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Mechanisms of Long-Term Potentiation: EPSP/Spike Dissociation, Intradendritic Recordings, and Glutamate Sensitivity

Jeffrey S. Taube[1,a] and Philip A. Schwartzkroin[1,2]

Departments of [1]Physiology and Biophysics and [2]Neurological Surgery, University of Washington, Seattle, Washington 98195

Synaptic efficacy is modified following a brief train of high-frequency stimulation (HFS) to a cell's afferent fibers (long-term potentiation; LTP). An alteration in the postsynaptic response to endogenous neurotransmitter, as a result of an increase in the number of postsynaptic receptors, has been proposed (Baudry and Lynch, 1980). We tested this hypothesis in the CA1 hippocampus by intracellularly recording the postsynaptic response to localized application of glutamate before and after induction of LTP. When LTP was produced, there was no corresponding change in neuronal sensitivity to glutamate application. These findings are not consistent with the hypothesis that HFS of fibers in CA1 stratum radiatum induces an increase in the number of postsynaptic glutamate receptors in CA1 pyramidal cells.

Previous reports concerning LTP have indicated a dissociation between the degree of potentiation in the population EPSP and population spike. Simultaneous recordings of the CA1 population EPSP and population spike in hippocampal slices confirmed that the degree of potentiation of the population spike was not predicted by the degree of potentiation in the population EPSP. Intradendritic impalements were obtained to more accurately assess changes in the intracellular EPSP following HFS. When the population EPSP was potentiated, there was also a potentiated intradendritic EPSP. When the population spike was potentiated following HFS, however, the intradendritic EPSP was often unchanged; in the same cell, there was an increased probability of action potential discharge to stimulation which was originally (i.e., pre-HFS) subthreshold for spike initiation. These results indicate that the EPSP (intracellular or extracellular) may be potentiated following HFS, but this potentiation is not a prerequisite for, or a correlation of, potentiation in the population spike. Furthermore, these findings suggest that LTP is composed of 2 independent components—a synaptic component and an EPSP-to-spike coupling component.

Enduring plastic changes occur in hippocampal neurons following brief periods of high-frequency stimulation (HFS) of the afferent fibers. One such change is the enhanced orthodromic response to a single test stimulus following HFS (Bliss and Lomo, 1973; Schwartzkroin and Wester, 1975; see Swanson et al., 1982, for review). This electrophysiological change has been termed long-term potentiation (LTP) and has received considerable interest because of its possible involvement in mediating neuronal mechanisms of learning and memory (Teyler and Discenna, 1984). Although some studies have focused on establishing a link between LTP and learning (and memory), investigative attention has been primarily on elucidating the underlying mechanisms of the LTP phenomenon.

Most workers believe that LTP is due to an increase in synaptic efficacy, and numerous studies now support a synaptic localization. The most compelling evidence for synaptic localization is the homosynaptic nature of the event; the potentiation appears to be confined to the pathway receiving the HFS (Schwartzkroin and Wester, 1975; Andersen et al., 1977; McNaughton and Barnes, 1977; Alger et al., 1978). Evidence has accumulated to support involvement of both pre- and postsynaptic control mechanisms in the development of LTP. On the presynaptic side, several investigations have shown an increased amount of neurotransmitter released in response to a single test stimulus following a period of HFS (Skrede and Malthe-Sorenssen, 1981; Dolphin et al., 1982). Presynaptic localization of control mechanisms would explain the high specificity observed for potentiated pathways. However, an increased liberation of transmitter cannot account for all the observations concerning LTP. Among the observations supporting a postsynaptic locus of control are (1) a calcium chelating agent (EGTA), injected postsynaptically, impairs LTP development (Lynch et al., 1983); (2) temporarily blocking synaptic transmission with glutamate antagonists (Dunwiddie et al., 1978; Krug et al., 1982) or removal of external calcium (Dunwiddie et al., 1978) prevents LTP development following HFS; (3) intracellular injection of depolarizing current during HFS to an initially weak afferent input (a pathway in which HFS alone does not lead to development of LTP) leads to the induction of LTP (Kelso et al., 1986); and (4) hippocampal LTP is induced by pairing single afferent volleys with intracellularly injected depolarizing current pulses (Gustafsson et al., 1987). Intracellular studies indicate that intrinsic cell properties monitored in the soma of the postsynaptic neuron (such as resting membrane potential, input resistance, and spike threshold) remain unchanged following induction of LTP (Andersen et al., 1980a).

LTP has been demonstrated most frequently in the hippocampus (Swanson et al., 1982). The neurotransmitter involved in the Schaffer collateral/CA1 pyramidal cell pathway has been intensely investigated; it is thought to be glutamate or a glutamate-like substance (see Crunelli et al., 1985, for review). Glu-

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Correspondence should be addressed to Jeffrey S. Taube, SUNY-Health Sciences Center, Department of Physiology, Box 31, 450 Clarkson Avenue, Brooklyn, NY 11203.

[a] Present address: Department of Physiology, SUNY-Health Sciences Center, Brooklyn, NY 11203.

tamate has been reported to activate several types of receptors in the hippocampus (Collingridge et al., 1983a; Fagni et al., 1983). One of these receptors, the *N*-methyl-D-aspartate (NMDA) receptor has been implicated in mediating LTP, since exposure of the tissue to 2-amino-5-phosphonovalerate (APV), an NMDA antagonist, will prevent induction of LTP without interfering with synaptic transmission (Collingridge et al., 1983b; Harris et al., 1984). Exogenously applied glutamate is known to depolarize CA1 pyramidal neurons in a manner similar to that of the orthodromically evoked EPSP (Schwartzkroin and Andersen, 1975; Segal, 1981; Hablitz and Langmoen, 1982). Baudry and Lynch (1980) have proposed that LTP occurs through an increase in the number of glutamate receptors in the postsynaptic membrane following HFS. Several biochemical and receptor binding experiments have supported this notion (Baudry et al., 1980; Lynch et al., 1982; also see Lynch and Baudry, 1984, for review). However, there is very little electrophysiological evidence to support this hypothesis. In fact, experiments from Lynch and colleagues (1976), in which extracellular recording techniques were used, demonstrated that the response to exogenously applied glutamate decreased following HFS. We have now used intracellular recording techniques to test this hypothesis.

The degree of LTP is often monitored either as an increase in the extracellularly recorded synaptic event (the population EPSP) or as an increase in the number of cells discharging action potentials in response to a stimulus (as measured by the population spike). Previous reports concerning LTP have shown that the degree of potentiation of the population EPSP could not account for the degree of potentiation in the population spike (Bliss and Lomo, 1973; Andersen et al., 1980a). Increased amplitudes of intracellular EPSPs have been reported following induction of LTP (Yamamoto and Chujo, 1978; Misgeld et al., 1979; Andersen et al., 1980a); however, the increases have been small and inconsistent. One possible explanation for difficulty in observing clear potentiation in the intracellular EPSP is that the recording electrode in most experiments is located remotely from the activated synapses. To test this hypothesis, we impaled cells in the apical dendritic region of CA1 hippocampus and monitored the evoked EPSP before and after a period of HFS that induced LTP.

The intradendritic experiments provided an opportunity to further characterize the relationships between potentiation of the population EPSP, population spike, and intracellular EPSP following an LTP conditioning train. Our findings supported the hypothesis that the LTP phenomenon was composed of at least 2 independent components—a synaptic component and an EPSP-to-spike coupling component. Intradendritic EPSP potentiation was not correlated with population spike amplitude or increased probability of cell discharge in response to a constant stimulus. Preliminary reports concerning some of these findings have been presented (Taube and Schwartzkroin, 1983).

Materials and Methods

Transverse slices, 400 μm thick, were prepared from adult guinea pig hippocampus and maintained *in vitro* at 35°C at a liquid–gas interface. Their undersurfaces were perfused with an oxygenated artificial CSF solution (pH 7.4) containing 124 mM NaCl, 5 mM KCl, 1.25 mM NaH_2PO_4, 2 mM $MgSO_4$, 26 mM $NaHCO_3$, 10 mM glucose, and 2 mM $CaCl_2$, and their upper surfaces exposed to a warmed, moistened 95% O_2/5% CO_2 atmosphere.

Microelectrodes used for intracellular (intrasomatic or intradendritic) and extracellular recording were made from fiber-filled glass micropipettes with tip resistances of 80 and 5 MΩ, respectively. Microelectrodes used for intracellular recording were filled with 4 M K^+ acetate and 0.01 M KCl; microelectrodes for extracellular recording contained 2 M NaCl. Intracellular recordings were obtained from CA1 pyramidal neurons for both the glutamate and intradendritic experiments. All pyramidal cells had resting membrane potentials >50 mV, input resistances >20 MΩ, and fired a train of 4–8 spikes in response to a 100 msec injection of 0.5 nA depolarizing current. Previous experiments have used these criteria to establish healthy intracellular impalements (Schwartzkroin, 1975). Amplified voltage signals were displayed on an oscilloscope and stored on an FM tape recorder for later off-line data analysis (Norland 3001 processing digital oscilloscope system).

Pyramidal neurons in the CA1 region were orthodromically activated by constant-current stimulation (50 μsec pulses) applied via bipolar tungsten electrodes placed in stratum radiatum. Field potentials were recorded with extracellular electrodes placed at the somatic (population spike) level for the glutamate sensitivity experiments, and at the somatic and/or dendritic level (population EPSP) for the intradendritic experiments. Stimulus intensity was adjusted until a small population spike (1–2 mV) was present in the field response. When the dendritic field potential was monitored in conjunction with the intradendritic recording, the stimulus intensity was adjusted to just below threshold for initiation of a spike in the intracellularly impaled cell. Baseline responses to stimulation were recorded at stimulation rates of 0.2 Hz for the glutamate experiments and 0.33 Hz for the intradendritic experiments. The population spike was quantified by measuring the difference between the peak negativity and the averaged values of the 2 peak positivities. The population EPSP was quantified by measuring the peak amplitude and the declining slope (i.e., rate of rise) of the negative-going waveform. LTP was elicited by stimulating the same pathway with a single 100 Hz, 1 sec train. Following HFS, population spike amplitude or population EPSP amplitude and slope were expressed as a percentage of control values; average post-HFS values for each experiment were then calculated based on 5 min intervals after HFS. LTP was defined as an average population spike amplitude (as computed at 5 min intervals) of at least 125% of control, or average population EPSP amplitude (as computed at 5 min intervals) of at least 110% of control. We have found that 80% of normal slices show LTP thus defined.

Glutamate experiments. Glutamate receptor sensitivity was tested before and after HFS by monitoring a pyramidal cell's response to a micropulse of glutamate. Sodium glutamate (1 mM; Sigma) was dissolved in saline solution and micropressure-ejected (pipette resistance, 15–30 MΩ; 30 psi applied to the pipette) at the apical dendrites of pyramidal cells in stratum radiatum. To activate the same part of the dendritic tree with glutamate as received the potentiating afferent volley, the stimulating electrodes were positioned the same distance from stratum pyramidale as the drug electrode. Pyramidal neurons were recorded at depths of 50–200 μm, where maximal field potential responses were observed.

The glutamate micropipette was lowered slowly into the tissue until a response to a micropulse of glutamate was observed. At this point the drug pipette was moved up and down in small (5 μm) steps until a maximum response was found. The drug pipette was then fixed at this position, and the same ejection duration and rate were used for the entire experiment. The duration of the glutamate pulse was adjusted (range, 5.5–50 msec) until it was subthreshold for spike elicitation. The glutamate response was very sensitive to small changes in the position of the drug pipette, and responses often varied over the course of a few minutes. Therefore, glutamate responses were monitored for at least 5 min; if, after this time, the glutamate response was stable, the afferent fibers were tetanized. Following HFS, the orthodromic and glutamate responses were monitored every 5 min for 30 min. The glutamate response was quantified with the following measurements: (1) peak depolarization amplitude, (2) slope of the rising phase of the depolarization, and (3) area beneath the depolarizing waveform. All 3 methods produced similar results; for clarity and simplicity of presentation, only the measurements for peak amplitude will be reported here. The intracellular EPSP was quantified by its peak amplitude and rate of rise (slope of initial depolarizing phase).

Intradendritic experiments. For intradendritic recording the intracellular microelectrode was visually positioned in the middle portion of stratum radiatum of the CA1 area. Recordings from pyramidal cell dendrites, as opposed to misplaced pyramidal cells or interneurons, were verified on the basis of response to a 0.5 nA, 100 msec depolarizing current injection. Intradendritic current injection produced a train of

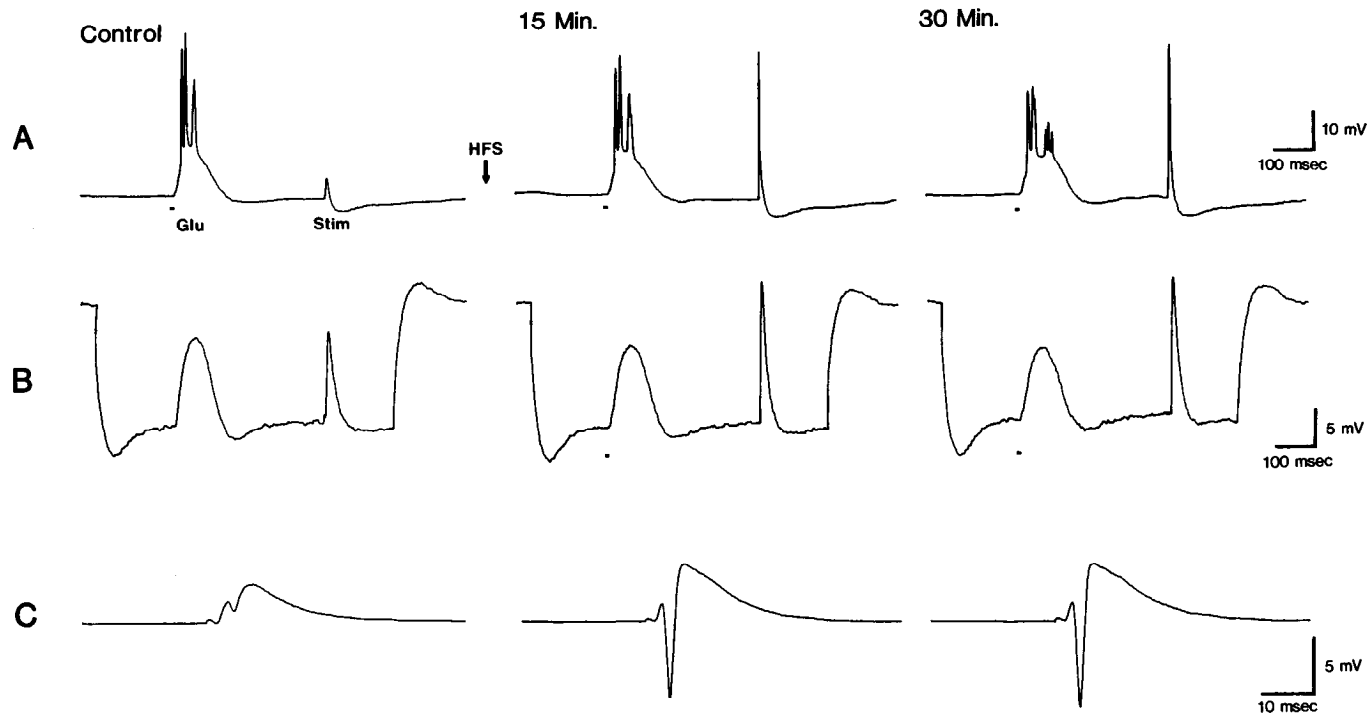

Figure 1. Intracellular response to glutamate before (*left column*) and after induction of LTP (*center* and *right columns*). *A*, Intracellular recording from a CA1 pyramidal neuron showing the response to stimulation of str. radiatum (*Stim*) and the response to a 6.8 msec micropressure pulse (30 psi) of 1 mM glutamate (*Glu*) applied in str. radiatum (150 μm from the cell-body layer). Compared with control responses, there is no change in the glutamate response at 15 and 30 min following development of LTP. *B*, Same as *A*, except responses are embedded in a 0.5 nA, 100 msec hyperpolarizing current pulse. Note that the EPSP is potentiated, whereas the response to glutamate is not. *C*, Extracellular field responses to stimulation of str. radiatum, recorded in str. pyramidale, showing an increase in the amplitude of the population spike following the HFS (2 trains of 100 Hz, 1 sec stimulation, spaced 5 sec apart). Note different time scales in *A*, *B*, and *C*. All traces are averages of 5 responses. Action potentials have been distorted by the averaging and plotting procedures.

4–8 spikes in which each spike was usually of lower amplitude and longer duration than spikes similarly evoked with intrasomatic (i.e., pyramidale) recordings. Simultaneous to the intradendritic recordings, field potentials were recorded at the somatic (population spike) or dendritic (population EPSP) level. Following HFS, there was often an evoked action potential in our intradendritic recordings, and the presence of a spike made it difficult to measure the intradendritic EPSP peak amplitude. Therefore, EPSPs were routinely (before and after HFS) embedded in a 100 msec, 0.5 nA hyperpolarizing current pulse to block action potential discharge.

Following stable recordings for 5 min with stimuli presented at 0.33 Hz, a 100 Hz, 1 sec tetanizing train was applied; occasionally 2 trains of HFS, spaced 3 sec apart, were given. Following HFS, the evoked response was monitored both intradendritically and extracellularly for as long as a healthy impalement could be maintained. Responses were recorded on an FM tape recorder and analyzed off-line. Five consecutive responses were averaged, and post-HFS averaged responses were expressed as a percentage of control values. Dendritic EPSPs were analyzed using 2 measures—peak amplitude and the rising slope of the EPSP. For the latter measurement, the EPSP slope was defined by the best-fitted line drawn through the points on the linear portion of the EPSP rising phase. Peak amplitude of the dendritically recorded IPSP was also measured.

Results

Glutamate sensitivity

A typical response of a pyramidal cell to a micropulse of glutamate applied in stratum radiatum is shown in Figure 1. A 10 msec ejection of glutamate was often sufficient to cause the pyramidal neuron to fire one or a series of action potentials. Because spike contamination made it difficult to measure the true amplitude of the glutamate response, cells were additionally monitored with the glutamate response and the EPSP embedded in a 0.5 nA hyperpolarizing pulse (Fig. 1*B*). This hyperpolarization was usually effective in preventing spike discharge and also served to monitor cell input resistance. Since the response to glutamate fluctuated slightly from trial to trial, 5 responses were averaged for a given time point.

Stable baseline glutamate responses were obtained for 5–10 min before applying HFS. In 20 slices showing LTP (Fig. 1*C*), as determined by an increase in population spike amplitude to at least 125% of control value, the population spike was potentiated to an average of 471 ± 291% over control values. Any slice that did not show potentiation in the population spike was excluded from analysis.

Figure 2 graphs the change in glutamate response amplitude over time for 12 cells in which stable impalements were maintained for 30 min and 8 cells in which stable impalements were maintained for 10–25 min. Depolarizing glutamate responses were essentiallly unchanged over a 30 min monitoring period. At 30 min post-HFS, 3 cells showed a consistent decrease in the response to glutamate, 6 cells were unchanged, and 3 cells showed an increase. For these latter cells, the change at 30 min was to 121, 116, and 106% of control values; for these same 3 cells, if the response was averaged across 30 min at 5 min intervals, the average percentage change was only to 96.1, 106, and 111% of controls. The amplitudes of the glutamate responses following HFS, averaged across all 20 cells at 5 min intervals and expressed as a percentage of control values, were 87.4, 83.8, 82.4, 88.6, 87.2, and 85.8 (Fig. 2, dotted line). Three

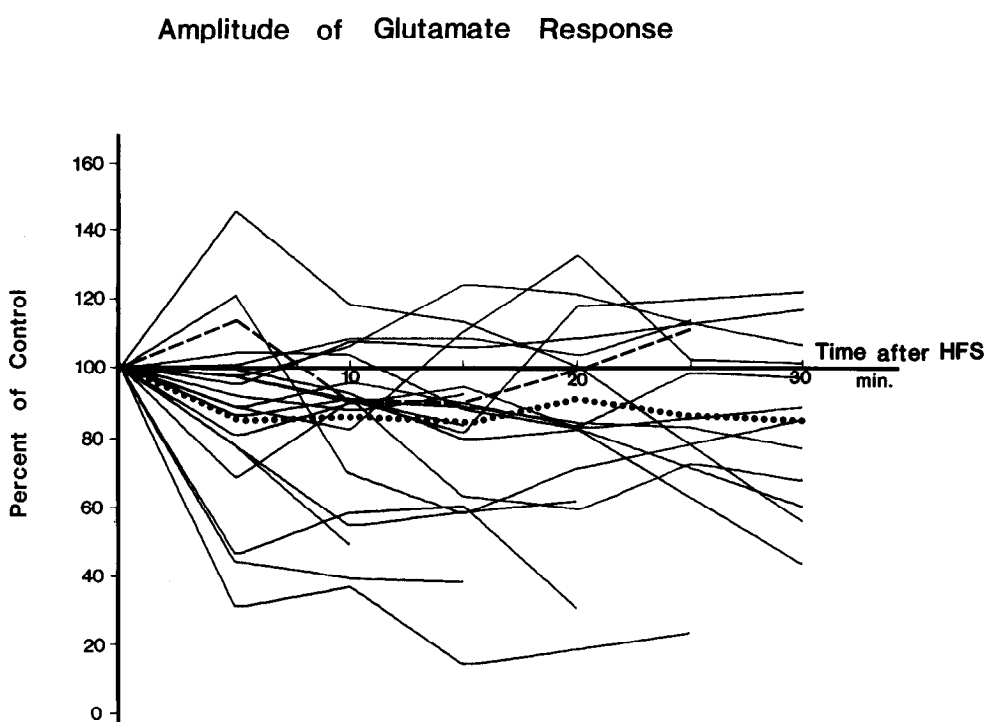

Figure 2. Graph plotting the amplitude of the intracellular glutamate response (percentage of control on ordinate) versus time after applying HFS (abscissa). Each line represents one cell. All cells shown were recorded from slices in which an enhanced population spike or intracellular EPSP was observed following HFS. Each point in each cell line represents an average of 5 responses. The *dotted line* shows the average glutamate response from all cells tested following induction of LTP. The *dashed line* shows the average responses to glutamate from 3 cells in slices that did not receive HFS (controls). Average pyramidal cell response to glutamate changed very little as a function of LTP.

"control" cells received no HFS, and the peak amplitude of the depolarizing glutamate response was monitored for 30 min. The average responses for these 3 cells are shown as the dashed line in Figure 2. When the slope and the area under the waveform of the depolarizing glutamate response were analyzed, no consistent post-HFS changes compared with control values were observed.

Following HFS, the peak amplitude of the intrasomatically-recorded EPSP varied considerably. In about half of the experiments (9 of 20 slices), the EPSP amplitude (as measured during a hyperpolarizing pulse) increased following HFS; the average percentage increase was to 131 ± 16.5% (range 112–164%). In these 9 experiments, only 2 cells demonstrated significant increases in the glutamate response above control values ($p < 0.05$; t test). In these 2 cells, the average glutamate response (calculated by averaging values over a 30 min period at 5 min intervals) had increased to 106 and 111% of controls. The other 7 cells showed either no change (e.g., Fig. 1*B*) or a decrease in the glutamate response.

Double field recordings

Initial experiments were designed to examine the relationship between the degree of potentiation in the population EPSP and the degree of potentiation in the population spike following HFS. Field potentials were simultaneously recorded in the area of the activated synapses in stratum radiatum (population EPSP) and at the level of the cell body layer in stratum pyramidale (population spike). The field potentials were monitored at 5 min intervals after HFS, and these values averaged over 20 min. The results from 6 experiments are shown in Table 1. In general, it was much easier to get potentiation of the population spike than potentiation of the population EPSP following HFS. With potentiation of the population spike, the population EPSP usually showed a small degree of potentiation; in 2 experiments, the level of population EPSP potentiation was below our criterion value of 110%. The degree of potentiation in the population EPSP (amplitude or slope) did not correlate with the degree of potentiation in the population spike ($R = 0.0497$ for potentiation of the population spike versus potentiation of the population EPSP amplitude; $R = 0.0857$ for potentiation of the population spike versus potentiation of the population EPSP slope).

In an additional 14 experiments carried out for other purposes, field potential recordings were obtained sequentially from somatic and dendritic locations. Since, in those studies, the recording electrode was moved during the course of the experiment, it was not possible to guarantee that the post-HFS field potential recordings were obtained from the exact location and depth as the pre-HFS field potential recordings. Nevertheless, the results of the field potential recordings from these latter

Table 1. Double field recordings and LTP

Experiment ($n = 6$)	Population spike amplitude (% Control)	Population EPSP amplitude (% Control)	Population EPSP slope (% Control)
1	572	113	139
2	432	132	132
3	343	124	128
4	325	155	200
5	288	104	104
6	241	108	126
Average	382.6 ± 125.7	126.4 ± 18.5	145.0 ± 31.1

Paired field potential recordings in CA1 hippocampus following HFS applied to the afferent fibers. One microelectrode was positioned in the cell body layer (str. pyramidale) to record the population spike, while the second microelectrode was positioned at the level of the apical dendrites (str. radiatum) to record the population EPSP. Following collection of baseline control values, HFS was applied to str. radiatum. Evoked responses were monitored and averaged at 5 min intervals post-HFS, and the average value expressed as a percentage of control.

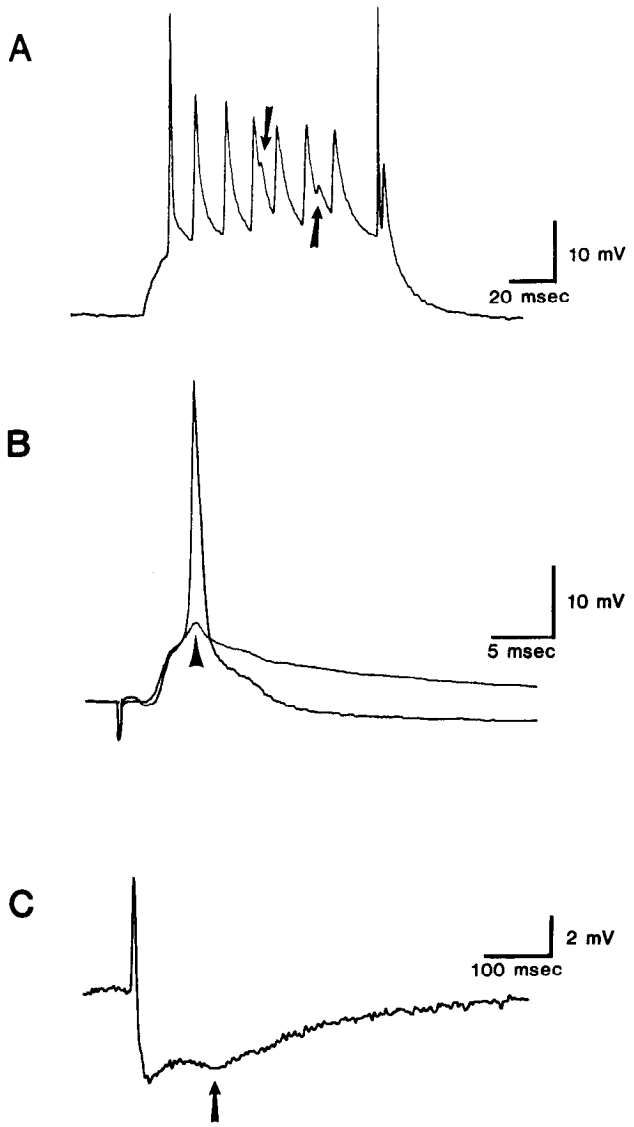

Figure 3. Intradendritic recordings in CA1 pyramidal neurons. *A*, Intradendritic response to a 100 msec, 0.5 nA depolarizing current pulse. Note the small amplitude of action potentials. Two action potentials appeared fractionated (*arrows*), suggesting multiple trigger zones. *B*, Intradendritic response to stimulation of stratum radiatum. Two superimposed traces are shown from the same neuron. In one trace, a subthreshold EPSP was triggered, with a small spikelike ("prepotential") depolarization forming the crest of the EPSP (*arrowhead*). In the second trace, stimulation evoked an EPSP that led to an action potential discharge. *C*, Biphasic IPSP recorded as part of the intradendritic response to stimulation of stratum radiatum. An EPSP/IPSP sequence was evoked in this cell. The *arrow* points to the peak of the late component of the biphasic IPSP.

experiments were similar to those from the 6 double field recording experiments described above. There was no correlation between the degree of potentiation in the population spike and the degree of potentiation in the population EPSP (amplitude and slope, $R = -0.018$ and -0.071, respectively).

These results, which confirm previous findings (Andersen et al., 1980a, 1984; Wilson, 1981; Wilson et al., 1981; Abraham et al., 1985), demonstrate that (1) there is an inconsistent relation between potentiation of the population spike and potentiation of the population EPSP, and (2) the population spike can show a high degree of potentiation without any clear potentiation in the population EPSP. We consequently examined the relationships between (1) the population spike and the intradendritic EPSP and (2) the population EPSP and the intradendritic EPSP.

Intradendritic experiments.

Intradendritic penetrations were made 100–200 μm from the apical border of stratum pyramidale. Stable dendritic impalements were more difficult to achieve than somatic impalements, presumably because of the smaller diameter of the dendrites compared to the soma. Dendritic resting membrane potentials were similar to those recorded intrasomatically (−55 to −65 mV), but dendritic input resistances (R_{in}) were generally higher. Input resistances were monitored by passing a 100 msec, 0.5 nA hyperpolarizing current pulse and calculating R_{in} by measuring the voltage deflection. Dendritic input resistances averaged 33.0 ± 8.82 MΩ (range, 19.0–48.1 MΩ; $n = 22$), compared with reported intrasomatic values from this laboratory of 27 MΩ (Turner and Schwartzkroin, 1980). Cell responses to a 100 msec, 0.5 nA injection of depolarizing current were also monitored. Current-evoked dendritic spikes had smaller peak amplitudes (30–40 mV) and broader profiles (half-amplitude duration of 2–3 msec) than current evoked somatic spikes (Fig. 3*A*). This finding is consistent with the idea that our dendritic recording sites are remote from the spike-generating zone (compared with intrasomatic recording sites), and see electrotonically conducted decremented signals.

Stimulation of stratum radiatum usually evoked an EPSP-IPSP sequence in intradendritic recordings. The amplitude of the EPSP was dependent on stimulation intensity. However, exceptionally large amplitude, subthreshold EPSPs could be evoked from intradendritic recordings—EPSPs that would have triggered spikes in somatic recordings. EPSP peak amplitudes prior to HFS ranged from 5.00 to 19.9 mV (average, 10.5 ± 3.43 mV). Subthreshold EPSPs often contained a small, all-or-none depolarizing potential rising from the peak of the EPSP envelope (arrowhead in Fig. 3*B*). An action potential could be evoked by increasing the stimulus strength (e.g., see Spencer and Kandel, 1961).

IPSPs were observed in 21/22 cells. In those cells in which IPSPs were present, the peak amplitude of the IPSP prior to HFS ranged from 0.644 to 9.38 mV (average, 4.46 ± 2.64 mV). In some cases, the IPSP appeared to be biphasic (Fig. 3*C*). Measurements of reversal potential for each component were consistent with the proposal that the early phase of the IPSP is due to a GABA-mediated chloride conductance (Andersen et al., 1980b; Dingledine and Langmoen, 1980; Alger and Nicoll, 1982; Mueller et al., 1984) and the later phase to a GABA-mediated potassium conductance (Newberry and Nicoll, 1985).

Stable baseline responses to orthodromic stimulation were monitored for 5 min before applying HFS to the orthodromic pathway. Following the HFS, cells were monitored for as long as healthy impalements could be maintained. All cells analyzed below were monitored for a minimum of 10 min post-HFS.

Intradendritic EPSPs/population spike experiments. Eight experiments were performed in which LTP was induced in the population spike. The average percentage potentiation in the population spike was to 370 ± 260% (range, 152–943%; Fig. 4*C*). Prior to HFS, none of the cells produced a spike in response to orthodromic stimulation at the stimulus strength used. Following development of LTP, 5/8 cells consistently discharged

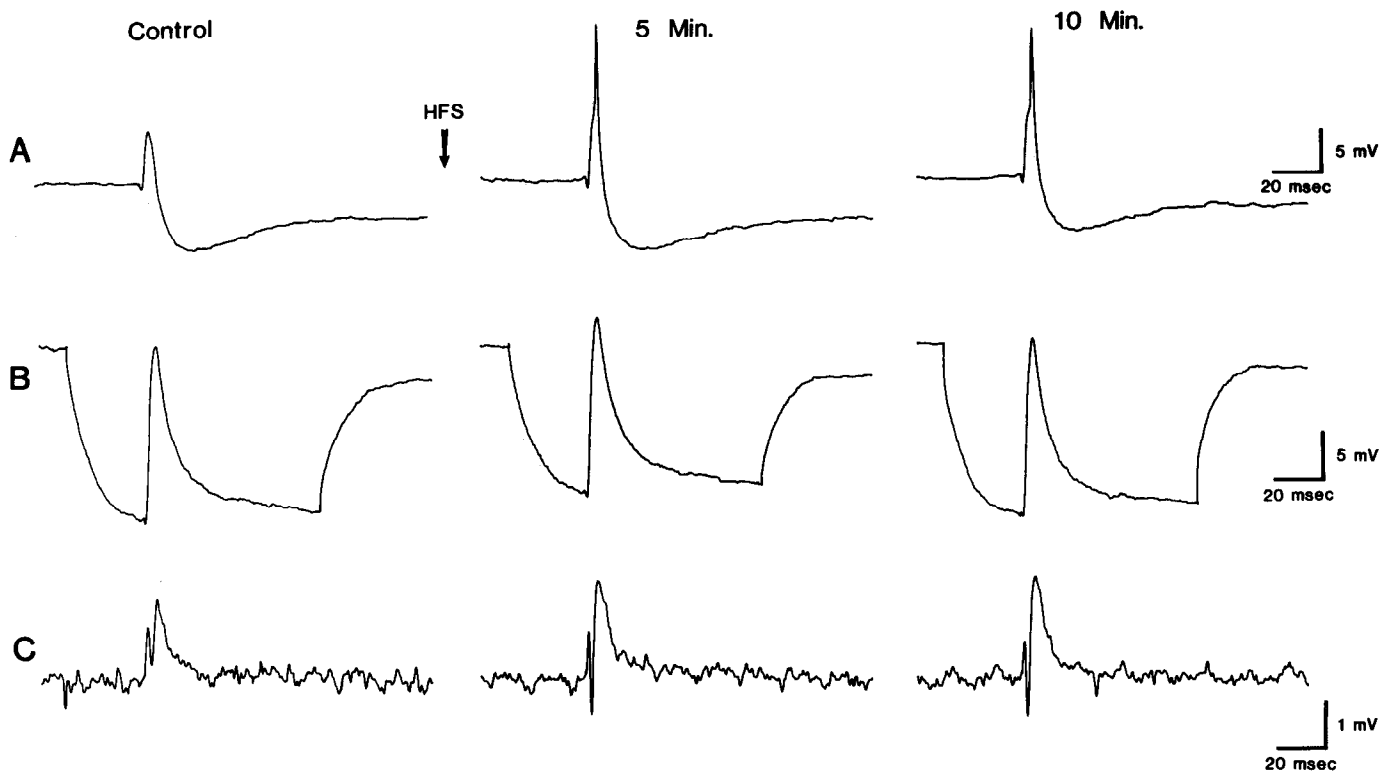

Figure 4. Unchanged intradendritic EPSP during potentiation of the population spike. *A*, Intradendritically recorded responses in a CA1 pyramidal neuron to stimulation of str. radiatum before (*left column*) and 5 and 10 min following HFS (*middle* and *right columns*, respectively). Before HFS, the stimulus elicited a subthreshold EPSP. Following development of LTP in the population spike (see traces in *C*), orthodromic stimulation consistently evoked an action potential. The rising slope of the intradendritic EPSP, however, remains unchanged. *B*, Same as *A*, except responses are embedded in a 100 msec, 0.5 nA hyperpolarizing current pulse in order to block spike discharge. This manipulation revealed that EPSP amplitude was also unchanged during population spike potentiation. *C*, Stratum pyramidale field potential responses to stimulation of str. radiatum showing a potentiated population spike at 5 and 10 min following HFS. All traces (*A–C*) are averages of 5 responses. HFS consisted of 2 trains, spaced 3 sec apart (100 Hz, 1 sec stimulation/train).

with orthodromic activation (Fig. 4*A*); 2 other cells produced an action potential about half the time.

With the evoked response embedded in a hyperpolarizing current pulse to block spike discharge, the peak amplitude of the intradendritic EPSP could be measured. EPSP amplitude remained virtually unchanged in most cells (Fig. 4*B*), even those cells that showed consistent spike firing following HFS. Figure 5*A* is a graph showing the change in the EPSP peak amplitude across time for 8 cells. Recordings monitored for several minutes before tetanizing indicated that the intradendritic EPSP peak amplitude could vary by as much as 10%, even when 5 responses were averaged. In 4/8 cells the EPSP peak amplitude remained within 10% of control values following HFS. In another cell, the EPSP amplitude increased to 114% of control values at 10 min following HFS. The average percentage change following HFS, computed for these 5 cells at 5 min intervals, was to 100.5 ± 11.3% of control values. In the remaining 3 cells (indicated by the solid steep-sloped line in Fig. 5*A*), it was not possible to determine the EPSP peak amplitude because a spike was consistently triggered from the EPSP, even when the EPSP was embedded in a hyperpolarizing current pulse.

Three cells were also monitored using intrasomatic recordings, and their EPSP changes are indicated by the dashed lines in Figure 5. Two of these cells did not show an increase in the EPSP, even though the population spike was potentiated in both cases (265 and 359% of control values). It was not possible to monitor the EPSP peak amplitude in the third cell because of spike contamination. The average percentage change in measurable intracellular EPSP amplitudes, averaged at 5 min intervals, from all cells (dendritic and somatic recordings) is shown as the diamond-demarcated line in Figure 5*A*.

Because change in the intracellular EPSP amplitude may be masked by the presence of a spike, and because our analysis of EPSP amplitude excluded those cells with spike discharge, we also used the rising slope of the intracellular EPSP as an index of EPSP potentiation. Figure 5*B* shows the slope of the rising phase of the EPSP (when embedded in a hyperpolarizing current pulse) plotted versus time after HFS. Three intrasomatic recordings are again shown with dashed lines. In general, the results parallel those found by measuring the EPSP peak amplitude. In most cells there was very little change in the rising slope of the intradendritic EPSP (average percentage change to 106.0 ± 10.4% of controls; $n = 5$) or the intrasomatic EPSP (average percentage change to 97.6 ± 6.61% of controls). However, the 3 cells in which a spike was present during the hyperpolarizing current pulse (and thus were not included in the initial analysis), did show an increase in EPSP rising slope. These 3 cells (indicated with an asterisk in Fig. 5*B*) had an average percentage change in their rising slopes to 125.8 ± 6.1% following HFS. The average percentage change in intracellular EPSP rising slopes, averaged at 5 min intervals, from all cells (dendritic and somatic recordings) is shown as the diamond-demarcated line in Figure 5*B*.

An analysis of the relationships between the average degree

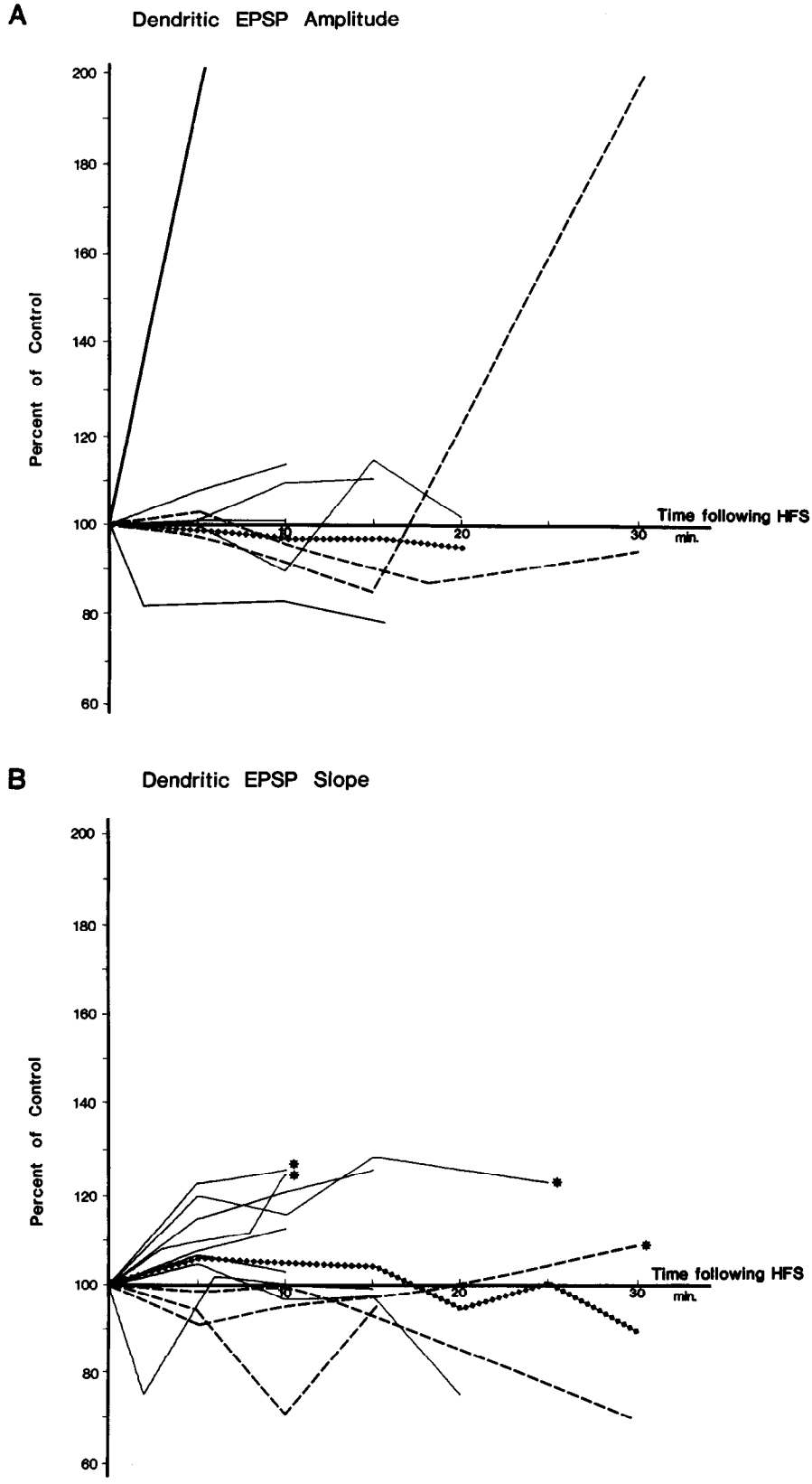

Figure 5. *A*, Graph plotting the change in intracellular EPSP amplitudes (expressed as percentage of control values) versus time following HFS. All cells shown were recorded from slices in which an enhanced population spike (in response to orthodromic stimulation) was observed following HFS. Orthodromic stimulation and HFS were applied to str. radiatum; HFS occurred at time 0. The *thin solid lines* are from CA1 pyramidal neurons impaled in their apical dendrites; the *dashed lines* are from intrasomatic penetrations. Cells that discharged an action potential in response to orthodromic stimulation (following HFS) are shown with steeply rising lines. The steep *solid line* at *left* presents 3 different intradendritic recordings, in which the triggered action potential could not be blocked by the 0.5 nA hyperpolarizing current pulse. The average of 5 intradendritic and 2 intrasomatic recordings in which no action potentials obscured peak EPSP amplitude is shown by the *diamond-demarcated line.* *B*, Same as *A*, except the ordinate gives the percentage change of the rising slope of the intracellular EPSP. The 4 lines labeled by *asterisks* denote neurons that discharged an actial in response to orthodromic stimulation following HFS. With the exception of the 3 intradendritic recordings in which action potentials occurred, the slope of intradendritic EPSP changed inconsistently, even with potentiation of the population spike. The average of all 8 intradendritic and 3 intrasomatic recordings is shown by the *diamond-demarcated line.*

of potentiation of the population spike and the intracellular EPSP (intradendritic and intrasomatic) showed none between the population spike and the amplitude of the intracellular EPSP (the slope of the best-fit line was −0.0906) and very little between the population spike and the rising slope of the intracellular EPSP (R = 0.414, slope of the best-fit line was 0.0248). These results indicate that a potentiated intradendritic EPSP does not necessarily correlate with a potentiated population

spike, nor does potentiation of the intradendritic EPSP appear to be a prerequisite for potentiation of the population spike.

Intradendritic EPSP/population EPSP experiments. To examine the relationship between the intradendritic EPSP and the population EPSP following HFS, we monitored 18 cells following a period of HFS. In 12 experiments the population EPSP amplitude was potentiated at least 110% following HFS; the average amount of potentiation was to 119.7 ± 16.3% (range, 110–151%). In the 12 experiments where the population EPSP was potentiated, 7 cells consistently discharged a spike to orthodromic stimulation following HFS; 2 cells discharged a spike occasionally. Prior to HFS, all 9 cells were subthreshold for spike initiation in response to the same orthodromic stimulation. Therefore, intradendritic EPSP amplitudes and rising slopes were again measured when the EPSP was embedded in a hyperpolarizing current pulse to block action potential discharge.

Figure 6*A* graphs the intradendritic EPSP peak amplitude (when embedded in a hyperpolarizing current pulse) versus time following HFS. In contrast to the studies examining the relationship of population spike to intradendritic EPSP, the peak amplitude of the intradendritic EPSP was frequently potentiated when the population EPSP was potentiated (Fig. 6*A*). When it was possible to measure the EPSP peak amplitude (i.e., no spikes were discharged), an average percentage change was computed for each cell, using 5 min post-HFS intervals. With this procedure, 7/10 cells showed potentiation to greater than 110% of control. The average percentage change of these 7 potentiated cells was to 120.1 ± 11.6% (range, 111–139%). The remaining 3 cells had EPSP responses that averaged 106, 105, and 88.4% of control. Figure 7 shows an example from one neuron that showed a potentiated intradendritic EPSP following HFS. The diamond-demarcated line in Figure 6*A* shows the average degree of potentiation of the dendritic EPSP peak amplitude averaged from all 10 cells at 5 min post-HFS intervals: The dotted line shows the average dendritic EPSP amplitude from 5 experiments where there was no change in the population EPSP following HFS. There was a significant difference in potentiation of intradendritic EPSP amplitudes between cells from slices showing potentiated population EPSPs and cells from slices where the population EPSP was not potentiated ($p < 0.05$, Mann-Whitney *U* test).

Following HFS, 5 cells that had previously responded to stimuli with subthreshold EPSPs discharged spikes in response to orthodromic stimulation, even when the EPSPs were embedded in a hyperpolarizing current pulse. We therefore assessed the intradendritic EPSP potentiation by measuring the slope of the EPSP when it was embedded in a hyperpolarizing current pulse. The slope of the linear portion on the rising phase of the intradendritic EPSP is plotted versus time after HFS in Figure 6*B*. The percentage change in slope, at 5 min post-HFS intervals, was calculated for cells in slices showing potentiation of the population EPSP amplitude; 10/12 cells showed an increase in EPSP slope of greater than 110%, with an average percentage change in slope to 128.6 ± 16.0% (range, 110–167%). In the remaining 2 cells, changes in their slopes were 107 and 89.9% of control. The diamond-demarcated line in Figure 6*B* shows the average change in the EPSP rising slope from all cells at 5 min post-HFS intervals. There was a significant difference ($p < 0.02$, Mann-Whitney *U* test) in the change of EPSP rising slope between cells from slices with a potentiated population EPSP (as described above) and cells from slices where the population EPSP was not potentiated (Fig. 6*B*, dotted line). When the degree of potentiation of the population EPSP was plotted versus potentiation in the amplitude or rate of rise of the intradendritic EPSP, there was a significant correlation (slope of the best-fit line = 1.004, $R = 0.778$ for amplitude; 1.084 and 0.803 for rising slope of EPSP). These results from the intradendritic EPSP/population EPSP experiments indicate that potentiation of the population EPSP amplitude is accompanied by an increase in the intradendritic EPSP amplitude and slope. The degree of potentiation in the intradendritic EPSP correlates well with the degree of potentiation in the population EPSP but not with the degree of potentiation in the population spike.

Threshold experiments. Threshold for spike initiation was examined in 13 intradendritic recordings. Depolarizing current pulses (100 msec duration) were passed through the electrode, and threshold was defined as the amount of current needed to elicit a spike 50% of the time. There was little change in cell firing threshold to intradendritic current injection following HFS. The average control threshold for the 13 cells was 0.115 ± 0.0819 nA. Ten of 13 cells showed no change in threshold; 2 cells showed a consistent decrease in threshold of greater than 10%. One of the latter cells had an increased R_{in} following HFS (and therefore less current was presumably needed to depolarize the cell to threshold). The second cell had an initial threshold of 0.11 nA; 30 min following HFS, the threshold had decreased to 0.07 nA. This cell fired spikes spontaneously for 30 min following HFS, after which the spontaneous firing ceased and the threshold increased to 0.10 nA. The 9 cells that were initially subthreshold for spike initiation in response to orthodromic stimulation, but responded with an evoked spike to orthodromic stimulation following HFS, had unchanged thresholds following development of LTP. These results indicate that the increased propensity for cell firing cannot be attributed to a change in spike threshold.

TTX experiments. Although we sometimes observed small changes in EPSP amplitude following HFS, large increases may have been obscured by action potential discharge. Experiments were therefore carried out in which somatic spikes were blocked with local application of TTX (Sigma) to the cell body region. TTX (10^{-4} M) was micropulsed into the pyramidal cell body layer until spikes evoked by intracellular depolarizing current were abolished. This procedure did not affect the amplitude of the control EPSP evoked in the cell dendrites. Four cells, in 4 different slices, were exposed to TTX application in this manner and monitored following a period of HFS. In 2 cells, small, but consistent decreases in EPSP amplitude and slope were observed following HFS; in the 2 other cells, there were small, but consistent increases in EPSP amplitude.

Picrotoxin experiments. The peak amplitude of the EPSP may be underestimated because its time course overlaps with that of the early IPSP. Therefore, 8 additional intradendritic experiments were performed with 50 or 100 μM picrotoxin (Sigma) introduced into the bathing medium. These concentrations of picrotoxin have been shown to inhibit GABA-mediated IPSPs in the hippocampus (Alger and Nicoll, 1979; Wigstrom and Gustafsson, 1983). Since cells exposed to picrotoxin often produced burst discharges in response to orthodromic stimulation, low-intensity stimuli were used. Even so, occasional bursts obscured the peak of the EPSP. The slope of the EPSP was therefore used as an index for EPSP change following LTP-inducing HFS. Only 2 of 8 cells showed a potentiation of the intradendritic EPSP. The 6 "nonpotentiated" cells were subsequently tested at higher stimulus strengths (approx. 1- to 3-fold increase) for

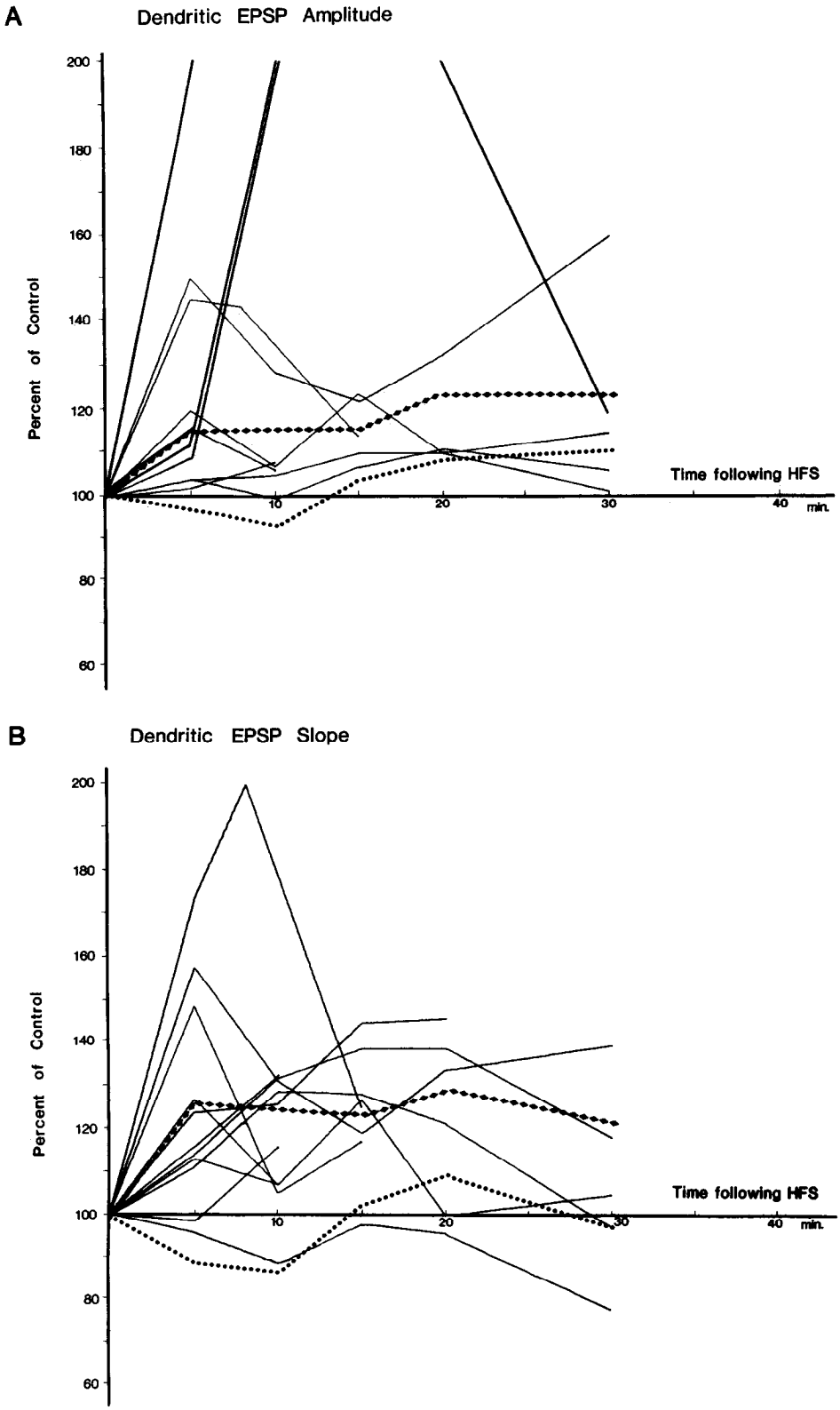

Figure 6. *A,* Graph plotting the percentage change in the intradendritic EPSP amplitude (expressed as percentage of control values) versus time following HFS. Cells shown were recorded from selected slices in which an enhanced population EPSP was observed following HFS. Cells that discharged an action potential which could not be blocked by the hyperpolarizing current pulse are represented by steeply rising lines that ascend to, or decline from, the 200% level. The steep *solid line* from 0 to 5 min represents 2 different cells. The *diamond-demarcated line* represents the average EPSP amplitudes from all cells measured (cells that discharged an action potential were not included in the averaging process). The *dotted line* represents the average EPSP amplitudes from 4 cells that did not show potentiation in the population EPSP after HFS. *B,* Same as *A,* except the ordinate represents the percentage change in the intradendritic EPSP rising slope. The average of all cells (including those discharging action potentials) is shown by the *diamond-demarcated line.* Potentiation of the intradendritic EPSP (both amplitude and slope) was seen more often when slices that showed a potentiated population EPSP were selected than when judgement of LTP was based solely on population spike enhancement (cf. Figs. 5 and 6).

their capacity to show potentiation. Under the higher stimulus strength condition, 2/6 cells that had not been potentiated by the lower stimulation intensity showed potentiation.

IPSPs. Changes in the "pure" IPSP following LTP induced by HFS was difficult to monitor in some experiments because spike discharge induced by HFS activates several intrinsic membrane conductances whose time courses coincide with the IPSP. In the absence of HFS-induced spike discharge, the IPSP was variable. Six of 9 cells showed an increased IPSP amplitude following HFS; the percentage changes in IPSP peak amplitude

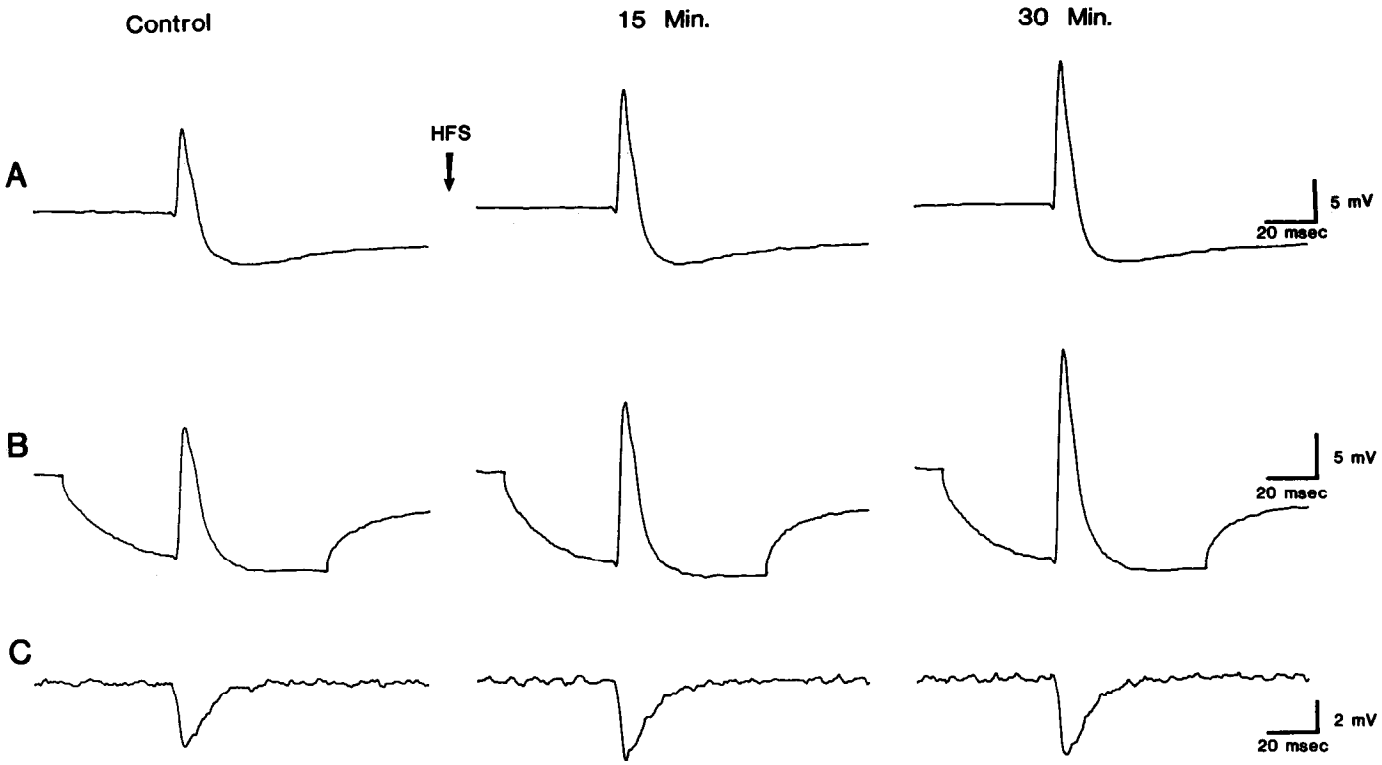

Figure 7. Enhanced intradendritic EPSP following potentiation of the population EPSP. *A,* Intradendritic EPSPs in a CA1 pyramidal neuron, in response to stimulation of str. radiatum before (*left column*) and 15 and 30 min after HFS (*middle* and *right columns,* respectively). Following development of LTP in the population EPSP (see traces in *C*), both amplitude and rising slope of the intradendritic EPSP were potentiated. *B,* Same as *A,* except responses are embedded in a 100 msec, 0.5 nA hyperpolarizing current pulse. *C,* Dendritic field potential responses to stimulation of str. radiatum, showing a potentiated population EPSP at 15 and 30 min following HFS. All traces (*A–C*) are averages of 5 responses. HFS consisted of 2 trains, spaced 3 sec apart (100 Hz, 1 sec stimulation/train).

averaged across these cells, at 5 min intervals, were to 230, 200, 180, 114, 109, and 108% of control values. There was also a trend for a small decrease in the latency to IPSP peak. The remaining 3 cells had decreases in IPSP amplitudes to averaged 94.0, 82.8, and 61.0% of control values. In those experiments, in which a post-HFS spike was evoked by orthodromic stimulation, the IPSP amplitude and latency to peak showed no consistent change; 4 cells showed an increase in IPSP amplitude, 4 cells showed no change in IPSP amplitude, and 4 cells showed a decrease in IPSP amplitude. These data indicate that there is little correlation between induction of LTP and a change in the intradendritic IPSP.

Discussion

Glutamate experiments

The primary finding of the glutamate study is that there is virtually no change in the CA1 pyramidal cell's sensitivity to glutamate following LTP-inducing tetanization. Even in the majority of cells where there was an amplitude increase in the orthodromically evoked EPSP, there was no change in their response to glutamate. These results are similar to those reported by Lynch et al. in an extracellular study examining the discharge frequency of pyramidal cells with application of glutamate (Lynch et al., 1976). In our experiments, several cells showed decreased response to glutamate following HFS. Critical in determination of the amplitude of the depolarizing glutamate response was the position of the drug electrode. Even small (5–10 μm) movements of the drug pipette could change the size of the response. The response decreases observed following HFS may therefore be due to slight movements of the tissue or drug electrode, rather than to an actual decrease in glutamate receptor sensitivity.

Several investigations have suggested that LTP—or at least a component of LTP—is due to events occurring on the postsynaptic side of the synapse (McNaughton et al., 1978; Levy and Steward, 1979; Andersen et al., 1980a; Robinson and Racine, 1982; Lynch et al., 1983; Kuhnt et al., 1985). For example, LTP does not develop when synaptic transmission is blocked by presumably postsynaptic glutamate antagonists such as glutamic acid diethyl ester (GDEE) (Krug et al., 1982) and D, L-2-amino-4-phosphonobutyric acid (APB) (Dunwiddie et al., 1978). The various possible postsynaptic changes that would lead to an enhancement of the response include (1) an increase in receptor sensitivity, i.e., receptor affinity for the binding of neurotransmitter; (2) an increase in the mean channel open time; (3) an increase in the conductance of a channel; or (4) an increase in receptor number or receptor density in the postsynaptic membrane. The amplitude and/or time course of the postsynaptic response would be enhanced if any one of these changes were to occur following high-frequency stimulation. Lynch and colleagues have suggested LTP occurs as a result of an increase in the number of glutamate receptors in the postsynaptic membrane (Baudry and Lynch, 1980; Lynch and Baudry, 1984). Our results are not consistent with this hypothesis, or any of the other possibilities listed above. If, indeed, there was an increase in glutamate receptor number, one would expect an increase in postsynaptic cell sensitivity to glutamate.

We do not conclude, however, that there is no change of the receptor/ionophore complex following LTP. Although numerous studies, including this one, have demonstrated that applied glutamate can "mimic" the orthodromically evoked EPSP in the Schaffer collateral to CA1 pyramidal cell pathway (Dudar, 1974; Schwartzkroin and Andersen, 1975; Segal, 1981; Hablitz and Langmoen, 1982), glutamate has not been established as the neurotransmitter at these synaptic junctions. Other excitatory amino acids and related compounds [such as *N*-methyl-D-aspartate, D,L-homocysteate, kainate, quisqualate, *N*-acetylaspartylglutamate (NAAG)] can produce similar responses (Collingridge et al., 1983a; Fagni et al., 1983; Bernstein et al., 1985). Furthermore, recent studies have pointed out differences in the actions of these compounds based on desensitization techniques (Fagni et al., 1983). The NMDA antagonist APV has been reported to block development of LTP without interfering with synaptic transmission (Collingridge et al., 1983b; Harris et al., 1984), suggesting that different receptors are involved in mediating synaptic transmission and LTP. Since the types of glutamate receptors activated by our microinjections of glutamate were not determined, we cannot rule out the possibility that our results (i.e., no change in the glutamate response following HFS) are attributable to the activation of a glutamate receptor subtype involved in LTP.

Double field recordings and intradendritic experiments

The results from our intradendritic recordings of EPSP potentiation can be separated into 2 categories, depending on whether (1) the population spike was potentiated or (2) the population EPSP was potentiated. When the population spike was potentiated, there was often no change in the intradendritically recorded EPSP amplitude; however, a previously subthreshold (for spike initiation) EPSP often triggered spike discharge following the HFS. In contrast, when the amplitude of the population EPSP was potentiated by HFS, the intradendritic EPSP amplitude and rise time were both increased. In this case, too, potentiation of the population EPSP was often correlated with the triggering of spike discharges at stimulus strengths previously subthreshold for spike initiation. The degree of potentiation of the intracellular EPSP correlated well with the degree of potentiation in the population EPSP but not with the degree of potentiation in the population spike. Consistent with these results was our finding, from the double field recording experiments, that the degree of potentiation in the population EPSP (either amplitude or slope) did not correlate well with the degree of potentiation in the population spike. It is important to note that potentiation of the population EPSP was always accompanied by potentiation of the population spike.

Previous extracellular studies of LTP have indicated that the population spike is potentiated more than can be explained simply on the basis of potentiation of the population EPSP (Andersen et al., 1980a; Wilson, 1981; Wilson et al., 1981; Abraham et al., 1985). In fact, the population spike is sometimes potentiated even in the absence of population EPSP potentiation (Bliss and Lomo, 1973). Input/output curves, which relate stimulus intensity to population EPSP amplitude and population EPSP amplitude to population spike amplitude, both show leftward shifts following HFS; these shifts indicate that the LTP phenomenon is composed of at least 2 components, labeled by Andersen et al. as "V-E" (volley to EPSP) potentiation and "E-S" (EPSP to spike) potentiation (Andersen et al., 1980a). The enhancement of the intradendritic EPSP following HFS represents the intracellular manifestation of the leftward shift observed in the graph relating stimulus strength versus population EPSP amplitude; the increased propensity for cellular discharge in response to orthodromic stimulation reflects the leftward shift of the plot relating population EPSP amplitude versus population spike amplitude (Andersen et al., 1980a; Wilson, 1981; Wilson et al., 1981; Abraham et al., 1985).

Support for the notion that LTP is composed of 2 independent components has been suggested by other investigations. Recent studies by Abraham et al. (1985) in the dentate region found that V-E potentiation occurred at a lower threshold level than E-S potentiation, suggesting that these 2 forms of potentiation have different underlying mechanisms and are probably independent of one another. A different study by Robinson and Racine (1982) showed that induction of LTP in one pathway was greater when the HFS was simultaneously paired with HFS in a second pathway; this "cooperativity" of afferent inputs effect was seen only when inducing LTP of the population spike, not of the population EPSP.

There are a number of factors that could contribute to the absence (or minimal amount) of potentiation of the intracellular EPSP, despite potentiation in the population spike. First, the recording electrode may have been electrotonically remote from the relevant, activated synapses. This possibility seems less likely than in past experiments, since recordings were made intradendritically (100–200 μm from the soma level). However, we cannot rule out the possibility that the recording electrode in these experiments was electrotonically remote from small dendritic processes or dendritic spines or was from a different dendritic branch in which most of the potentiated activity occurred. A second possibility is that post-HFS changes were masked by changes in the IPSP. This interference does not explain all results, however, since similar data were obtained when picrotoxin was added to the bathing medium to block IPSPs. Third, the absence of intradendritic EPSP potentiation could be attributed to a sampling problem. Our studies defined LTP as an enhancement in the field potential response, but the number of synaptically activated/discharging pyramidal neurons that contribute to a population EPSP/population spike is unclear. What percentage of pyramidal neurons must show a potentiated response (either in EPSP or in probability for discharge) in order for potentiation to become evident in field potential recording? It appears that a potentiated EPSP in all neurons of a population is not necessary to produce a potentiated population spike. Our observation that the intradendritic EPSP may be only minimally potentiated is consistent with previous findings that the potentiation of the population EPSP is often small (Bliss and Lomo, 1973; Swanson et al., 1982) even when the population spike potentiation is large.

A major component of the enhanced population spike, therefore, is due to a still to be elucidated process. This process may involve EPSP-to-spike coupling or may be due to other factors such as ephaptic interactions (Turner et al., 1984). The intracellular manifestation of the E-S potentiation is seen in our experiments as an increased propensity for spike discharge in response to a constant orthodromic stimulus following HFS. This increased probability of spike discharge was often observed despite absence of potentiation of the intracellularly recorded EPSP and is consistent with the results reported by Andersen et al. (1984) using intrasomatic recordings. Furthermore, our

intradendritic recordings also showed no alterations in membrane potential, input resistance, or spike threshold following development of LTP (Andersen et al., 1980a). Our results do not rule out, of course, the possibility that alterations in membrane properties may be occurring at the level of the synapses (e.g., in the dendritic spines). Alterations in the EPSP (intracellular or extracellular) are not a prerequisite for potentiation of the population spike.

The intradendritically recorded IPSP was not consistently altered following HFS, and thus cannot explain the dissociation of dendritic EPSP and population spike potentiation. However, several cells did show enhanced dendritic IPSPs post-HFS, an increase that occurred concomitantly with a large enhancement of the population spike. Our experiments cannot identify the cause of the IPSP potentiation since we could not distinguish between (1) actual enhancement of the IPSP due to alterations in interneuron function or (2) increased recurrent inhibition resulting from a prior increased discharge of pyramidal cells. Previous studies, however, have shown that basket cells can be potentiated following an LTP-conditioning train (Buzsaki and Eidelberg, 1982; Taube and Schwartzkroin, 1987), suggesting that some of the IPSP potentiation seen in our dendritic recordings can be attributed to direct alterations in interneuron excitability. The IPSP was not consistently decreased in our recordings, confirming previous findings that loss of IPSP efficacy can play only a minor role (if any) in generating LTP (Haas and Rose, 1982, 1984; Wigstrom and Gustafsson, 1983).

In conclusion, some postsynaptic changes must occur during LTP. However, the EPSP enhancement does not appear to be due to changes in cell responsiveness to glutamate. Further, alterations in the EPSP (measured intra- or extracellularly) are not prerequisite for potentiation of the population spike. The results of our intradendritic experiments confirm that LTP is composed of at least 2 independent components.

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