Long-Term Plasticity at Excitatory Synapses on Aspinous Interneurons in Area CA1 Lacks Synaptic Specificity

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Cowan, A. I., C. Stricker, L. J. Reece, and S. J. Redman. Long-term plasticity at excitatory synapses on aspinous interneurons in area CA1 lacks synaptic specificity. *J. Neurophysiol.* 79: 13–20, 1998. The synaptic specificity of long-term potentiation (LTP) was examined at synapses formed on aspinous dendrites of interneurons whose somata were located in the pyramidal cell layer of hippocampal area CA1. Intracellular recordings from slices prepared from rats were used to monitor excitatory postsynaptic potentials (EPSPs) elicited by extracellular stimulation in stratum radiatum. Two synaptic inputs were evoked at 0.5 Hz by stimulating axons adjacent to stratum pyramidale and s. lacunosum-moleculare. After obtaining baseline recordings ($\geq$10 min), one of the EPSPs was conditioned. The protocol involved tetanic stimulation, sometimes combined with somatic depolarization. Low-frequency stimulation of the two pathways was then resumed and EPSPs were recorded for $<$30 min. We observed both homosynaptic and heterosynaptic changes in synaptic strength. LTP and long-term depression (LTD) were seen in both pathways and all possible combinations of changes in the two EPSPs were observed, including heterosynaptic LTP associated with either homosynaptic LTP or LTD. Intracellular 1,2-bis (2-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (10 mM) abolished alterations in synaptic strength. When axons in s. radiatum synapse onto a spiny pyramidal cell, synaptic specificity of LTP is preserved. However the results obtained from aspinous interneurons show that synaptic specificity of LTP is lost. These results are consistent with the hypothesis that spines provide postsynaptic mechanism(s) for conferring specificity to LTP.

INTRODUCTION

The mechanisms underlying long-term potentiation (LTP) of excitatory transmission have been studied mostly at synapses on principal neurons in the hippocampus (reviewed in Bliss and Collingridge 1993; Kuba and Kumamoto 1990). These synapses form on spines, or as synaptic clusters on large spinous processes in the special case of mossy fiber synapses on CA3 pyramidal cells. It has been proposed that the spines provide an electrical compartment that promotes the entry of calcium into the spine via NMDA receptors and voltage-dependent calcium channels after synaptic activation and a chemical compartment that localizes and concentrates the calcium influx to the immediate vicinity of the activated synapses (reviewed in Wickens 1988). An increase in intracellular calcium is essential to trigger the induction of LTP (Lynch et al. 1983; Malenka et al. 1988) and the role of spines in enhancing and localizing the increased calcium concentration at the activated synapses is believed to be important in making LTP synapse specific.

The interneurons whose somata lie in or on the edge of the pyramidal cell layer in CA1 (basket, axo-axonic and bistratified cells) have all been reported to have largely aspinous dendrites (with only sparse or occasional spines). Of these interneurons, the basket cell is the most common (Buhl et al. 1994, 1995; Han 1994; Schwartzkroin and Kunkel 1985; Schwartzkroin et al. 1990; Sik et al. 1995). LTP has been induced in these interneurons by high-frequency stimulation of axons in stratum radiatum (Stelzer et al. 1994; Taube and Schwartzkroin 1987). LTP has also been reported in excitatory post-synaptic potentials (EPSCs) evoked in interneurons located in s. oriens near the alveus (Maccaferri and McBain 1995, 1996; Ouardouz and Lacaille 1995) with different interpretations on its site of origin, but some of these interneurons have spiny dendrites (McBain et al. 1994; Pitkänen and Amaral 1993). As the axons in s. radiatum make synaptic connections with pyramidal cells on spinous processes and with interneurons in the pyramidal cell layer on smooth dendrites, this arrangement provides an experimental opportunity to test the hypothesis that spines confer synaptic specificity to LTP.

We have examined some properties of LTP induced at excitatory synapses formed on the interneurons in the CA1 pyramidal cell layer by high-frequency stimulation of axons in s. radiatum, with and without superimposed somatic depolarization. In particular, we have examined the specificity of LTP induced at these synapses by conditioning one group of synapses and recording the subsequent responses evoked at this synaptic input and at a second, unconditioned synaptic input to the same neuron. Heterosynaptic interactions indicating lack of input specificity were regularly observed, some resulting in LTP and others resulting in long-term depression (LTD). These findings support the hypothesis that spines confer synaptic specificity to LTP.

METHODS

Slice preparation

Conventional 400-μm-thick hippocampal slices obtained from 17–25 day old Wistar rats were prepared with the methods described in Stricker et al. (1996). Ethical approval for the experimental procedures was given by the Animal Experimentation Ethics Committee of the Australian National University. After incubation at 34°C for 1 h, the slices were kept at room temperature. One slice was transferred to a recording bath and superperfused with artificial cerebrospinal fluid (ACSF) at a rate of about 1 ml/min and maintained at 30 ± 1°C. The ACSF contained 124 mM NaCl, 26 mM $NaHCO_3$, 3 mM KCl, 1.3 mM $MgSO_4$, 2.5 mM NaH_2PO_4, 2.5 mM $CaCl_2$, 10 mM glucose, and either 10 μM bicuculline or 100 μM picrotoxin (Sigma Chemical, St. Louis, MO). The ACSF (pH 7.4) was continuously bubbled with 95% O_2-5% CO_2.

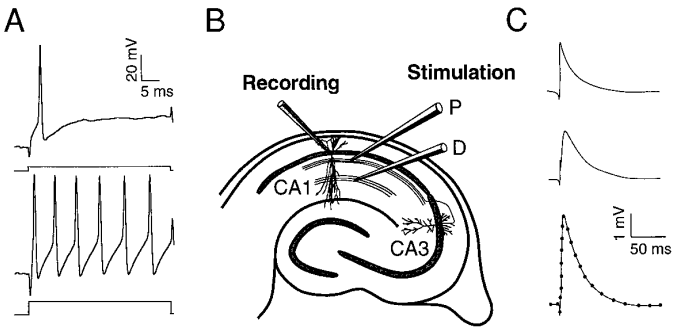

FIG. 1. *A*: action potentials recorded from a CA1 interneuron. Note short spike duration, pronounced afterhyperpolarization (AHP), and lack of spike adaptation during a prolonged current pulse (0.1 for *top* and 0.4 nA for *bottom*). *B*: hippocampal slice indicating positions of 2 stimulating electrodes in stratum radiatum. *C*: excitatory postsynaptic potential (EPSPs) recorded after stimulation at P (proximal) and D (distal) sites. *Bottom*: EPSP recorded when both sites were stimulated simultaneously and linear sum of EPSPs (●) recorded when P and D sites were stimulated separately.

Recording and stimulation

Intracellular recordings were made from interneurons and pyramidal cells in s. pyramidale of CA1. Fast-spiking interneurons were distinguished from pyramidal cells (PC) with criteria described by Schwartzkroin and Mathers (1978), Lacaille et al. (1989), and Buhl et al. (1995). These are a brief spike duration (0.8 vs. 1.5 ms for PCs), a pronounced afterhyperpolarization, and a rapid discharge with only small adaptation in response to small depolarizing current pulses (Fig. 1*A*). Intracellular electrodes were filled with 3 M KCl and had resistances of 70–140 MΩ. When intracellular 1,2-bis (2-aminophenoxy)-ethane-*N*, *N*,*N*′,*N*′-tetraacetic acid (BAPTA, 10 mM, Sigma) was used, it was added to the electrode solution. When interneurons were to be labeled, 4% Neurobiotin [*N*-(2-aminoethyl) biotinamide hydrochloride, Vector Laboratories, Burlingame, CA] was added to the electrode solution.

Recordings were considered acceptable when the spike amplitude remained >60 mV. Hyperpolarizing currents of 0.1–0.5 nA were often needed to suppress background discharge of these neurons. Two monopolar sharpened tungsten electrodes were positioned in stratum radiatum within 300 μm of the recorded interneuron, as shown in Fig. 1*B*. One electrode (P for proximal) was placed adjacent to the PC layer; the other electrode (D for distal) was placed near the border of stratum radiatum and stratum lacunosum-moleculare. The stimulating electrodes were normally positioned adjacent to the recorded interneuron and never further than 300 μm from it. Control and test EPSPs were evoked at 0.5 Hz with brief (100 μs) constant current pulses (10–70 μA). Stimuli to the P and D electrodes were separated by 115 ms to avoid overlap of the EPSPs. Stimulus intensities were adjusted to evoke large EPSPs (peak amplitudes between 1 and 7 mV) or small EPSPs (peak amplitude between 150 μV and 1 mV). To ensure that the two inputs did not have synaptic contacts in common, the two inputs were stimulated simultaneously, and the resulting EPSP averaged over 50 responses. This EPSP was then compared with the arithmetic sum of the average of the two EPSPs, evoked separately (Fig. 1*C*). The difference was never >10%, and for 40/46 EPSPs, no difference was observed.

Conditioning stimulation consisted of four sequences of 40 stimuli at 100 Hz, each sequence separated by 10 s. In some experiments a depolarizing current of 0.4–0.8 nA was maintained throughout the conditioning stimulation, causing the interneuron to discharge at >100 Hz. For each input, at least 300 control EPSPs were recorded (10 min). Then either the P or D input was conditioned and the other input was not stimulated. This was followed by a period of at least 30 min when conditioned responses were recorded for both inputs (0.5 Hz).

As a control on the stability of the EPSPs when evoked at 0.5 Hz for prolonged periods, the proximal and distal EPSPs were recorded over periods of 30–40 min, without any conditioning stimulation. Another control was designed to evaluate the effects of somatic depolarization alone on the EPSPs. In these experiments, the EPSPs were evoked at 0.5 Hz for 10 min, followed by somatic depolarization with currents in the range 0.4–1.0 nA for 40 s and neither EPSP was evoked. This was followed by a further 30 min of recording the EPSPs evoked at 0.5 Hz.

In some neurons, spontaneous EPSPs (sEPSPs) were continuously recorded for 10 min before recording control EPSPs. After conditioning of one of the inputs and recording conditioned responses for 30 min, a further 10 min of continuous recording of sEPSPs was taken. Tetrodotoxin (TTX; 1 μM; Sigma) was then added to the ACSF. When the action potential could no longer be evoked by intracellular current pulses and high stimulating currents could no longer evoke EPSPs, miniature EPSPs (mEPSPs) were recorded over a 10-min period. sEPSPs were also recorded in the control experiments described above.

When neurobiotin was present in the electrode, depolarizing current pulses (0.5–3 nA) of 500-ms duration with a 50% duty cycle were applied for 20 min after completion of recording. The electrode was then withdrawn from the neuron and the slice was transferred to an incubation chamber where it remained in oxygenated ACSF for at least a further 60 min before fixing.

Data analysis

All records were filtered at 1 kHz and digitized at 5 kHz (12 bits). Sequential EPSPs were averaged in groups of 60 for each input and the peak amplitudes of these sequential averages (calculated from a 1.6 ms window in the baseline and a similar one straddling the peak) were plotted against time. If the EPSP triggered an action potential, that record was omitted from the group of 60, and the average was made from those EPSPs that were subthreshold.

A difference in the EPSP amplitude before and after conditioning was determined by a comparison (Student's *t*-test) of the amplitudes during the 10-min control period and the period 20–30 min after conditioning.

Histology

Each slice in which an interneuron was labeled with neurobiotin was placed between two pieces of millipore filter to keep it flat and fixed in a solution containing 4% paraformaldehyde, 4% sucrose, and 0.1 M phosphate buffer. After fixation for at least two hours, the slice was soaked in a 25% sucrose/phosphate buffer solution until it sank and then embedded in a 3–5% gelatin/agarose block. After the block hardened the slice was sectioned at 60 μm by using a vibratome. The slices were then washed in 0.1 M phosphate buffer and incubated with avidin and biotin conjugated horseradish peroxidase (HRP) (ABC Elite Kit, Vectastain) for 48 h in a 0.1% TWEEN 20 (polyoxyethylene-sorbitan monolaurate, Sigma), 4% sucrose, 0.1 M phosphate buffer solution. The slices were then reacted for 2 h with 0.05% diaminobenzidine (Sigma) and 0.02% $NiCl_2$ in phosphate buffer. Next, 0.003% hydrogen peroxide was added and the slices left until the desired staining was achieved. Finally, the slices were mounted on slides and dehydrated through an alcohol series.

RESULTS

The results are based on intracellular recordings from 46 interneurons that were sufficiently stable to allow at least 40 min of recording. The resting membrane potential of these neurons was −63.0 ± 0.9 (SE) mV, the input resistance was 50.4 ± 4.4 MΩ, and the amplitude of the action potential

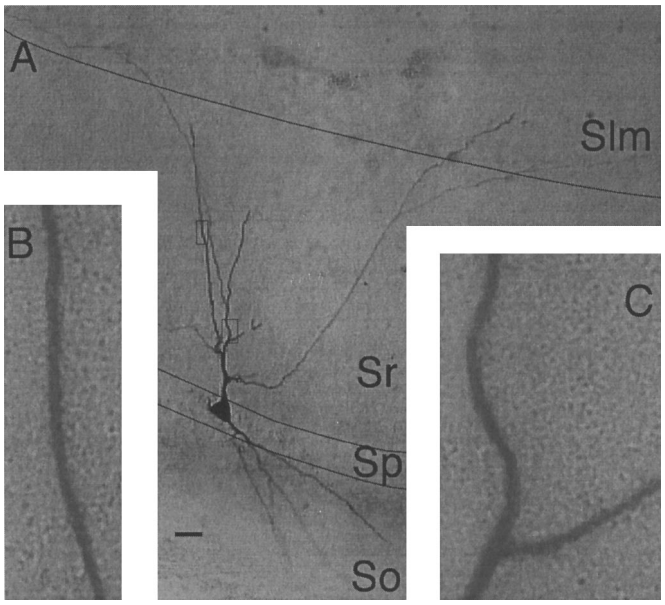

FIG. 2. Morphology of an interneuron in CA1 pyramidal cell layer. *A*: composite photomicrograph of an interneuron showing all labeled dendrites. Note that some dendrites were probably chopped off during slicing. *B* and *C*: 2 photomicrographs of sections of dendrites. Rectangles in *A* indicate positions from which *B* and *C* were taken. Slm, s. lacunosum-moleculare; Sr, s. radiatum; Sp, s. pyramidale; So, s. oriens. Scale bar in *A* is 20 μm and for *B* and *C* it is 2 μm.

evoked by a brief current pulse was 72.3 ± 1.2 mV (n = 46). The mean rise time and half-width of the proximal and distal EPSPs were 3.5 ± 0.4 ms and 24.7 ± 2.2 ms and 4.7 ± 0.4 ms and 29.8 ± 2.0 ms, respectively (n = 42). These two time courses were significantly different from each other ($P < 0.05$, paired t-test).

Some of the interneurons used in these experiments were filled with neurobiotin and their morphology was subsequently fully reconstructed (n = 5). Figure 2 shows one reconstruction (Fig. 2*A*) and two photomicrographs of sections of dendrites (Fig. 2, *B* and *C*). The interneuron lies in the pyramidal cell layer (as did all others that were successfully stained). The dendrites of the interneuron are devoid of spines (Fig. 2, *B* and *C*) and so were the dendrites of all the other reconstructed interneurons. However at the same magnification spines could be clearly seen on pyramidal cells. This result is consistent with the numerous reports (see INTRODUCTION) that the dendrites of interneurons in the CA1 pyramidal cell layer are aspinous.

Results are described for experiments where one synaptic input was conditioned by a tetanization (n = 11) or by tetanization in combination with somatic depolarization (n = 35). Somatic depolarization combined with tetanization induced synaptic plasticity more frequently than tetanization alone and thus the combined conditioning protocol was most commonly used. Control experiments (n = 15) are also described where conditioning consisted of somatic depolarization with no concurrent stimulation (n = 4), where no conditioning protocol was used and low frequency stimulation was maintained for 30–40 min (n = 6), and where BAPTA was included in the electrode (n = 5).

The results in Fig. 3 summarize the most important observations from these experiments. High-frequency stimulation, with or without somatic depolarization, altered synaptic transmission at one or both synaptic inputs (Fig. 3, *A* and *B*). In the result shown in Fig. 3*A*, a tetanus alone caused no change in the conditioned EPSP but LTP (increase of 36%) was induced in the unconditioned EPSP. Somatic depolarization combined with tetanic stimulation, resulted in LTP for both the conditioned and unconditioned synapses in the result illustrated in Fig. 3*B*. When measured (n = 18), little change was observed in either input resistance (54.3 ± 7.3 vs. 50.9 ± 6.3 MΩ, P = 0.3) or action-potential characteristics (67.9 ± 2.8 vs. 66.6 ± 2.5 mV, P = 0.2) throughout these experiments and in all the experiments described, changes in membrane properties could not account for the observed changes in EPSP amplitude.

Two more examples of common outcomes are illustrated in Fig. 4. One is another example where LTP occurred in both the conditioned and unconditioned EPSP (Fig. 4*A*). In this example, and in Fig. 3*B*, there was a slow onset of the EPSP enhancement. Transmission at both synapses was depressed in the result shown in Fig. 4*B*. In other results (not illustrated) mixed effects occurred with heterosynaptic LTP coupled to homosynaptic LTD and the reverse, or in some cases only one or neither of the two EPSPs altered. A summary of all the results is given in Table 1. In 46 experiments on CA1 interneurons, we observed heterosynaptic LTP (n = 11), heterosynaptic LTD (n = 21), and no hetero-

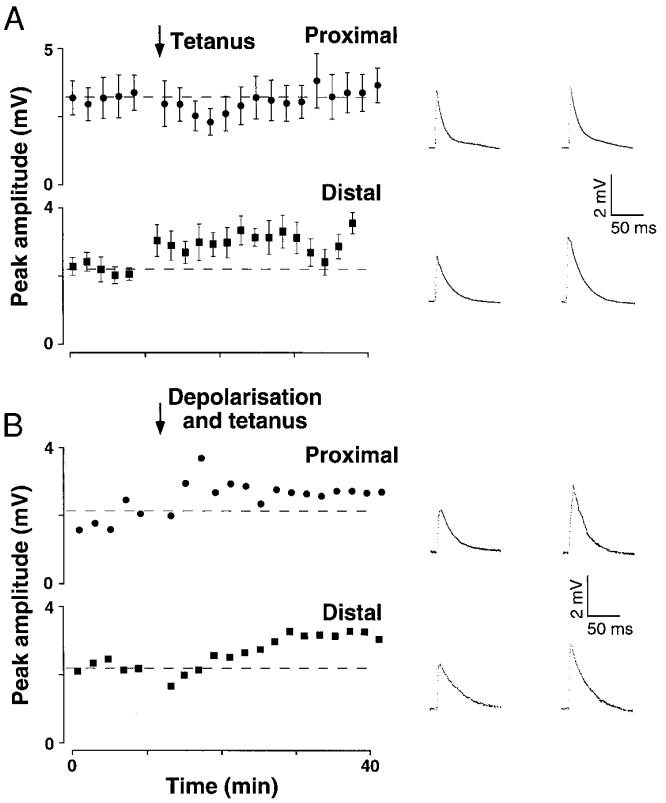

FIG. 3. Examples of results from different conditioning protocols. *A*: heterosynaptic LTP could be induced by a conditioning stimulus that involved only a tetanus to proximal input. *B*: heterosynaptic long-term potentiation (LTP) could also be induced when conditioning stimulus involved a tetanus combined with somatic depolarization. In this example there was also homosynaptic LTP. *Left*: each point represents average amplitude (± SE) of 60 consecutive EPSPs (2 min). *Top* of each panel shows conditioned EPSP. Averaged EPSPs shown to right of each record were obtained over 10 min of recording control EPSPs and over last 10 min of recording of conditioned EPSPs. Error bars in *B* were smaller than symbols.

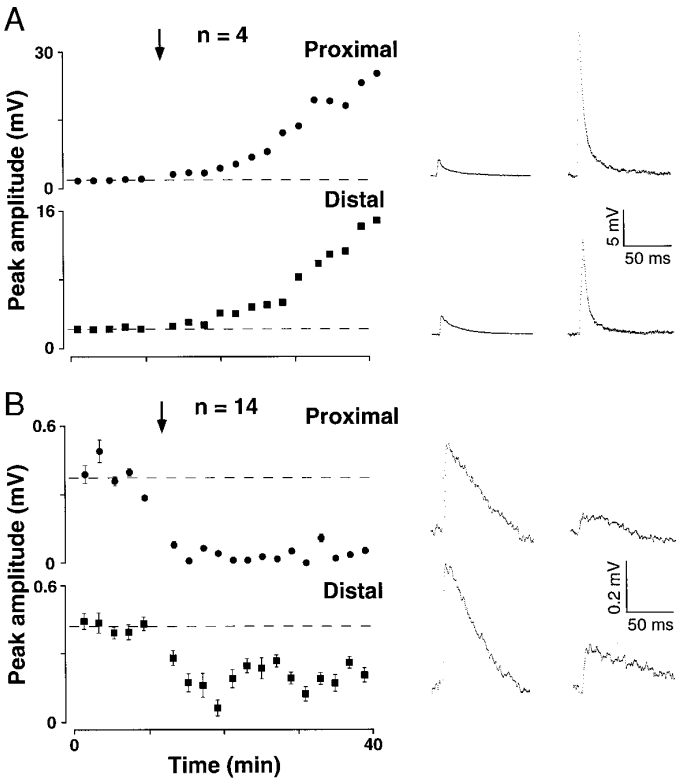

FIG. 4. Examples of 2 of possible outcomes where both EPSPs demonstrated a change in amplitude after conditioning. *A*: both EPSPs potentiated. *B*: both EPSPs depressed. *Left*: each point represents average amplitude (± SE) of 60 consecutive EPSPs (2 min). *Top* of each panel shows conditioned EPSP. Numbers indicate times that particular outcome of conditioning was observed (total number of experiments was 46). *Right*: averaged EPSPs of each record were obtained over 10 min of recording control EPSPs and over last 10 min of recording of conditioned EPSPs. Note that scale bar for EPSPs is very different for the 2 examples and this is the reason that EPSPs in *B* are more noisy. Error bars in parts of figure were smaller than symbols and are therefore not evident.

synaptic change ($n = 14$). When the results are separated by the homosynaptic outcome and whether the tetanus was paired with depolarization or not, the results are shown in Table 1, *B* and *C*.

To determine whether or not synaptic specificity of LTP in CA1 pyramidal neurons was preserved under our recording conditions, we examined the effects of the same conditioning protocol (tetanus plus depolarization) on pyramidal neurons. Homosynaptic LTP was observed in 7 of 10 pyramidal neurons and there was no change in the conditioned EPSP for the remaining three. Heterosynaptic LTD was observed in 3 of 10 neurons whereas heterosynaptic LTP was never observed (Table 1*A*). The difference in the heterosynaptic effects for CA1 interneurons and pyramidal cells was significant ($P < 0.02$; χ^2 test). A recording showing synaptic specificity of LTP in a pyramidal neuron is shown in Fig. 5.

In the recordings from interneurons we searched for differences in outcome depending on which input (proximal or distal) was conditioned and whether or not the EPSPs were large (1–7 mV) or small (0.15–1 mV). The averaged changes in the conditioned and the unconditioned inputs were not significantly different (unpaired *t*-test) when either the proximal or the distal input was conditioned. Similarly there was no significant difference in the averaged changes at both inputs when either small or large EPSPs were evoked. For this reason we have combined all results in Table 1, regardless of which input was conditioned, and regardless of the amplitudes of the EPSPs.

To demonstrate that we had stable stimulation and recording conditions we performed a number of control experiments. In Figure 6*A*, no conditioning stimulation was used and continuous stimulation at 0.5 Hz was maintained for both inputs for 30 min. There was no difference in EPSP amplitude averaged over the first and last 10 min of the recording, for either the distal or proximal EPSP. An identical result was obtained for all six experiments of this kind. For proximal EPSPs the average amplitude in the first 10 min was 3.7 ± 0.2 mV and in the final 10 min it was 3.4 ± 0.2 mV. These amplitudes are not significantly different ($P = 0.3$). Similarly the amplitude of the distal EPSPs was unchanged during this recording period (3.5 ± 0.5 vs. 3.4 ± 0.5 mV, $P = 0.3$). Thus any change in EPSP amplitude after conditioning procedures can be attributed to alterations in synaptic strength as a result of the conditioning protocol. A second type of control experiment (Fig. 6*B*) showed that somatic depolarization, in the absence of stimulation, also resulted in no change in EPSP amplitude. The same result was obtained in all experiments of this kind ($n = 4$, proximal EPSPs 3.1 ± 0.4 mV to 3.1 ± 0.6 mV, $P = 0.8$; distal EPSPs 2.9 ± 0.7 mV to 2.8 ± 0.5 mV, $P = 0.7$).

Effect of intracellular BAPTA on changes in synaptic strength

To determine whether or not the synaptic plasticity observed in these interneurons was dependent on elevating intracellular calcium concentration and whether or not it occurred at synapses on the interneurons, we conditioned synapses while recording with electrodes containing 10 mM BAPTA. The result obtained for one interneuron is illustrated in Fig. 7. Tetanic stimulation was applied to the proximal input in combination with somatic depolarization. No change was observed in either the distal or proximal EPSP. This experiment was repeated in five different interneurons in which large (>1 mV) EPSPs were elicited. No heterosynaptic changes were observed (2.0 ± 0.6 mV vs. 2.0 ± 0.5 mV, $P = 0.8$). In one cell, a homosynaptic depression (32%) occurred. These results are significantly different ($P < 0.05$, χ^2 test) from those where the electrode did not contain BAPTA.

Possible disynaptic contamination of the interneuronal EPSP

Recurrent collaterals of pyramidal cells form excitatory contacts with basket cells (Buhl 1994; Knowles and Schwartzkroin 1981). If the stimuli to stratum radiatum resulted in suprathreshold EPSPs in some pyramidal cells, the EPSP in the interneuron could be partially disynaptic. This matter was investigated by recording distal and proximal EPSPs in an interneuron and sequentially in numerous nearby pyramidal cells by using the same stimuli (strengths and positions). The pyramidal cells were located between the stimulating electrodes and the recorded interneuron (≈300 μm separation). In 3 of 4 experiments, recordings were made of pyramidal cell EPSPs before conditioning and

TABLE 1.

	C ↑, Homosynaptic LTP			C ↓, Homosynaptic LTD			C ↔		
	n	ΔC	ΔUC	*n*	ΔC	ΔUC	*n*	ΔC	ΔUC
					A. Pyramidal neurons				
UC ↑, Heterosynaptic LTP									
UC ↓, Heterosynaptic LTD	2	51 ± 2	−44 ± 23				1	6	−75
UC ↔	5	160 ± 58	−5 ± 9				2	8 ± 21	−9 ± 18
					B. Interneurons: tetanus alone				
UC ↑, Heterosynaptic LTP							2	−5 ± 0	35 ± 17
UC ↓, Heterosynaptic LTD				3	−56 ± 12	−36 ± 6			
UC ↔				3	−27 ± 13	−3 ± 10	3	7 ± 7	−5 ± 4
					C. Interneurons: tetanus and depolarization				
UC ↑, Heterosynaptic LTP	4	267 ± 112	179 ± 85	4	−32 ± 5	56 ± 26	1	−13	51
UC ↓, Heterosynaptic LTD	4	86 ± 23	−82 ± 9	11	−72 ± 3	−53 ± 8	3	1 ± 5	−80 ± 10
UC ↔	2	49 ± 12	2 ± 4	2	−76 ± 11	3 ± 8	4	−6 ± 6	0 ± 4

Values are means ± SE. Number of cells showing various possible outcomes after conditioning of either proximal or distal excitatory postsynaptic potential (EPSP). *A:* results for CA1 pyramidal neurons. *B:* data accumulated for CA1 interneurons when conditioning involved a tetanus alone. *C:* data for CA1 interneurons where conditioning involved a tetanus combined with somatic depolarization. C and UC are the conditioned and unconditioned EPSPs, respectively; ΔC and ΔUC represent average percent change after conditioning, where 0 is no change, a positive number an increase, and a negative number a decrease in EPSP average amplitude. LTP, long-term potentiation; LTD, long-term depression.

after conditioning stimulation was applied to the slice. In the fourth experiment, pyramidal cell EPSPs were only recorded after conditioning. The results recorded from one slice are shown in Fig. 8. The two interneuron and five pyramidal cell recordings were obtained in close proximity to each other. The interneuron EPSPs are shown in the left columns and the EPSPs in nearby pyramidal cells are on the right. This particular example was chosen because a second interneuron was impaled while searching for pyramidal neurons. In five interneurons, the ten EPSPs had amplitudes between 1.2 and 3.7 mV, a range similar to the large amplitude EPSPs used throughout these experiments. Recordings from pyramidal cells yielded a total of 68 EPSPs (two per PC), with amplitudes in the range 0–2.3 mV. No EPSP was suprathreshold nor was a field population spike detectable in any of the EPSPs used in this paper. Many of the EPSPs

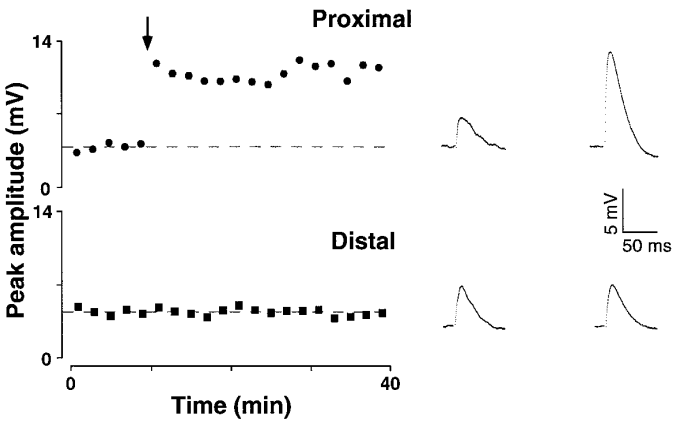

FIG. 5. Example of EPSPs recorded from a CA1 pyramidal neuron showing synaptic specificity of LTP. Proximal (conditioned) EPSP potentiated, whereas there was no change in amplitude of unconditioned EPSP. Error bars in parts of figure were smaller than symbols and are therefore not evident. *Right*: averaged EPSPs were obtained over first and last 10 min of recording.

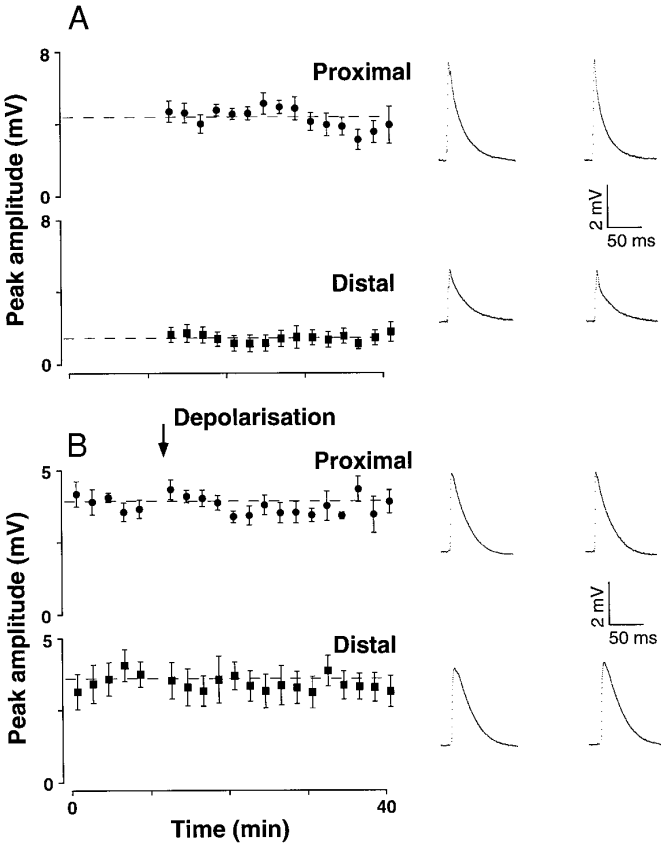

FIG. 6. Examples of control experiments. *A*: 30 min of recording of 2 EPSPs during which time no conditioning stimuli were given. There was no change in average amplitude of either proximal or distal EPSP during this time. *B*: similarly there was no change in either proximal or distal EPSP amplitude after a period of somatic depolarization sufficient to make neuron discharge at a high-frequency (100 Hz). *Right*: averaged EPSPs were obtained over first and last 10 min of recording.

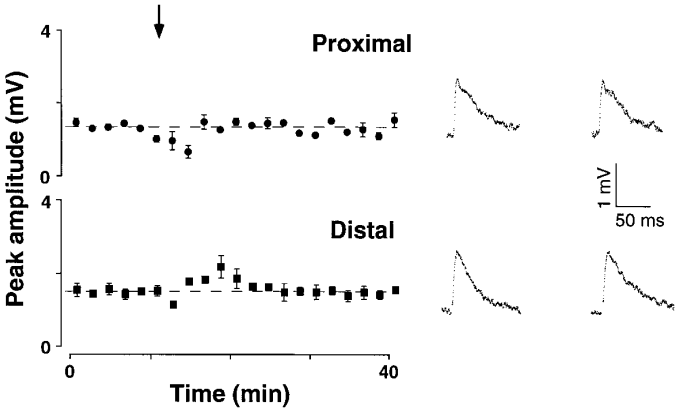

FIG. 7. Example of EPSPs recorded when 10 mM 1,2-bis (2-aminophenoxy)-ethane-N,N,N',N'-tetraacetic acid (BAPTA) was present in electrode. There was no change in either proximal or distal EPSP after tetanic stimulation of proximal input combined with somatic depolarization.

were recorded with sufficient gain to detect very small population spike fields.

Delay in onset of conditioned responses

In some results, there was an obvious delay between the time of conditioning stimulation and the time of onset of potentiation or depression, for both the conditioned and unconditioned inputs. This delay can be observed in the responses illustrated in Figs. 3*B* (distal EPSP) and 4*A* (both EPSPs). The delay measured from the end of the conditioning stimulation until the mean response (changed by 1 SD of the control value) was 7.4 ± 1.3 min ($n = 19$) for potentiated responses, compared with 5.0 ± 0.9 min ($n = 36$) for depressed responses. This difference is only significant at $P = 0.1$. The greater delay in onset of potentiation compared with depression was more obvious at distal synapses (8.3 ± 2.8 min vs. 3.7 ± 1.0 min, respectively, $P < 0.005$). For comparison, differences in onset time were not significant at proximal synapses (6.8 ± 1.3 min vs. 6.1 ± 1.4 min, respectively).

Spontaneous EPSPs

During the course of these experiments, we noticed that the amplitude and frequency of sEPSPs often changed after conditioning of one input. As changes in sEPSPs should be a useful assay of heterosynaptic effects, large sets of sEPSPs (1000–7000) were recorded from 14 interneurons before conditioning and after 30 min of recording conditioned EPSPs. Other sets of sEPSPs were recorded during the control experiments. We wanted to determine whether or not the changes that occurred in the conditioned and unconditioned EPSPs would be reflected in changes to the amplitudes of sEPSPs. We were unable to obtain stable amplitude distributions of sEPSPs in control experiments extending over 40 min in which no conditioning stimulation was used. For this reason we were unable to place any reliability in the changes we observed in sEPSP amplitudes and frequencies after conditioning stimulation.

DISCUSSION

The results indicate that the strength of a synapse can be altered by conditioning stimulation to another synaptic input of the same neuron when the synapses are not on spines. Heterosynaptic LTP was observed and this was linked to either LTP, LTD, or no change at the conditioned synapse. Heterosynaptic LTD was most commonly observed and this was also linked with either LTP or LTD at the conditioned synapse.

Heterosynaptic LTD has previously been observed in CA1 field EPSPs and population spike potentials (Abraham and Wickens 1991; Lynch et al. 1977), in field EPSPs in the dentate gyrus (Abraham and Goddard 1983; Levy and Steward 1979), and in EPSCs evoked in CA3 pyramidal cells (Bradler and Barrionuevo 1990). In contrast, heterosynaptic LTP has not been detected in CA1 until recently (Muller et al. 1995), when it was shown that prior induction of LTD in one pathway could be reversed by the induction of LTP in a second pathway. However Muller et al. (1995) confirmed that LTP was restricted to the conditioned input when the other synapses were not previously depressed.

The new result arising from the present investigation is that LTP can be induced at synapses on aspinous interneurons when these synapses has not been conditioned. Lack of input specificity has also observed in CA1 pyramidal neurons in organotypic cell culture (Engert and Bonhoeffer 1997). However in this case input specificity breaks down only when the synapses are <70 μm apart, whereas the EPSPs evoked in interneurons were separated by a much larger distance. Although our data are consistent with the hypothesis that spines provide the mechanism for synaptic specificity of LTP, other postsynaptic factors could also be responsible. These could include active properties of dendritic membrane or the type and/or distribution of cellular

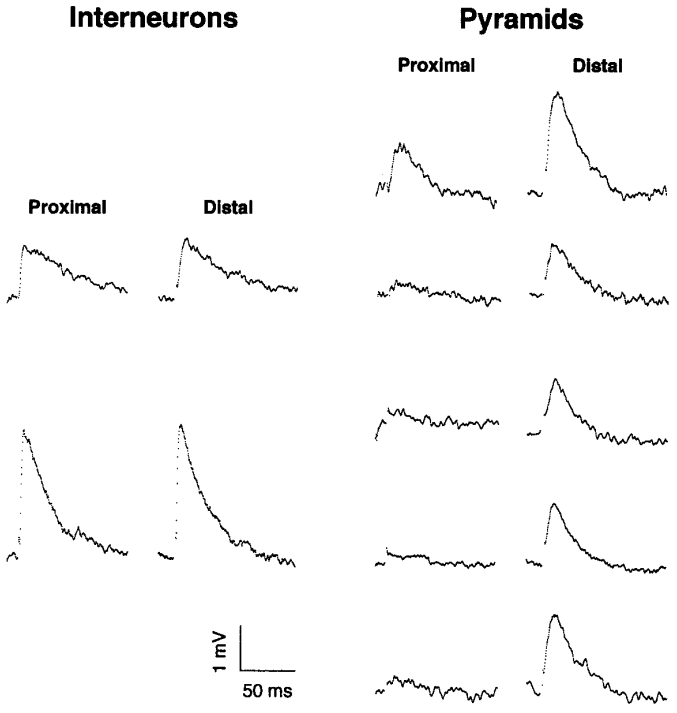

FIG. 8. Examples of EPSPs recorded in interneurons and nearby pyramidal neurons. Two interneuron and 5 pyramidal cell recordings were obtained in close proximity to each other without changing position of stimulating electrodes or altering stimulus strengths. *Left*: interneuron EPSPs. *Right*: EPSPs in nearby pyramidal cells. No EPSP in pyramidal cells was suprathreshold.

characteristics, such as postsynaptic receptors, second messengers, and calcium-binding proteins.

In making the claim that synaptic specificity of LTP in aspinous interneurons is lost we needed to demonstrate that the EPSPs were generated at monosynaptic connections between axons in stratum radiatum and the interneurons, and that these synapses were made on smooth dendrites.

Evidence for monosynaptic EPSPs

We were concerned that the synaptic plasticity observed may have occurred at synapses on pyramidal cells made by axons in stratum radiatum, as was described by Maccaferri and McBain (1996) for EPSPs evoked in interneurons in stratum oriens. If transmission at the synapses on pyramidal cells in either CA1 or CA3 was suprathreshold, or became suprathreshold after conditioning, the EPSPs recorded in interneurons would contain a component arising from recurrent excitation. Furthermore some (or all) of the plasticity observed could be caused by changes to synaptic strength on the pyramidal cells (Maccaferri and McBain 1995, 1996). We have several reasons to believe this did not happen. Firstly, changes in synaptic strength were abolished in all but one interneuron when the electrode contained BAPTA. It is highly unlikely that in the other BAPTA experiments ($n = 4$) no changes would have been observed in either EPSP after conditioning if BAPTA had not been present in the electrode (no change at either input occurred in 7 of 46 experiments; Table 1). Furthermore, it is unlikely that synapses formed on or by CA1 pyramidal cells were involved in the plasticity because no suprathreshold EPSP was found in any CA1 pyramidal cell at the stimulus strengths used, for both conditioned and unconditioned slices. Finally, a population spike field potential was never observed in any EPSP, even though many of them were recorded with high gain, again suggesting that at the stimulus strengths used, most EPSPs evoked in pyramidal cells were subthreshold.

Evidence for aspinous dendrites

The dendrites of interneurons whose somata lie in the CA1 pyramidal cell layer are largely aspinous (Buhl et al. 1994, 1995; Han 1994; Schwartzkroin and Kunkel 1985; Sik et al. 1995). These interneurons can be distinguished from pyramidal cells by their response to depolarizing current, especially by their lack of impulse frequency adaptation and their short impulse duration. For those neurons that were reconstructed, no spines could be seen under high power light microscopy (×1000) and we confirmed that the cells were interneurons lying in the pyramidal cell layer.

Postsynaptic mechanisms for synaptic plasticity

The prevailing hypothesis for the induction of homosynaptic LTP and LTD at CA1 synapses is that both forms of plasticity require an elevation in postsynaptic calcium concentration adjacent to the activated synapse, with LTP requiring a greater rise in concentration than LTD (Bear and Malenka 1994). The results obtained with BAPTA are consistent with this hypothesis. In this investigation, the conditioning protocol caused either LTP or LTD to be induced in the conditioned synapses, regardless of whether these synapses were proximal or distal, and regardless of whether the evoked EPSP was large or small. If a threshold concentration of calcium must be achieved to induce LTD and an even higher threshold exists for the induction of LTP, there must be factors determined by dendritic geometry, location of dendritic calcium channels, calcium buffering, and the magnitude of the evoked EPSPs at the synaptic sites, that make the rise in dendritic calcium concentration a highly variable factor at different synapses. Factors other than the change in calcium concentration could also play a role in the variability of the change in synaptic strength. These could include type and dendritic distribution of second messengers, identity of the interneuron, or postsynaptic receptor distribution. Retrograde messengers or extracellular factors could also play a role. The variety of outcomes obtained may also reflect the induction protocol used. Alhough this mostly involved somatic depolarization with sufficient current to evoke impulses at a high frequency, the extent to which the impulses propagated into the dendrites and depolarized them at the site of the conditioned synapses may have been highly variable (Spruston et al. 1995).

The mechanisms that couple the induction of LTP or LTD with plasticity at nonactivated synapses are less well understood. The induction of heterosynaptic LTD in CA1 pyramidal cells (reviewed in Linden 1994) requires activation of NMDA receptors and is facilitated by blocking GABAergic inhibition (Abraham and Wickens 1991). It is prevented by blockade of voltage-gated Ca^{2+} channels, particularly L-type, according to Wickens and Abraham (1991) (but c.f. Scanziani et al. 1996). These results might suggest that heterosynaptic LTD is a consequence of the induction of homosynaptic LTP, but the two phenomena were dissociated (Abraham and Goddard 1983; Bradler and Barrionuevo 1990). The heterosynaptic reversal of previously induced LTP or LTD by inducing the opposite change in a second pathway was blocked by D-2-amino-5-phosphonopentanoic acid (D-AP5) or by cyclosporin A (which inhibits a calcineurin/inhibitor-1 phosphatase cascade; Muller et al. 1995). The implication to draw from these results on heterosynaptic LTD is that it is generated by mechanisms similar to those involved in inducing homosynaptic LTD, though there is little information on the processes that contribute to heterosynaptic LTP.

One variable that may influence whether the heterosynaptic effect is LTD or LTP may be the length of the diffusion path between the conditioned and unconditioned synapses for an intracellular messenger. For example, basket cells in CA1 s. pyramidale have four or more long dendrites extending into s. radiatum, each with negligible branching (Buhl et al. 1994, 1995; Thurbon et al. 1994). It is possible that in some experiments, the conditioned synapses were on different dendrites from the unconditioned synapses. If this occurred, the presumed intracellular message would have a longer diffusion path than when both sets of synapses are mainly on the same dendrite. The diffusion distance may also be relevant to the delayed onset of the heterosynaptic effect, although in one result (Fig. 4*A*) the delayed onset occurred for homosynaptic LTP as well. These long delays were also present in the results obtained by Taube and Schwartzkroin (1987) on LTP and in the heterosynaptic reversals of LTP and LTD investigated by Muller et al. (1995).

In conclusion, heterosynaptic LTP was observed at aspinous synapses made on interneurons by Schaffer collaterals, whereas LTP at synapses formed by these same collaterals on dendritic spines of CA1 pyramidal cells is known to be synapse specific. These results indicate that synaptic specificity of LTP is determined by postsynaptic mechanisms. They support the hypothesis that dendritic spines provide sufficient electrical and chemical isolation between synapses to confer synaptic specificity to LTP in pyramidal cells.

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