed, it has been shown that LTP cannot be induced in this pathway, even in the presence of bicuculline, if the initial response is too small (Colbert and Levy 1993). [LTP of the temporoammonic pathway in the slice preparation in the presence of intact inhibition has been shown only in a situation where the temporoammonic input was stimulated so as to elicit a population spike recorded in stratum pyramidale (Doller and
Weight 1985); we never observed population spike activity in response to temporoammonic stimulation.[1] The LTP that was induced by HFS of the temporoammonic pathway was dependent on the activation of NMDA receptors (Fig. 7), just like LTP in many other hippocampal pathways (Bliss and Collingridge 1993).

The entorhinal cortex is not the only brain region to send a projection to SLM of area CA1. Fibers from nucleus reuniens thalami (Dollemen-Van Der Weel and Witter 1996; Wouterlood et al. 1990), the amygdala (Petrovich et al. 1997), and area TE of inferotemporal cortex (Yukie and Iwai 1988) also terminate in SLM, raising the possibility that activity in these areas might also serve to modulate or gate information flow through the trisynaptic circuit. It is worth noting that extracellular stimulation electrodes placed in SLM may well activate these fibers as well as temporoammonic axons.

The function of the temporoammonic pathway and of other inputs to SLM is not yet well understood. In vivo studies suggest that the temporoammonic input plays a role in the generation of \( \theta \) oscillations (Buzsáki et al. 1995) and 40-Hz oscillations (Charpak et al. 1995); models of the hippocampus as a heteroassociative learning network include the temporoammonic pathway and the Schaffer collateral pathway as two distinct information-bearing inputs to CA1 (Hasselmo and Scholl 1994). The inhibition activated by temporoammonic input may serve to gate the output of the hippocampus (Dvorak and Schuman 1997; Empson and Heinemann 1995). Temporoammonic activity has also been shown to be capable of modulating the induction of LTP at the Schaffer collateral input to CA1 (Levy et al. 1998); it will be interesting to examine the impact of plasticity of the temporoammonic pathway on this heterosynaptic modulatory effect.

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