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

B. R. SASTRY, J. W. GOH, A. AUYEUNG

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

Department of Pharmacology and Therapeutics, University of British Columbia, Vancouver, B.C. V6T 1W5, Canada.

ten trains (100 Hz, ten pulses in each train, one train every 5 seconds). If the test EPSP was not evoked during conditioning, a depression rather than PTP or LTP of this response was observed. This finding strengthens the conclusion that test fibers are not activated by the conditioning stimulation. If each of the conditioning trains was paired with a single stimulation of the weak input given 1 msec after the onset of the conditioning train, PTP (with one or five trains) or LTP (with ten trains) could be induced (Fig. 1A). The PTP was 130 to 200 percent of control (60 seconds posttetanus) in six of eight experiments; there was no change in two of eight experiments. The LTP was 120 to 170 percent of control (15 minutes posttetanus) in six of eight experiments; there was no change in two of eight experiments. PTP, which lasted 2 to 3 minutes, could be repeatedly induced (with no LTP), usually with one to five paired trains. In the same experiment, if the number of paired trains was increased, LTP could eventually be induced (Fig. 1A).

To confirm that associative interactions can occur between two separate inputs, the above experiments were repeated using stratum oriens stimulation for conditioning. Results that were similar qualitatively and quantitatively to those seen with the stratum radiatum were obtained in five slices.

The necessity for the presence of extracellular Ca^{2+} for associative interactions between Schaffer collaterals (*5*) suggested the participation of CA1 neurons. We therefore tested whether depolarization of CA1 neurons mimicked the conditioning effects of stratum radiatum or stratum oriens stimulation. For this purpose, EPSP's evoked by stratum radiatum and stratum oriens stimulation were recorded from individual CA1 neurons with intracellular electrode filled with 1*M* KCl and 1.6*M* potassium citrate. The stimulation strengths of the test inputs were chosen to evoke EPSP's that were 30 percent of maximum. Unpaired conditioning depolarizations (75- to 200-msec duration, 3 to 10 nA; one, five, or ten depolarizing commands at 0.2 Hz) produced a 10 to 30 percent depression of the EPSP's (eight of nine cells, no change in one of nine) (Fig. 2). When each of the conditioning commands was paired with a single stratum radiatum stimulation, PTP of 2 to 3 minutes duration (with one or five trains) or LTP (with ten trains) of the stratum radiatum–induced (paired) but not stratum oriens–induced (unpaired) EPSP was observed depending on the number of paired conditioning commands (Fig. 2). The PTP was 140 to 220 percent of control (60 seconds postconditioning) in seven of nine cells; there was no change in two of nine cells. The LTP was 130 to 180 percent of control (15 minutes postconditioning in six of nine cells; there was no change in three of nine cells.

To determine whether the PTP observed in our study was associated with a change in the excitability of presynaptic terminals, as in the spinal cord (*7, 8*), a test stimulating electrode was positioned in the apical dendritic areas of CA1 neurons to activate terminal regions of Schaffer collaterals so that all-or-none antidromic action potentials could be recorded from CA3 cell bodies (Fig. 1B). The excitability of the Schaffer collateral–terminal region was assessed by monitoring the amount of current required to induce an action potential (*5*). A conditioning stimulating electrode was positioned in stratum radiatum or in stratum oriens to deliver the conditioning trains (100 Hz, 10 pulses; one, five, or ten trains, one train every 5 seconds). The conditioning trains did not activate the test CA3 neuron. The unpaired conditioning trains themselves did not produce any postconditioning excitability changes in the test fiber. As in the case of

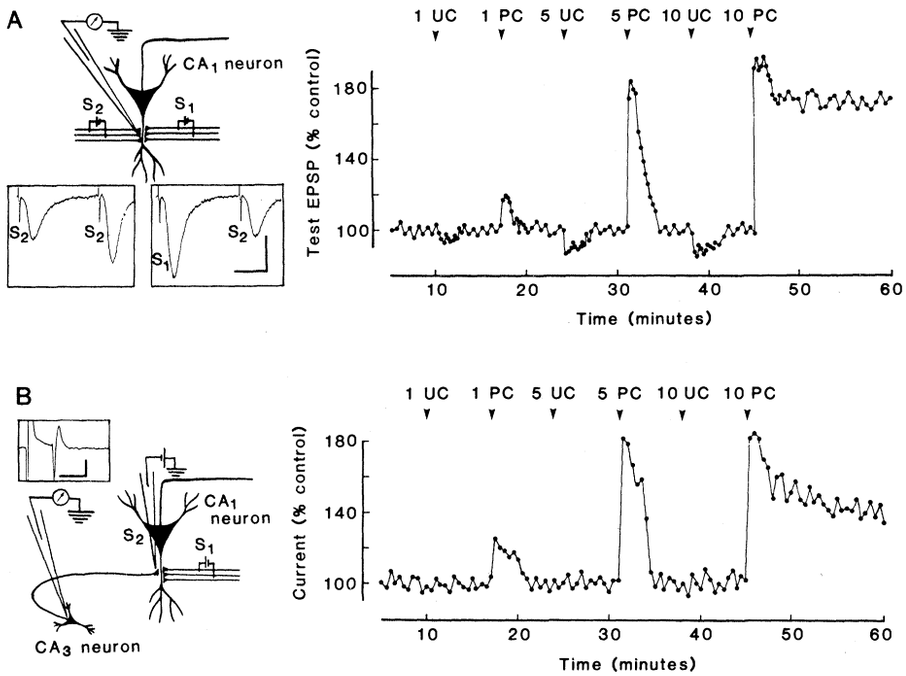

Fig. 1. Associative induction of PTP and LTP. (A) A bipolar stimulating test electrode (S_2) was positioned in the stratum radiatum and a bipolar conditioning stimulating electrode (S_1) was positioned in another area of the stratum radiatum (left). A recording microelectrode (containing 4*M* NaCl) was positioned in the apical dendritic area of CA1 neurons to monitor the test EPSP evoked at 0.2 Hz (stimulation strength was adjusted to obtain a response between 300 and 600 μV). The conditioning stimulation strength was adjusted to evoke a population EPSP of 1 to 3 mV. If a double stimulation of S_2 (50-msec interval) resulted in a facilitation of the second population EPSP (inset, left) and if a stimulation of S_1 preceding S_2 stimulation by 50 msec resulted in no facilitation of the second population EPSP (inset, right), then the S_1 and S_2 stimulations were presumed to activate separate input fibers. The calibrations (inset, right) represent 20 msec and 0.5 mV. In all experiments, the effect of unpaired conditioning trains (UC) (the test stimulation was off during the conditioning, each conditioning train consisted of ten pulses at 100 Hz) and of the paired conditioning trains (PC) (the test stimulation was on 1 msec after the onset of each train) were examined on the postconditioning test population EPSP. During the first 3 minutes after UC or PC the response was monitored every 15 seconds and at all other times at 30-second intervals. The graph on the right shows results from one experiment. PTP occurs after one and five PC's, and LTP after ten PC's. (B) Effects of conditioning on the excitability of the terminal region of a Schaffer collateral. A monopolar stimulating test electrode (S_2) was positioned in the apical dendritic area of the CA1 neurons to activate (0.2-msec negative pulses; 3 to 10 μA; 0.2 Hz) the terminal regions of Schaffer collaterals so that antidromic all-or-none action potentials (see inset; the calibration bars represent 6 msec and 0.2 mV) could be recorded from the CA3 cell bodies. A conditioning stimulation electrode (S_1) was positioned in the stratum radiatum and the unpaired (UC) and paired (PC) conditioning trains were applied as in (A). The conditioning stimulation did not activate the test Schaffer collateral (*5*). During the PC, the stimulation strength to antidromically activate the test Schaffer collateral was increased to twice that of control to make sure that the fiber was activated during PC. (A similar activation of the test fiber without the presence of the conditioning produced no changes in the excitability of the test fiber.) The amount of current required to produce an all-or-none action potential was that which induced a spike in one to two of three consecutive attempts. Recordings taken at 30-second intervals are at right. Note that one and five PC's induced a 3-minute decrease when ten PC's induced a prolonged decrease in the excitability of the test fiber terminal. Results in (A) and (B) were from different experiments.

PTP of the EPSP, however, a pairing of the conditioning trains with the test stimulation did cause a postconditioning decrease in the excitability. The amount of current required to elicit an action potential was 160 to 210 percent of control (60 seconds after five-train paired conditioning by radiatum) in six of seven fibers (Fig. 1B); there was no change in one of seven fibers. At 60 seconds after five-train paired conditioning by the oriens, 130 to 180 percent of the control current was required in five of six fibers; there was no change in one of six fibers. An increase in the number of paired conditioning trains eventually led to a prolonged decrease in the Schaffer collateral terminal excitability, a phenomenon associated with LTP (*9*) (Fig. 1B). The amount of current required was 130 to 160 percent of control (15 minutes after ten-train paired conditioning by stratum radiatum) in five of six fibers; there was no change in one of six fibers.

Although PTP is produced by a tetanic stimulation of the test input (*7, 10*), LTP can be induced either as in Figs. 1A and 2 or by a tetanic stimulation of the test input concurrent with a tetanic stimulation of other inputs that terminate in the same region (*3, 4*). Our results show that a tetanic stimulation of the test input is not absolutely required to induce PTP or LTP. A mere association of the normal stimulation of the test input with a conditioning tetanic activation of other inputs to the same postsynaptic neurons (that do not necessarily terminate in the same area) can also induce PTP or LTP. We have also shown that a postsynaptic depolarization can induce LTP (*11*) and PTP when paired with an activation of the test input.

Posttetanic potentiation, which has been described in various excitable systems including hippocampus (*3*), is generally thought to be presynaptic (*3, 7, 10, 12, 13*). During PTP in the spinal cord, the presynaptic terminal excitability is decreased, presumably because of a hyperpolarization (*7, 8, 10*). Similarly, in our study, the presynaptic terminal excitability is decreased. We do not yet know how such a presynaptic change can be caused by postsynaptic or other input conditioning. The conditioning by stratum radiatum or stratum oriens tetanization and intracellular depolarization of the postsynaptic neurons may all induce an "adequate" depolarization of the CA1 neuronal dendrite at the test synaptic zone. CA1 neurons "communicate" with each other through ephaptic interactions (*14*). Perhaps a similar ephaptic coupling occurs between the postsynaptic neuron and the presynaptic terminal. Other possible causes for the conditioning effects are a release of potassium or a chemical signal by the depolarization of the dendrite that affects the presynaptic terminal. The necessity for pairing conditioning with an action potential in the presynaptic terminal to induce PTP or LTP indicates that the conditioning facilitates, rather than is responsible for, the change required for PTP or LTP. Presumably, a subliminal facilitatory process for transmitter release occurs following a single action potential in the presynaptic terminal; when this facilitation occurs concomitantly with the presynaptic effect of the postsynaptic depolarization, it is greatly amplified and leads to PTP or LTP. Even though evidence presented here supports a presynaptic involvement for associative induction of PTP and LTP, an additional postsynaptic mechanism selectively localized to the test synaptic zone cannot be excluded.

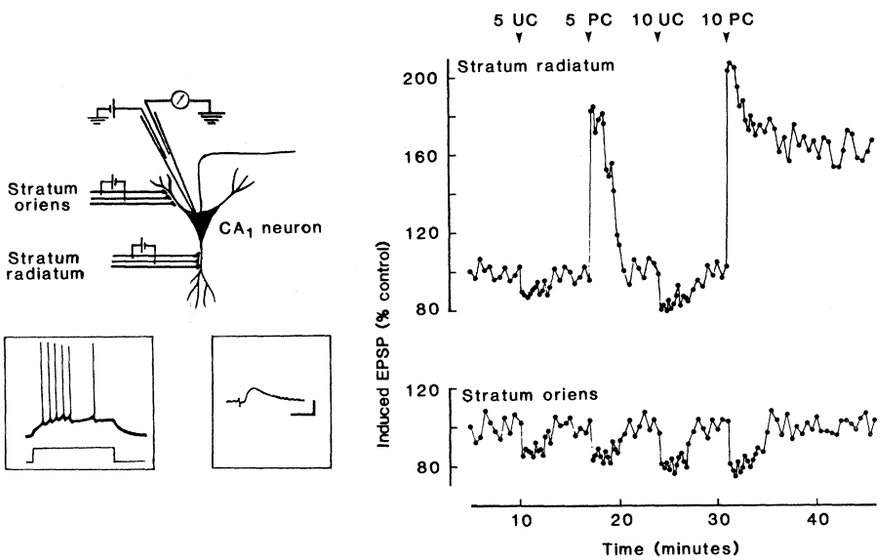

Fig. 2. Induction of PTP and LTP by paired conditioning depolarization of a CA1 neuron. To evoke EPSP's in the CA1 neuron, bipolar test stimulating electrodes were positioned in stratum radiatum and stratum oriens. A recording intracellular microelectrode in the CA1 neuron recorded the test EPSP's (inset, right; the calibrations represent 10 msec and 5 mV) and applied the conditioning depolarizing commands (3 to 10 nA, 75 to 200 msec, one to ten commands at 0.2 Hz) (inset, left; the square wave is 1 nA and 75 msec). The stimulation strengths were adjusted to evoke EPSP's at 30 percent of maximum size. The stimulation of stratum radiatum (every 15 seconds) and stratum oriens (every 15 seconds) was arranged so that there was a 7.5-second delay between the two stimulations. During the unpaired conditioning depolarization (UC), the test EPSP's by stratum oriens and stratum radiatum were not evoked and during the paired conditioning depolarization (PC) the stratum radiatum–induced EPSP was evoked 1 msec after the onset of the depolarizing command, while the stratum oriens was not stimulated. When more than one UC or PC was applied, they were given at 0.2 Hz. During this PC, stratum radiatum was stimulated at 0.2 Hz. (Stratum radiatum stimulated at 0.2 Hz without the presence of the conditioning depolarization of the CA1 neuron did not result in a change in the size of the EPSP.) Note PTP and LTP of the stratum radiatum–induced, but not of the stratum oriens–induced, EPSP following the PC. EPSP's were recorded at 30-second intervals. After UC and PC, however, recordings were taken at 15-second intervals for 3 minutes. The resting membrane potential of the neuron at the beginning of the experiment was −65 mV and at the end of the experiment was −61 mV. This is a typical experiment; similar results were found for six of nine cells.

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