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13. Each experiment was conducted at least three times, variation in results was ±20 percent. The data presented in the figures represents results of one experiment. Experiments were carried out with the following: MS* medium (*p*H adjusted to 5.7) containing MS salts [T. Murashige, F. Skoog, *Physiol. Plant.* **15**, 473 (1962)] (4.3 g/liter, available from Gibco), thiamine (1 mg/liter), *myo*-inositol (0.1 g/liter), K_2HPO_4 (0.18 g/liter), 2,4-dichlorophenoxyacetic acid (2,4-D) (0.2 mg/liter). MS⁻ medium is MS* (less the 2,4-D) with 0.6 percent (w/v) agar and 0.1 mg/ml X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside). MG/L medium is from J. E. Beringer [*J. Gen. Microbiol.* **84**, 188 (1974)]. *Nicotiana tobacum* cells were grown in MS* in 50-ml cultures. The tobacco cells were incubated in continuous light at 28°C on a rotary shaker (120 cycles per minute). Fresh cell cultures were started each week by using a 1-ml inoculum from the previous week's culture. Three-day-old tobacco cells were used in the cocultivation experiments. Conditioned medium was the supernatant from 3-day-old tobacco cell cultures.
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Induction of Synaptic Potentiation in Hippocampus by Patterned Stimulation Involves Two Events

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Electrical stimulation of axons in the hippocampus with short high-frequency bursts that resemble in vivo activity patterns produces stable potentiation of postsynaptic responses when the bursts occur at intervals of 200 milliseconds but not 2 seconds. When a burst was applied to one input and a second burst applied to a different input to the same target neuron 200 milliseconds later, only the synapses activated by the second burst showed stable potentiation. This effect was observed even when the two inputs innervated completely different regions of the postsynaptic cells; but did not occur when the inputs were stimulated simultaneously or when the second burst was delayed by 2 seconds. Intracellular recordings indicated that the first burst extended the decay phase of excitatory postsynaptic potentials evoked 200 milliseconds later. These results suggest that a single burst of axonal stimulation produces a transient, spatially diffuse "priming" effect that prolongs responses to subsequent bursts, and that these altered responses trigger spatially restricted synaptic modifications. The similarity of the temporal parameters of the priming effect and the theta rhythm that dominates the hippocampal electroencephalogram (EEG) during learning episodes suggests that this priming may be involved in behaviorally induced synaptic plasticity.

EXCITATORY POSTSYNAPTIC POTENTIALS (EPSP's) in hippocampal neurons exhibit a persistent potentiation, termed long-term potentiation (LTP), following high-frequency stimulation (*1*). Similar effects are seen in other parts of the brain (*2*) and nervous system (*3*), and it has been proposed that the underlying mechanisms might be involved in memory storage (*4*). LTP is triggered by transient events in the postsynaptic neuron (*5–7*) and requires cooperative action among many synapses (*8, 9*). We recently reported that short bursts of high-frequency axonal stimulation (four pulses at 100 Hz) do not induce LTP unless repeated at intervals of less than 2 seconds; the optimal interburst interval proved to be 200 msec (*10*). These short bursts mimic a discharge pattern commonly seen in hippocampal pyramidal cells (*11*), and the optimal interburst interval corresponds to the theta EEG frequency found in exploring rats (*12*). The mechanisms through which naturally occurring activity patterns might produce synaptic modifications of the type represented by LTP are not known. One possibility is that each burst of high-frequency synaptic activity produces an effect at the activated synapses that accumulates maximally when the bursts are repeated at 200-msec intervals; this effect is then directly involved in producing LTP. Alternatively, a burst of synaptic activity might produce a diffuse "priming" effect, which is maximal 200 msec after the burst and alters the postsynaptic response to high-frequency activity at any synapses on the target neuron; these altered responses would then serve to trigger LTP. Our results support the priming mechanism and, in addition, demonstrate that primed responses directly induce LTP without the need for further stimulation-dependent processes. The findings clarify the nature and number of events that translate bursting activity into LTP and suggest that LTP is caused by a relatively simple physiological mechanism.

The experiments were conducted on slices of rat hippocampus with stimulating electrodes activating separate groups of Schaffer-commissural projections converging on a common set of CA1 pyramidal neurons. This preparation is used widely in studies of synaptic interactions in the hippocampus (*9, 13, 14*). A short burst was delivered to one set of fibers (S1) every 2 seconds, with each burst followed by an identical burst to the other set (S2) after a 200-msec delay (Fig. 1A). If LTP is caused by a specific accumulation mechanism, similar effects should have been seen on subsequent synaptic responses to each input (that is, LTP on each or no LTP on each) because each pathway received the same pattern of stimulation. If, however, induction of LTP

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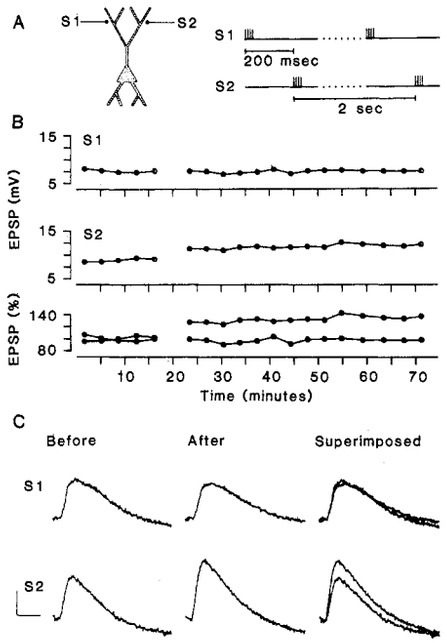

Fig. 1. Demonstration of separate priming and consolidation stages in the induction of LTP. (A) Two stimulating electrodes, one placed in stratum radiatum of CA1c, the other in stratum radiatum of CA1a, were used to activate separate sets of Schaffer-commissural inputs to a pyramidal cell in CA1b. The stimulation pattern consisted of four-pulse bursts (100 Hz) applied to each set of fibers at 2-second intervals with each burst on S2 occurring 200 msec after a burst on S1. The burst pairs were repeated ten times. (B) Data are shown for one neuron in which intracellular EPSP's were recorded alternately on each input at 10-second intervals before and after the burst stimulation episode. The upper panel shows the amplitude of the EPSP's evoked by S1, the middle panel those evoked by S2 (each point is an average of six successive responses). Burst stimulation was applied at the gap in the graphs. LTP only occurred on the input in which bursts occurred 200 msec after a burst on the other input. The bottom panel shows the EPSP amplitudes for both pathways expressed as a percent of their respective sizes before the burst episode. (C) EPSP's recorded 5 minutes before and 40 minutes after burst stimulation. Each record is an average of three successive responses. Calibration bar: 5 mV, 5 msec.

involves sequential priming and consolidation stages, then only the responses evoked by the delayed burst input should have been potentiated.

A very robust and stable LTP appeared in responses evoked by the second (delayed) electrode with no detectable changes in the responses elicited by the first electrode (Fig. 1, B and C). We have obtained this result in five different experiments with intracellular recording, and shown it to be true for extracellular responses as well. The results of 11 experiments in which population EPSP's were recorded in the apical dendritic field are summarized in Table 1. The stimulation paradigm was as in Fig. 1A, except that the burst pairs were separated by 5 seconds. In ten of these experiments, the synaptic responses evoked by the delayed electrode (S2) remained potentiated by at least 10 percent for a 30-minute test period after the final stimulation burst; responses to the first electrode (S1) were potentiated in only one case (*15*). Thus, we conclude that the first stimulation burst produced a spatially diffuse priming event that is distinct from a subsequent, spatially restricted consolidation event produced by the delayed burst.

Because the first (or priming) input, which was stimulated with bursts separated by 2 seconds, was not potentiated, we assumed that whatever effect underlies priming must dissipate between 200 msec (that is, the interval between stimulation of S1 and S2) and 2 seconds (the interval between bursts to the same input). The possibility that the second burst blocks the development of LTP in the first can be dismissed because bursts delivered at 2-second intervals without a follower burst do not elicit stable potentiation (*10*). Moreover, delaying the burst on the second input by 2 seconds rather than 200 msec (when bursts on the first input are separated by 5 seconds) does not result in LTP. It also appears that priming requires a delay to be effective. When short bursts are applied to two inputs simultaneously, LTP does not develop in either (*16*). Moreover, bursts delivered to a single input at 100-msec intervals were not as effective as the same stimulation with interburst intervals of 200 msec (*10*). To study the spatial distribution of the priming effect, we carried out experiments in which the priming stimulation was applied to fibers terminating in the apical dendrites, with each burst followed 200 msec later by a burst to fibers terminating in the basal dendrites (*17*). This paradigm produced LTP of the basal dendritic synapses in five of seven experiments [46 ± 15 (SEM) percent increase 30 minutes after stimulation]. Priming is thus diffusely distributed, delayed in onset, and short-lived.

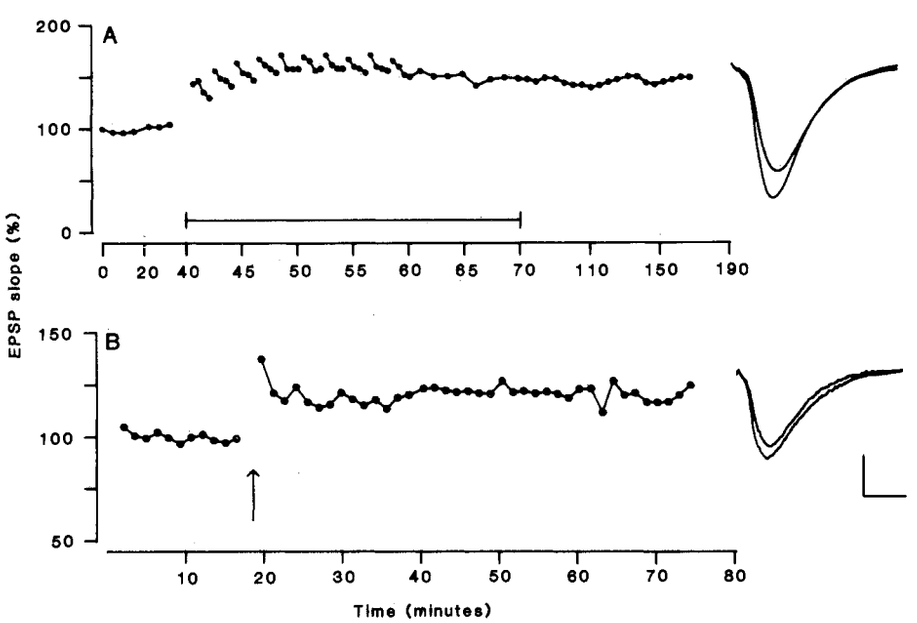

Fig. 2. Each pair of priming and delayed bursts produces LTP of the delayed input without requiring other stimulation-dependent events. (A) Ten pairs of bursts to S1 and S2 (with S2 following S1 by 200 msec) were given at 2-minute intervals. The graph shows the slope of the population EPSP evoked by test pulses to S2 (each point is an average of three successive responses evoked at 20-second intervals). Each discontinuity in the graph indicates the presentation of a single burst pair. Note that the potentiation present 2 minutes after a burst pair increases for the first five pairs but then saturates; the potentiation after the tenth pair did not decrease appreciably after 2 minutes. The time scale is expanded in the middle of the graph (that is, during presentation of burst pairs). Population EPSP's (each trace an average of four successive responses) recorded 5 minutes before the first burst and 60 minutes after the last burst are superimposed at the right. (B) The responses to S2 in another slice are shown before and after a single pair of S1-S2 bursts (arrow). For comparison, the LTP induced by one burst was about 15% while that after ten pairs was about 45%. Records (each trace is an average of four successive responses) taken 5 minutes before and 60 minutes after the burst pair are shown at right. Calibration bar: 1 mV, 5 msec.

The above experiments lead to the question of whether bursts of synaptic activity in primed dendrites directly produce LTP or whether they activate an intermediate process that summates across repeated primed bursts until a threshold is reached for an additional event that then causes stable potentiation. If repetitive pairings were producing a short-lasting process that continued to build until the actual LTP-eliciting mechanism was activated, we would expect that longer intervals between pairs should

produce less LTP. To test this, we separated the pairs by 2 minutes. This pattern resulted in LTP that was as large as that observed when successive priming and follower pairs were given every 5 seconds (Fig. 2A). This indicated that either the hypothetical intermediate event had a very long duration, or that each pair produced LTP and that LTP itself was additive across successive pairings. The experiment illustrated in Fig. 2B points to the latter conclusion. A single pairing of priming-follower bursts resulted in a measurable and stable LTP.

It thus appears that a single burst of four pulses to an input to a previously primed dendrite is sufficient for the production of LTP and that further stimulation-dependent events are not required. Additional studies with pairs separated by 2 to 10 minutes indicated that maximal LTP is reached after only three to five pairings.

These results suggest that repetitive burst stimulation produces LTP by a simple two-step sequence in which a burst primes the postsynaptic neuron such that synapses activated by a subsequent burst cause a stable modification step. Some clues about how priming alters subsequent inputs so that they produce this effect can be gained from analyses of single EPSP's in primed dendrites. Such potentials have more prolonged falling phases than those evoked under control conditions (Fig. 3). Comparable effects can be detected in extracellular recordings of the population EPSP, where the prolongation is maximal 200 msec after a burst and absent after 2 seconds. The prolongation of the intracellular EPSP is not mimicked by hyperpolarizing current applied under control conditions (that is, no priming) nor is it blocked by depolarizing the cell during the priming period (Fig. 3). This suggests that priming causes either the blockade of a normally present outward current or the activation of a normally absent inward current. Possibly relevant to the latter hypothesis, activation of the *N*-methyl-D-aspartate (NMA) receptor produces a prolongation of the population EPSP much like that seen with priming (*18*). This receptor is inactive under normal conditions, becoming functional only upon depolarization or removal of magnesium (*19*). Moreover, antagonists of the receptor suppress the development of LTP (*20*). Therefore, priming may transform the NMA receptor so that it is stimulated by released transmitter, and the resulting alterations in synaptic potentials may serve as triggers for LTP. Alternatively, priming might block an inhibitory postsynaptic potential or other hyperpolarizing current that normally truncates the EPSP. Appropriate pharmacological manipulations should distinguish between these alternatives.

Table 1. Average percentage increase (mean ± SEM, $n = 11$) of the slope of the population EPSP evoked by S1 and S2 at various times after burst stimulation. The baseline value for S1 was 1.30 ± 0.05 (SEM) mV/msec and for S2 was 1.25 ± 0.06 mV/msec.

Stimulus	Percentage increase in EPSP slope*				
	0.5 min	5 min	10 min	20 min	30 min
S1	4 ± 5	2 ± 4	2 ± 3	2 ± 3	1 ± 3
S2	49 ± 5	39 ± 4	33 ± 4	31 ± 4	30 ± 4

*Percent increase 30 minutes after stimulation was significantly greater for S2 than for S1 ($P < 0.01$, two-tailed t test).

These results provide new insights into the mechanisms responsible for the production of LTP and how these mechanisms might relate to the naturally occurring theta bursting pattern. They support the argument (*5–7*) that the pertinent processes are located postsynaptically. It is difficult to imagine that priming in the apical dendrite influences synapses in the basal dendrites by axo-axonic interactions between afferents separated by a cell body layer. The widespread distribution of priming makes it likely that a physiological change in the postsynaptic cell is involved. In fact, hippocampal pyramidal cells are electrotonically compact (*21*). The induction of LTP by pairs separated by minutes, and indeed even by a single pair, indicates that an appropriately primed input can activate the process that causes long-term modification of the synapses. This somewhat simplifies the search for the triggering mechanism and emphasizes the importance of determining exactly how primed responses differ from normal potentials. One correlate of priming is a prolongation of EPSP's. In that LTP is blocked by intracellular injections of calcium buffering agents (*5*), it seems plausible that an increased calcium influx due to the opening of unusual numbers of voltage-sensitive or receptor-linked calcium channels by burst stimulation of primed synapses may be the cause of LTP. However, we have no experimental evidence that the observed EPSP prolongation is the agency through which priming is effected and other, more subtle factors could be involved.

If priming does facilitate the induction of

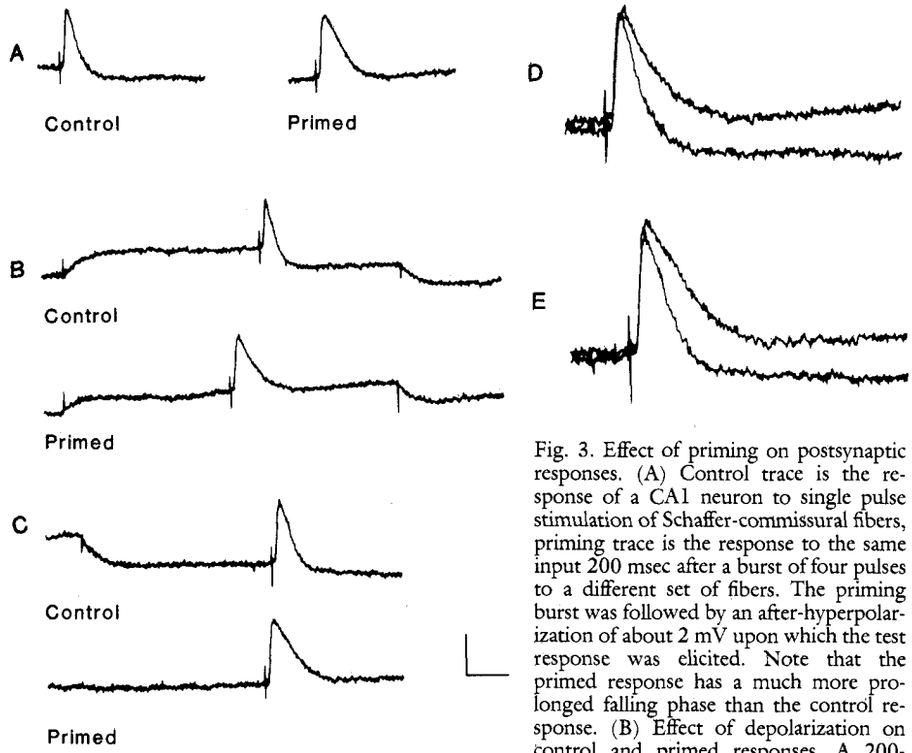

Fig. 3. Effect of priming on postsynaptic responses. (A) Control trace is the response of a CA1 neuron to single pulse stimulation of Schaffer-commissural fibers, priming trace is the response to the same input 200 msec after a burst of four pulses to a different set of fibers. The priming burst was followed by an after-hyperpolarization of about 2 mV upon which the test response was elicited. Note that the primed response has a much more prolonged falling phase than the control response. (B) Effect of depolarization on control and primed responses. A 200-msec, 0.1-nA depolarizing current pulse, sufficient to reset the membrane potential to the level present for the control response in (A) did not alter the prolongation of the EPSP produced by priming. The current pulse itself did not prolong the EPSP evoked without priming (control). (C) Hyperpolarization of the membrane (200-msec, 0.1-nA current pulse) does not prolong the control EPSP to the same extent as a priming burst. Both responses were evoked at the same somatic membrane potential. (D) Control and primed responses evoked at the same membrane potential. The control record from (A) and the primed record from (B) are superimposed. (E) Control and primed responses evoked at the same membrane potential. Control and primed records from (C) are superimposed. Calibration bar for (A) to (C), 10 mV, 25 msec; for (D) and (E), 5 mV, 12.5 msec. All records are from the same CA1 neuron with resting membrane potential of −62 mV.

LTP by so simple an action as prolonging postsynaptic responses, then it should be possible to reproduce its effects with a number of manipulations. Comparisons of priming with other conditions [for example, pharmacological blockade of inhibitory postsynaptic potentials (*6*)] reported to increase the likelihood of LTP should be useful in identifying the final events that potentiate synapses.

Finally, our observations indicate that certain aspects of naturally occurring physiological activity in hippocampus can be expected to produce LTP-like phenomena. The brief trains of three to four action potentials exhibited by hippocampal pyramidal cells in behaving animals, when used as stimulation bursts, prove to be fully capable of producing robust LTP if spaced apart so as to form a pattern like the theta EEG rhythm found in freely moving rats. Thus analyses of the priming and execution processes may lead to a greater understanding of how the physiological characteristics of hippocampal networks are actually translated into synaptic changes during learning.

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14. Hippocampal slices were prepared from male Sprague-Dawley rats and maintained at 35° ± 1°C in medium containing 124 m*M* NaCl, 5 m*M* KCl, 1.25 m*M* KH_2PO_4, 26 m*M* $NaHCO_3$, 3.4 m*M* $CaCl_2$, 2.5 m*M* $MgSO_4$, 10 m*M* D-glucose, and 2 m*M* L-ascorbic acid. Intracellular recordings were made with microelectrodes filled with 2*M* potassium methylsulfate (40 to 80 megohms). Population EPSP's were recorded in the dendritic zones with electrodes filled with 2*M* NaCl (1 to 5 megohms).
15. The absence of potentiation of responses evoked by the first electrode (S1) provides additional support for the conclusion drawn from paired-pulse experiments (*13*; also tested in several of our experiments) that two stimulating electrodes spaced widely apart in stratum radiatum activate separate groups of Schaffer-commissural fibers.
16. It has been reported that long high-frequency stimulation trains (50 to 100 pulses) given to two convergent sets of Schaffer-commissural fibers simultaneously are more effective in inducing LTP than stimulation of one input alone (*9*). When bursts of four pulses (repeated ten times at 5-second intervals) were applied to separate inputs simultaneously, only 4 of 18 pathways tested showed evidence of LTP (that is, an increase in the EPSP slope of at least 10% persisting for 20 minutes).
17. Stimulating electrodes were placed in stratum radiatum and stratum oriens of field CA1c and population EPSP's were recorded in stratum oriens of CA1b. In each slice, we ensured that short bursts (two to four pulses at 100 Hz, repeated ten times at 20-second intervals) to the basal input alone did not induce LTP (mean ± SEM, 5 ± 3% increase 10 minutes after stimulation, $n = 7$). We then proceeded to give the same pattern of stimulation to the basal input but with each burst preceded by a priming burst to the apical input.
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Associative Induction of Posttetanic and Long-Term Potentiation in CA1 Neurons of Rat Hippocampus

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Electrical stimulation of fibers in the stratum radiatum causes an excitatory postsynaptic potential in CA1 neurons of the hippocampus. Other excitatory inputs to or direct depolarization of these CA1 neurons during stimulation of the stratum radiatum caused a subsequent increase in the excitatory postsynaptic potential. This enhancement was characterized as a brief potentiation (2 to 3 minutes, similar to posttetanic potentiation) and a long-term potentiation (presumed to be involved in learning and memory). These potentiations are probably induced by an interaction of the postsynaptic cell or other presynaptic terminals with the test presynaptic terminals.

A REPETITIVE HIGH-FREQUENCY activation of an excitatory input in the hippocampus results in a posttetanic potentiation of short duration (PTP) followed by a long-term potentiation (LTP) of synaptic transmission evoked by the same input (*1*). LTP has been implicated as a mechanism involved in learning and memory (*2*). Some investigators reported that a coactivation of several input fibers is needed to induce LTP and suggested that the necessity for a coactivation of fibers and the associative nature of the induction of LTP could be explained if LTP is postsynaptic (*3, 4*). However, presynaptic terminals in the hippocampus interact with each other (*5*); this raises the possibility that these interactions could be involved in associative induction of LTP. Our results indicate that LTP and PTP (which is thought to be presynaptic) can be induced without a tetanic stimulation of the input, but only if activation of the test input occurs during either a tetanic stimulation of other excitatory inputs or a depolarization of the postsynaptic neuron.

Experiments were conducted on transversely sectioned rat hippocampal slices prepared and maintained as described (*5*). The slices were continuously perfused with a medium containing 120 m*M* NaCl, 3.1 m*M* KCl, 26 m*M* $NaHCO_3$, 4 m*M* $MgCl_2$, 4 m*M* $CaCl_2$, 10 m*M* dextrose, and 10 μ*M* picrotoxin. Picrotoxin was added to the medium to block inhibition and facilitate the associative induction of LTP (*6*). To examine the associative interactions between afferent fibers in the stratum radiatum leading to changes in synaptic transmission, a test electrode was positioned in the stratum radiatum and stimulated at 0.2 Hz (200 to 600 μV) to evoke a small population excitatory postsynaptic potential (EPSP) in the apical dendritic area of CA1 neurons. A conditioning stimulating electrode was positioned in another part of stratum radiatum to evoke a large population EPSP (1 to 3 mV) monitored through the same recording electrode. If the weak input was stimulated twice in succession (50-msec interval), the response to the second stimulation invariably increased in size. If the conditioning (strong) input was stimulated 50 msec prior to the test (weak) input, there was no such facilitation, an indication that these two inputs did not share common fibers (Fig. 1A). Conditioning was given as one, five, or

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