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Associative long-term potentiation in hippocampal slices

(synaptic transmission/neuronal plasticity/pyramidal neurons/membrane properties)

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ABSTRACT **Interactions between two excitatory monosynaptic inputs to hippocampal neurons of the CA1 region were examined in the *in vitro* slice. By adjusting the strengths of the electrical stimuli delivered to the two input pathways, one was made to generate a weak and the other a strong synaptic response. Simultaneous tetanic stimulation of both input pathways resulted in a subsequent long-term enhanced synaptic efficacy in the weak input under conditions in which the same tetanic stimulation of either input alone failed to have this effect. This form of long-term synaptic potentiation (LTP), known as associative LTP, was shown in some cases to last hours without decrement. The plastic changes were localized within the CA1 region and appear to reside in the pre- or postsynaptic elements of the monosynaptic excitatory input to the pyramidal neurons. The increased synaptic efficacy could not be accounted for by any of several measured postsynaptic passive membrane properties.**

Associative long-term synaptic potentiation (LTP) has been suggested as a mechanism for aspects of learning and memory (refs. 1–4, see also ref. 5). The essential phenomenon was clearly described in a series of *in vivo* studies of the interaction between two sets of synaptic inputs to the dentate gyrus (1–4). One input was arranged to generate a weak and the other a strong extracellularly recorded synaptic response. Concurrent tetanic stimulation of both inputs was found to result in a long-term enhanced synaptic efficacy in the previously weak input under conditions in which tetanic stimulation of either pathway alone failed to have this effect.

One major breakthrough in the effort to understand such use-dependent forms of cortical synaptic plasticity was the development of the *in vitro* hippocampal slice preparation. The advantage of the hippocampal slice is that the circuitry is relatively simple and well-defined, and it is possible to apply to the slice powerful neurochemical (6, 7) and biophysical (8–12) techniques that are impracticable *in vivo*. In the present study, we report that associative LTP can be reliably induced in the CA1 region of the hippocampal slice. The enhanced synaptic efficacy was shown in some cases to persist for an hour or more with little or no decrement. Results pertinent to the locus and origin of the underlying plastic changes are also presented.

MATERIALS AND METHODS

Preparation and Maintenance of Slices. Rat hippocampal slices were prepared and maintained *in vitro* in the conventional manner (8–10). To begin localizing the observed plastic changes, the CA3 region was sometimes removed (see *Results*). To restrict further the locus of the plastic changes, in some experiments picrotoxin was added to the standard saline (8–10). Picrotoxin is known to block the synaptic inhibition that normally accompanies the monosynaptic excitatory input (refs. 8 and 10; see also refs. 11–15). This concomitant synaptic inhibition is believed to be mediated by interneurons that use γ-aminobutyric acid as their neurotransmitter (15). Whenever picrotoxin was added to the bath, the magnesium ion concentration was increased to 4 mM to prevent epileptiform activity (8, 10–12).

Electrophysiological Techniques. Standard techniques were used for extracellular (16) and intracellular electrophysiological recordings (17), the results of which were stored on FM magnetic tape for subsequent off-line computer analysis. The arrangement of the stimulating and recording electrodes is shown in Fig. 1*A*. The extracellular recording microelectrode was placed in the stratum radiatum, and the intracellular recording microelectrode was placed in the stratum pyramidale of the CA1 region. Bipolar electrodes were used to stimulate two sets of synaptic inputs. One stimulating electrode activated a set of Schaffer collateral/commissural fibers orthodromically and was placed either in the stratum pyramidale of the CA3 region or in the stratum radiatum of the CA1 region. The second stimulating electrode was placed in the stratum radiatum of the CA1 region on the other side of the recording micropipette. This stimulating electrode activated a different set of Schaffer collateral/commisural fibers (see below; see also ref. 16) antidromically. One synaptic input was made to generate a weak synaptic response, and the other input was made to produce a strong synaptic response. In the experiments involving intracellular recordings (Fig. 1*B*), the stimulus currents delivered to the weak input were adjusted (usually 5–20 μA) to produce 2- to 5-mV excitatory postsynaptic potentials (EPSPs) in the postsynaptic cell (left trace), and the stimulus currents delivered to the strong input were adjusted (usually to 30–60 μA) to yield responses that were 2–5 times larger (right trace). The synaptic response produced by the strong input was sometimes suprathreshold for eliciting an action potential; the synaptic response produced by the weak input was always well below threshold.

In the experiments involving extracellular recordings (Fig. 1*C*), the stimulus currents for the weak input were adjusted (usually 10–40 μA) to produce extremely small population EPSPs with amplitudes in the range of 100–300 μV (left trace). For the strong input, the stimulus currents were adjusted (usually 50–80 μA) to yield population EPSPs that were $\approx$10 times larger than those produced by the weak input (right trace).

Possible changes in the postsynaptic passive membrane properties were assessed before and after the induction of associative LTP. Hyperpolarizing constant-current steps were injected into the cells and the input resistance and membrane time constant were measured in the usual manner (see *Results*; see also ref. 17). The value of the resting membrane potential, at the end of the experiment, was verified by rapidly with-

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Abbreviations: LTP, long-term synaptic potentiation; EPSP, excitatory postsynaptic potential; PTP, posttetanic potentiation.

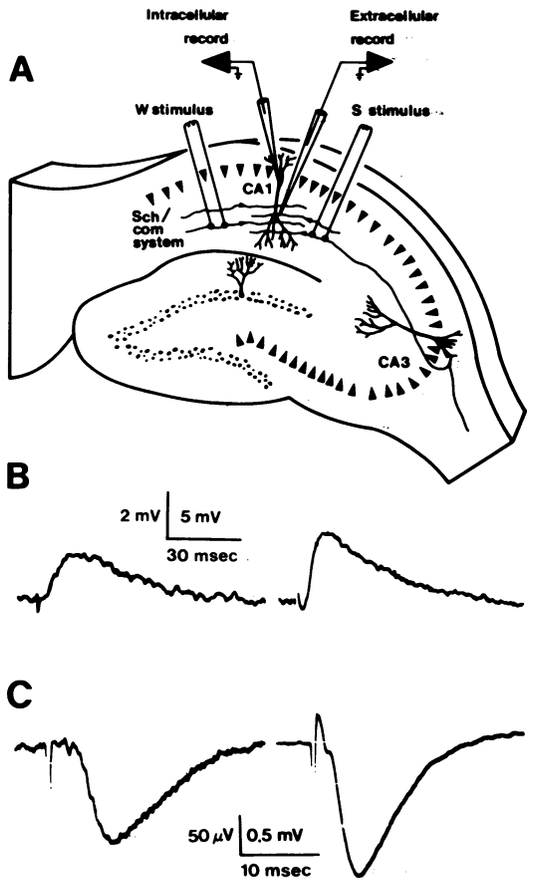

FIG. 1. Relationships between the two afferent inputs (W, weak input; S, strong input). (*A*) Location of the stimulating and recording electrodes relative to the Schaffer collateral/commissural (Sch/com) fiber trajectory and the various layers of the hippocampus. (*B*) Typical intracellular recording of the weak (left trace) and strong (right trace) synaptic responses. (*C*) Typical extracellular recording of the weak (left trace) and strong (right trace) population EPSPs.

drawing the micropipette from the impaled cell.

Experimental Design. A demonstration of associative LTP requires that conjoint tetanic stimulation of both sets of synaptic inputs produces LTP in the weak input and that the same tetanic stimulation of either input alone fails to have this effect. The complete experimental protocol involved a sequence of five tests. A successful experiment consisted of satisfying the first four tests; in addition, a demonstration of associative LTP required satisfying the fifth test. First, we tested the weak input at 0.2 Hz for several minutes to ensure that the response amplitudes remained temporally stationary and that the coefficient of variation in the response amplitudes was <10% in the extracellular recordings and <30% in the intracellular recordings. In the latter experiments we also measured the input resistance and membrane time constant at this point. Second, we performed standard paired-pulse experiments, to confirm (cf. ref. 16) that the two stimulating electrodes were in fact activating separate sets of Schaffer collateral/commisural fibers (see also the fourth test below). This test was not always performed in the intracellular experiments where long-term stability, and therefore the passage of time, was more of a problem. Third, we tetanically stimulated the weak input alone to verify that this was not sufficient to generate LTP in this pathway. The tetanic stimulation consisted of a 100-Hz train for 1 sec, and this stimulus train was repeated 5 sec later. The posttetanic effects were then assessed by stimulating the weak input at 0.2 Hz to verify that the response amplitude returned to control level within 1–5 min. To ensure that the weak input would not generate LTP on subsequent tetanic stimulation of this pathway, we usually repeated this test two or three times. Fourth, we tested for heterosynaptic LTP to show that tetanic stimulation of the strong input by itself would not produce a subsequent enhanced response in the weak input (cf. ref. 16). The strong input was tetanically stimulated with the same stimulus frequency parameters previously used to test for LTP in the weak input, and the posttetanic heterosynaptic effects were then assessed by stimulating the weak input at 0.2 Hz. Fifth, we tested for associative LTP by tetanically stimulating both inputs simultaneously, again using the same stimulus frequency parameters, after which the weak input was stimulated at 0.2 Hz for 15 min to several hours. If the weak response amplitude was enhanced for at least 15 min, we considered this a successful demonstration of associative LTP. In the intracellular experiments, we repeated the measurements of the input resistance and membrane time constant at this point.

RESULTS

Extracellular Recordings. Paired-pulse experiments were performed to verify the separateness of the weak and strong synaptic inputs. Results from such an experiment are shown in Fig. 2. The upper oscilloscope traces are superimposed paired-pulse stimulations of the weak input at interpulse intervals of 50, 100, 200, 300, and 400 msec. The second response is clearly facilitated at interpulse intervals between 50 and 300 msec. The lower traces show the result of paired-pulse experiments in which the first pulse was presented to the strong input and the second pulse was presented to the weak input. As indicated, there was no paired-pulse facilitation. In principle, the failure to observe

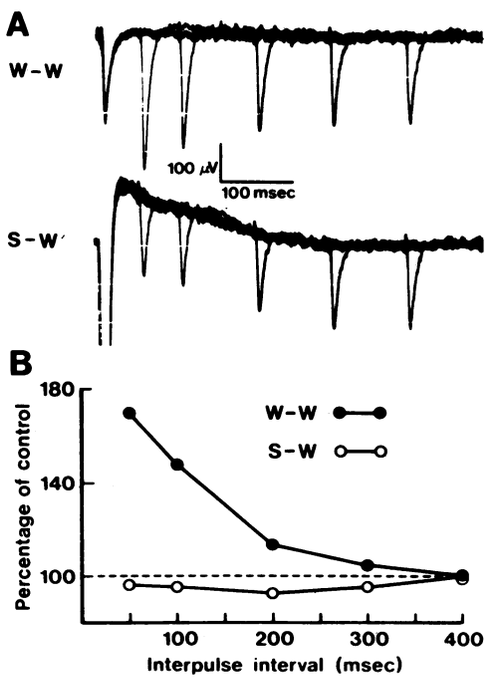

FIG. 2. Interactions at short time intervals within and between the two synaptic inputs using extracellular recordings. (*A*) Superimposed oscilloscope traces of population EPSPs from a representative paired-pulse experiment in which both pulses were presented to the weak input (W-W), or the first pulse was presented to the strong input and the second pulse was presented to the weak input (S-W). (*B*) Plot of the paired-pulse data from the same slice. Each data point is derived from the mean amplitude of the second response averaged over three trials.

heterosynaptic paired-pulse facilitation could result from a masking effect of concomitant inhibition (8). This possibility was ruled out by performing parallel experiments in slices bathed in 10 μM picrotoxin (8). Under these conditions, we also failed to observe heterosynaptic paired-pulse facilitation.

The results from the remainder of this experiment are shown in Fig. 3. After the first control sequence we see that tetanic stimulation of the weak input alone resulted in posttetanic potentiation (PTP), which lasted for about 2 min, but failed to produce LTP. When tetanic stimulation was repeated, again we observed PTP but not LTP. The PTP duration ranged from 1 to 4 min (cf. ref. 18). As shown, tetanic stimulation of the strong input alone failed to increase the amplitude of the weak responses and in fact produced a transient heterosynaptic depression. However, in the next stage of the experiment we were able to demonstrate associative LTP. Note that simultaneous tetanic stimulation of both inputs resulted in a sizeable and persistent enhancement in the subsequent response amplitude of the weak input.

Associative LTP was found to occur in 85% of the 28 slices in which we carried out successful extracellular experiments that satisfied the first four tests in our experimental protocol. In these 24 slices, the change in the population EPSP amplitude, expressed as a percentage of the pretetanic control value, averaged 149; the range was from 110 to 190. In 3 of the above slices, the experiments were performed on the isolated CA1 region. All 3 of these slices showed associative LTP, with a mean percentage of 143.

To determine whether the inhibitory circuitry (8, 13–15) plays an essential role in associative LTP, the same experiments were repeated in slices bathed in 10 μM picrotoxin. Successful experiments were carried out on five slices, four of which showed associative LTP. In these four slices, the mean percentage was 169, and the range was from 140 to 190. Similarly, in intracellular studies (discussed below), the induction of associative LTP was not prevented by bath application of 10 μM picrotoxin.

Intracellular Recordings. The results from intracellular measurements of the EPSP amplitudes paralleled those obtained from the extracellular recordings. Averaged EPSP waveforms, taken from selected stages of a typical experiment, are shown in Fig. 4*A*. Results from the complete experiment are plotted in Fig. 4*B*. As indicated, tetanic stimulation of the weak input alone produced PTP but not LTP. Similarly, tetanic stim-

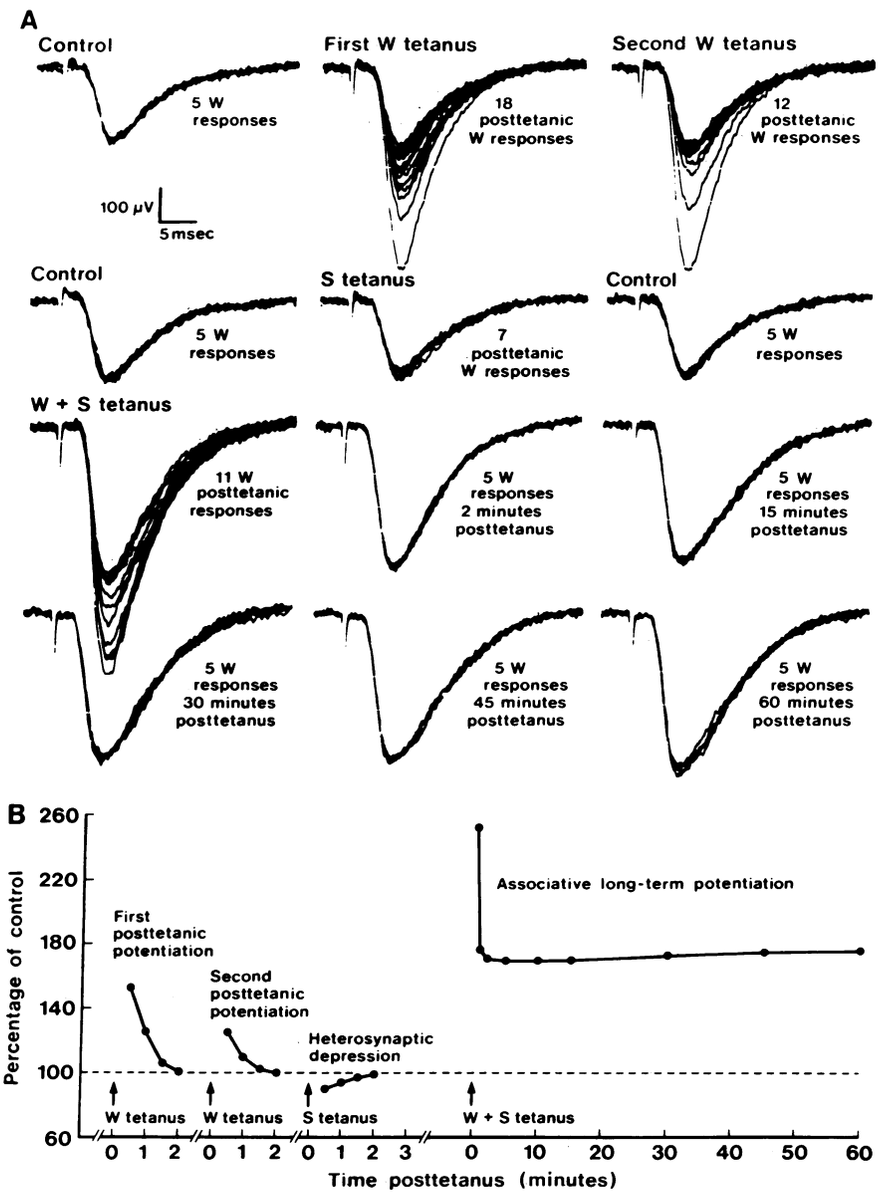

FIG. 3. Associative LTP in extracellular experiments (W, weak input; S, strong input). (*A*) Superimposed population EPSPs showing the indicated number of responses to stimulation of the weak input (described below each set of traces), which was tested at 0.2 Hz at the specified stages of the experimental protocol (stated above the traces). The sequence proceeds from left to right, beginning with the top row. The first response, obtained 5 sec after the concurrent W + S tetanic stimulation, was off-scale and is not shown. (*B*) Plot of the data from the same slice. Data points represent either single response measurements (when the W-response amplitude was changing rapidly after a tetanus) or mean of five responses (when the W-response amplitudes had relaxed to a relatively constant value). The time scale changes after concurrent tetanic stimulation of both inputs (W + S tetanus).

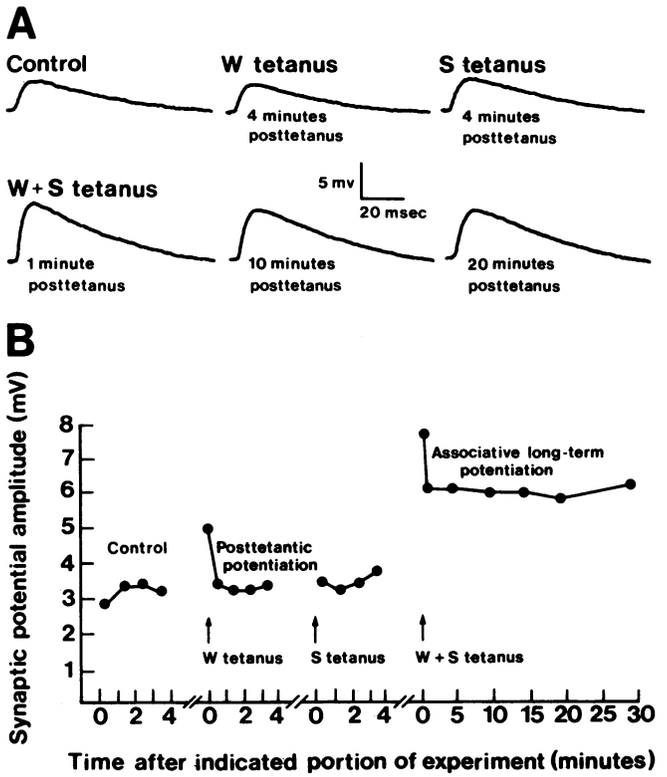

FIG. 4. Associative LTP in intracellular experiments (W, weak input; S, strong input). (*A*) Averaged intracellular EPSPs produced by stimulating the weak input at 0.2 Hz at the indicated stages in the experimental protocol. The times shown below the traces indicate the end of the 1-min averaging interval. Tetanic stimulation of neither the weak nor the strong input alone produced a long-term enhancement of the weak-response amplitude, but concurrent tetanic stimulation of both inputs produced a sizeable long-term enhancement of the weak-response amplitude. (*B*) Plot of the data from the same cell. Each data point represents the mean of either 6 EPSPs (when the response amplitudes were rapidly changing after a tetanus) or 12 EPSPs (when the response amplitudes had relaxed to a relatively constant value). The EPSP amplitudes are plotted at the midpoint of the averaging interval. Tetanic stimulation of the weak input alone (W tetanus) produced PTP but not LTP. Tetanic stimulation of the strong input alone (S tetanus) had no heterosynaptic effect on the weak input. However, concurrent tetanic stimulation of both inputs (W + S tetanus) resulted in associative LTP that persisted for the duration of the experiment (note the change in the time scale at this point).

ulation of the strong input alone produced no significant heterosynaptic effect on the response to the weak input. However, simultaneous tetanic stimulation of both inputs almost doubled the amplitude of the EPSP produced by the weak input. This increase in EPSP amplitude lasted for the duration of the testing period.

To determine whether changes in the passive membrane properties of the cells might be responsible for associative LTP, we measured the resting membrane potential, input resistance, and membrane time constant at the beginning and end of the experiments. The passive membrane properties of the cell illustrated in Fig. 4 remained fairly constant throughout the experiment. The resting membrane potential was −81 mV at both the beginning and end of the experiment, while the input resistance decreased slightly—from 87 MΩ at the beginning to 82 MΩ at the end of the experiment (Fig. 5*A*). The membrane time constant was 14 msec at the beginning of the experiment and 13 msec at the end (Fig. 5*B*).

Successful intracellular experiments that satisfied our first four criteria were performed on 16 well-impaled pyramidal

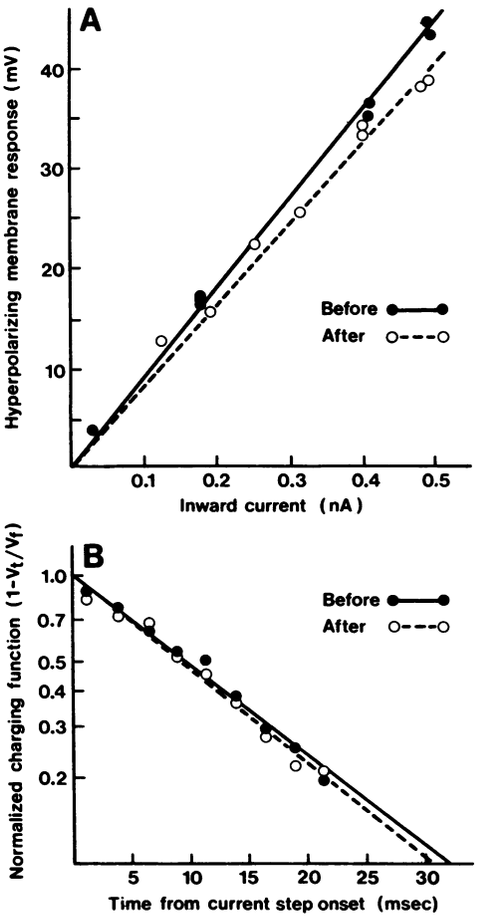

FIG. 5. Passive membrane properties before and after the induction of associative LTP (from the same cell shown in Fig. 4). (*A*) Input resistance determined from the current–voltage relationship during the control period and again after the induction of associative LTP. (*B*) Membrane time constant determined during the control period and after the induction of associative LTP. The ordinate represents the normalized charging function $(1 - V_t/V_f)$, where V_t is the time-dependent potential produced by the applied current step and V_f is the final asymptotic value of V_t.

neurons. Of these, 75% displayed associative LTP. In 10 of these 12 cells, the average change in the size of the EPSP amplitudes, expressed as a percentage of the control values, was 181 (Table 1); the range was from 135 to 293. In the remaining two cells, the EPSPs produced by the weak input were suprathreshold after the induction of associative LTP so that the EPSP amplitudes could not be determined. Ten of these 12 cells were from slices bathed in 10 μM picrotoxin. Mean (±SEM) values

Table 1. Intracellular measurements before and after associative LTP

Physiological measurement	Before (mean ± SEM)	After (mean ± SEM)	Sample size	Percentage of control
Resting potential, mV	76.4 ± 2.0	75.0 ± 2.2	12	98
Input resistance, MΩ	50.5 ± 5.7	46.5 ± 5.1	12	92
Time constant, msec	15.4 ± 1.2	14.1 ± 1.2	12	92
EPSP amplitude, mV	2.6 ± 0.2	4.7 ± 0.4	10	181

for the passive membrane properties measured at the beginning and end of the experiment are given in Table 1.

DISCUSSION

Our results show that associative LTP occurs *in vitro* and is not unique to the dentate gyrus. The fact that associative LTP occurs even in slices in which the CA3 region has been removed indicates that the underlying plastic changes can occur entirely within the delimited circuitry of the CA1 region of a thin slice. The known circuitry of the CA1 region includes the pyramidal neurons, their monosynaptic excitatory inputs, and inhibitory interneurons (19).

We were interested in knowing whether the inhibitory interneurons play an essential role in the induction of associative LTP. Stimulation of the various inputs to hippocampal neurons normally evokes a mixed synaptic response, consisting of an early excitatory conductance increase and a delayed, but temporally overlapping, inhibitory conductance increase (8, 10). The former component represents the monosynaptic excitatory response, and the latter component is thought to arise from recurrent or feed-forward inhibition mediated by the inhibitory interneurons (10, 12–15). The inhibitory response can be entirely blocked by picrotoxin (8, 10, 12). Because both our extracellular and intracellular recordings show that the induction of associative LTP is not blocked by 10 μM picrotoxin, the essential plastic changes appear to reside in either the pre- or postsynaptic elements of the monosynaptic excitatory input.

Both pre- and postsynaptic mechanisms have been proposed to underlie the various long-term forms of plasticity that are observed in the mammalian central nervous system (6, 7, 20–25). Several investigators (22–24) have suggested that increases in the postsynaptic input resistance may be important. This explanation cannot account for the present results. The changes that we observed in the postsynaptic passive membrane properties (Table 1) were quantitatively small and in the wrong direction to increase the EPSP amplitudes.

The technical advantages of the *in vitro* slice (8, 10) should facilitate further analysis of the cellular and biophysical mechanisms underlying associative LTP. In particular, the feasibility of performing quantal and voltage-clamp analysis in the slice (8–10, 26) may permit a determination of whether the essential plastic changes are pre- or postsynaptic in origin.

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