Associative EPSP–spike potentiation induced by pairing orthodromic and antidromic stimulation in rat hippocampal slices

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1. Pairing low-frequency orthodromic stimulation with high-frequency antidromic conditioning of pyramidal cells in area CA1 of the rat hippocampus resulted in long-lasting potentiation of the extracellular population spike of the cells, without an accompanying increase in the extracellular excitatory postsynaptic potential (EPSP), indicating an increase in EPSP–spike (E–S) coupling, also called E–S potentiation.

2. The amplitude of the antidromically conditioned E–S potentiation took up to 60 min to reach its peak, much longer than synaptic long-term potentiation (LTP) induced by orthodromic tetanic stimulation.

3. The population spike amplitude of a control orthodromic input, which stimulated a separate set of fibres and which was inactive during the pairing, was also increased in over half the slices tested. That it can affect a silent pathway suggests that antidromically conditioned E–S potentiation is not generated locally at tetanized synapses.

4. Bath application of 50 μM D,L-2-amino-5-phosphonovaleric acid (AP5) blocked induction of antidromically conditioned E–S potentiation. After washing out the AP5, the same stimulation resulted in population spike increases. This suggests that activation of the NMDA subtype of glutamate receptor is necessary for the induction of this form of E–S potentiation.

5. Application of 10 μM picrotoxin and/or 10 μM bicuculline, which block inhibition mediated by γ-aminobutyric acid A ($GABA_A$) receptors, did not reduce antidromically conditioned E–S potentiation. Thus, plasticity in $GABA_A$-mediated inhibition cannot account for the increased population spike amplitude.

6. E–S potentiation did not increase the amplitude of either extracellular or intracellular EPSPs recorded at the cell body.

Long-term potentiation (LTP) is a persistent increase in evoked responses following a high-frequency synaptic input (Bliss & Lømo, 1973). LTP manifests both a synaptic component which increases intracellular and extracellular excitatory postsynaptic potentials (EPSPs) and a component that results in a larger population spike amplitude for a given EPSP size. This second component is called potentiation of EPSP–spike coupling or E–S potentiation (Andersen, Sundberg, Sveen, Swann & Wigström, 1980). Although E–S potentiation was noted in the earliest report of LTP (Bliss & Lømo, 1973) and has often been observed in studies of conventional synaptic LTP (Andersen *et al.* 1980; Wilson, Levy & Steward, 1981; Abraham, Bliss & Goddard, 1985), its mechanism is poorly understood.

According to Hebb's postulate of memory formation (Hebb, 1949), the strength of the connection between two neurons is increased when a presynaptic neuron is repeatedly involved in firing a postsynaptic neuron. Similarly, repetitive synaptic input to a depolarized cell induces LTP. The postsynaptic depolarization can be provided by a high-frequency tetanus in a separate pathway paired with low-frequency or low-intensity stimulation that alone would not cause LTP (McNaughton, Douglas & Goddard, 1978; Levy & Steward, 1979; Barrionuevo & Brown, 1983). This has been called associative LTP and has been noted as a possible mechanism for associative memory (Levy & Steward, 1979). In studying associative LTP, it has been found that depolarization of the postsynaptic membrane during low-

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frequency synaptic input is sufficient for associative LTP to occur (Kelso, Ganong & Brown, 1986; Sastry, Goh & Auyeung, 1986; Gustafsson, Wigström, Abraham & Huang, 1987); action potentials in the postsynaptic neuron are not required (Kelso *et al.* 1986; Gustafsson *et al.* 1987). Therefore, postsynaptic firing is not a necessary condition for associative LTP. A more natural way to depolarize is by antidromic stimulation, which causes postsynaptic firing and less steady-state depolarization than a long step of intracellular current injection. The present study investigates whether antidromic stimulation in conjunction with presynaptic stimulation is a sufficient condition for associative LTP.

Two other groups have previously addressed this issue by pairing antidromic conditioning with orthodromic stimulation. Lee (1983), measuring only the field EPSP, examined co-operativity of different types of stimuli in area CA1 of rat hippocampus slices. He used a low-intensity orthodromic stimulation which resulted in a small, short-lasting potentiation of the population EPSP when given alone. This stimulation was paired with either orthodromic or antidromic high-frequency conditioning stimulation. Whereas pairing with orthodromic conditioning caused an increase in the amplitude and duration of the potentiation, antidromic conditioning did not affect potentiation of the EPSP. In contrast, in guinea-pig hippocampus slices, Ito, Miyakawa & Kato (1986) paired single orthodromic shocks with antidromic bursts while measuring only the population spike. They reported a slowly developing increase of the population spike. In our experiments, we sought to clarify this discrepancy by simultaneously measuring changes in both the population EPSP and the population spike in response to pairing orthodromic and antidromic stimulation. We found that the pairing resulted in a pure form of E–S potentiation with properties that distinguish it from that induced by high-frequency orthodromic stimulation. Some of these results have been previously published in abstract form (Jester & Sejnowski, 1991).

METHODS

Preparation of slices

Standard methods were used to record extracellularly and intracellularly from slices of rat hippocampus. Adult male Sprague–Dawley rats (100–200 g) were decapitated under deep ether anaesthesia. The hippocampi were dissected from the brain and cut into transverse slices of 400 μm thickness with a Vibratome. The slices were transferred to a modified Haas-type interface recording chamber. They were maintained at 30–34 °C. The slices were continuously perfused with a solution containing (mM): NaCl, 126; KCl, 5; NaH_2PO_4, 1·25; $MgCl_2$, 2; $CaCl_2$, 2; $NaHCO_3$, 26; and glucose, 10. The perfusion buffer was continuously bubbled with 95% O_2–5% CO_2. Humidified air mixture was also passed over the slices throughout the recording session.

In some experiments, 50 μM D,L-2-amino-5-phosphonovaleric acid (AP5) (Sigma, USA) was added to the perfusing solution to test the role of *N*-methyl-D-aspartate (NMDA) receptors in this phenomenon. In another series of experiments, the effect of blocking inhibition was investigated using bath application of 10 μM picrotoxin (Sigma) and/or 10 μM bicuculline (Sigma) to block γ-aminobutyric acid A ($GABA_A$) receptors. An example of the depression following unpaired antidromic conditioning is shown with inhibition blocked by 20 μM bicuculline (Fig. 3). In order to reduce hyperexcitability and the spread of seizure-like activity due to disinhibition, the picrotoxin/bicuculline experiments and their controls were conducted in slices with a knife cut between CA3 and CA1.

Stimulating and recording

Two extracellular recording electrodes, glass micropipettes with resistance of approximately 1–5 MΩ when filled with 2 M NaCl, were placed in area CA1 (Fig. 1). One was positioned in the cell body layer, the stratum pyramidale, in order to measure population spikes (Population spike recording), and the other (EPSP recording) in the apical dendritic layer, stratum radiatum, to measure dendritic population excitatory postsynaptic potentials (EPSPs). The two recording electrodes were placed in a line perpendicular to the cell body layer so that each would tend to record responses from the same set of neurons.

In each slice, a stimulating electrode was placed in the alveus to elicit antidromic population spikes used as the conditioning input (Fig. 1, Antidromic conditioning input). This was a platinum–iridium side-by-side bipolar electrode (Frederick Haer Co., Brunswick, ME, USA) with pole diameter of 25 μm and tip separation of approximately 50 μm. Figure 1 (lower left) shows sample population spikes activated by the orthodromic and antidromic inputs. The antidromic spike is recognizable by its short latency and the lack of an initial upward phase, which reflects the population EPSP in an orthodromic stimulation. Antidromic responses were accepted if they showed these two criteria and did not also have an orthodromic spike at a longer latency.

For orthodromic stimulation, concentric bipolar electrodes (Frederick Haer Co.) with inner diameter of 25 μm and outer diameter 100 μm were used. The paired input was placed in the stratum radiatum approximately 1 mm closer to the CA3 region than the recording electrodes (Fig. 1, Orthodromic paired input). The intensity of stimulation was adjusted so that the population spike was approximately half-maximal. This intensity was then used throughout the experiment for testing, control and paired stimulation. In later experiments, this stimulation was alternated with a lower intensity stimulation to elicit EPSPs which were not contaminated by a population spike.

In some experiments, a third stimulating electrode was placed in the stratum radiatum on the subicular side of the recording electrodes (Fig. 1, Orthodromic control input). This electrode was used as a control input to test the specificity of the induced changes. In some cases, paired-pulse facilitation tests were performed to verify that the control orthodromic input did not stimulate a set of synapses which significantly overlapped the test input (McNaughton & Barnes, 1977). A single shock to one pathway was followed 20 ms later by a shock to the other pathway. In no case tested did the pathway receiving the second shock exhibit significant facilitation of the population spike ($-9·2 \pm 3·1$%, $n = 14$ for CA3 side followed by CA3 side

electrode and $-4{\cdot}8 \pm 1{\cdot}6\%$, $n = 14$, for CA3 side followed by subiculum side). However, at the same 20 ms interval, both of the pathways always exhibited paired-pulse facilitation ($+56{\cdot}4 \pm 5{\cdot}5\%$, $n = 14$ for CA3 side and $+60{\cdot}8 \pm 13{\cdot}7\%$, $n = 14$ for the subicular side). The lack of cross-facilitation indicates that the two electrodes were stimulating populations of fibres which did not overlap (McNaughton & Barnes, 1977). Another test was performed to ensure that the pathways were converging on an overlapping population of cells. A single shock was given to each pathway separately to elicit a small population spike. The two pathways were then jointly activated at the same intensities and the resulting population spike amplitude was measured and compared with those resulting from independent applications of each shock. In every case tested, the simultaneous application of the shocks resulted in a population spike that was at least 135% of the sum of the two independently activated population spikes ($248{\cdot}4 \pm 32{\cdot}1\%$, $n = 15$). This spatial summation has been taken as evidence that the two pathways converge on an overlapping population of cells (McNaughton & Barnes, 1977).

Throughout the experiment, single 50 μs pulses were given to each orthodromic input at intervals of 5 s and the resulting population spike and EPSP recorded. This recording was interrupted by the tetanization. In some experiments, input–output curves were done over a range of stimulus intensities in order to generate a more complete relationship between the EPSP and the population spike. In these cases, four to five different stimulus intensities were tested at various times during the experiment. The stimuli ranged from 0·01 to 0·3 mA and were adjusted so that the lowest intensity was subthreshold for population spikes and the highest generated a nearly maximal population spike, usually around 10 mV in amplitude.

Intracellular recordings were made in some slices from the CA1 cell layer near the extracellular population spike recording electrode. The recording electrodes had resistances of 80–100 MΩ when filled with 2 M potassium acetate. A vinyl spray coating (Los Angeles Research Packaging, Los Angeles, CA, USA) was used above the shoulder of the electrode to reduce moisture condensation. Electrodes were mounted on a Leitz mechanical

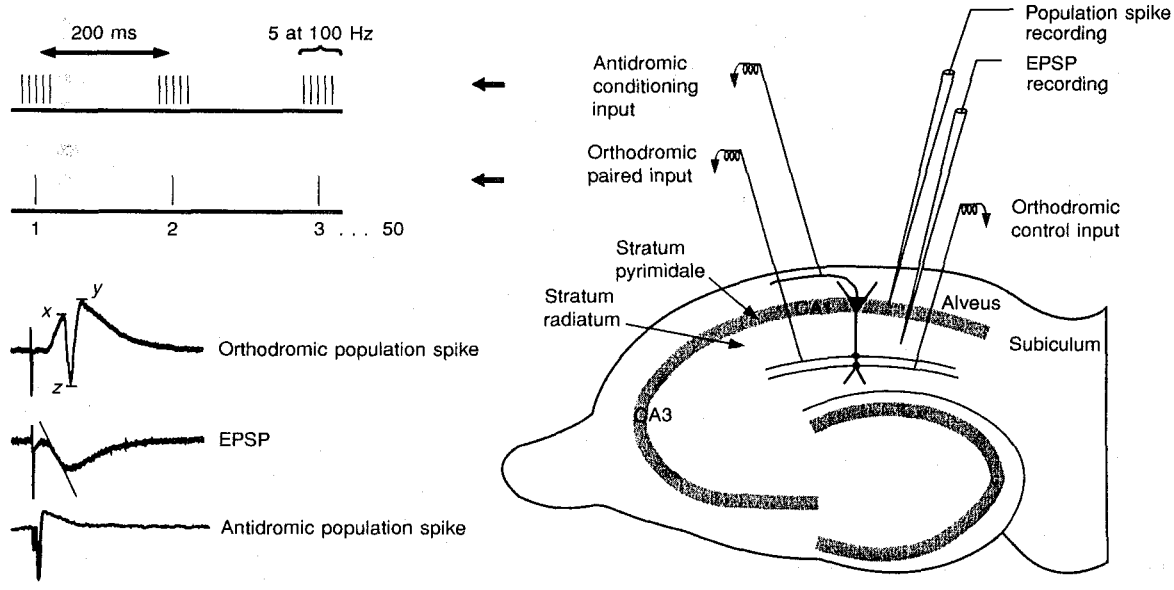

Figure 1. Schematic drawing of the slice preparation showing electrode placements and stimulation protocols

For orthodromic stimulation, a stimulating electrode (Orthodromic paired input) was placed in the stratum radiatum on the CA3 side of the recording electrodes. Stimulation of this electrode was used for measuring the population spike and for pairing with antidromic conditioning. For experiments with a second orthodromic input, the control stimulating electrode (Orthodromic control input) was placed in the stratum radiatum on the subicular side of the recording electrodes. This electrode activated a separate group of fibres. For antidromic stimulation, a stimulating electrode (Antidromic conditioning input) was placed in the alveus to stimulate the axons of the CA1 pyramidal cells. Population spike recording was from the stratum pyramidale, and the population EPSP was recorded from the stratum radiatum. The stimulus pattern used for pairing of orthodromic and antidromic stimulation is shown to the left of the appropriate stimulating electrode. The antidromic input received 50 bursts of 5 impulses at 200 ms intervals and the orthodromic input received 50 single impulses at 200 ms intervals (5 Hz). For paired stimulation, the orthodromic single shock was given simultaneously with the middle shock of the burst of 5 to the antidromic input. For controls, the orthodromic and antidromic stimuli were applied separately. The population spike amplitude was calculated as $(x + y)/2 - z$. The lower left part of the figure shows examples of orthodromic and antidromic population responses to single 50 μs stimuli.

micromanipulator and guided with a Zeiss stereo dissecting scope. Cell penetration was facilitated with 2 ms applications of an Axoclamp-2A remote 'buzz' circuit. Hyperpolarizing constant current (0·2–1 nA) was injected immediately following penetration to stabilize the cell. Intracellular signals were amplified using an Axoclamp-2A amplifier (Axon Instruments, Burlingame, CA, USA).

Tetanization protocols

Figure 1 (upper left) shows a schematic diagram of the protocol used for tetanization. The conditioning stimulus applied to the antidromic input consisted of fifty bursts of five impulses at a frequency of 100 Hz. Each impulse in a burst had a duration of 50 μs. The bursts were separated by an interval of 200 ms, corresponding to a frequency of 5 Hz. This pattern is similar to the hippocampal theta rhythm (5–7 Hz). Larson, Wong & Lynch (1986) have explored a similar pattern of stimulation applied orthodromically and found that it reliably produced LTP. Furthermore, they reported that the interval of 200 ms (corresponding to the theta frequency of 5 Hz) was the optimum separation between the bursts for LTP induction.

Paired stimulation consisted of the 5 Hz burst stimulation for 10 s to the antidromic input temporally paired with the orthodromic test input (Orthodromic paired input, Fig. 1) which received a single impulse coinciding with each burst to the antidromic input, i.e. a 5 Hz train. For control stimulations, one of the inputs was stimulated in the same pattern used for the paired stimulation without concurrent stimulation of the other input.

For each slice, a baseline period of at least 10 min was recorded before applying any tetanization. Next, a control stimulation of the orthodromic input alone was given. This was followed by a 10–40 min rest period. Then a control antidromic stimulation was given, followed by another rest period. Finally a paired stimulation was applied and the field potentials were recorded for a minimum of 15 min up to 2·5 h. Half of the slices that displayed potentiation were monitored for 15–30 min following the paired stimulation and the remaining half were monitored for over 40 min.

In some slices, intracellular recordings were made throughout the control and test periods. Once a stable intracellular recording was established, the membrane potential was set to −70 mV using DC current; no further changes to the holding current were made. Throughout the recording session, the input resistance and time constant of the membrane were measured using a 50 ms 0·2 nA hyperpolarizing current injection. These current injections were also used to ensure that the bridge balance was properly adjusted throughout the recording.

Data acquisition and measurement

The extracellular response of the slice was assessed by measuring the amplitude of the population spike in the cell body layer and the peak initial slope of the dendritic EPSP. The population spike amplitude was calculated as $(x + y)/2 - z$, with x, y and z as illustrated in Fig. 1 (lower left). To approximate the peak initial slope of the EPSP, the segment of recording between onset of the EPSP and its first minimum was approximated by a second-order polynomial which was differentiated. The (absolute value) maximum of the differentiated polynomial was taken as the peak initial slope of the EPSP. Analysis marks indicating points x, y and z of the population spike and a line indicating the slope of EPSP were plotted during the experiment (as shown in Fig. 1), so that it was possible to monitor visually the success of the analysis program in finding the population spike amplitude and EPSP slope.

Data were collected and analysed using the ASYST programming language on IBM compatible 386 and 486 computers. To quantify changes in the population spike and EPSP following a stimulation, the responses to test shocks were averaged over a period of 5 min at two time points, one immediately preceding the stimulation and the other 10–30 min after a control or paired stimulation. The data points used for summary data were taken approximately 20 min after the control or paired stimulations. For each slice, the percentage change due to a particular stimulation was found by dividing the mean following the stimulation by the mean immediately preceding the stimulation. Changes are summarized as the mean ± S.E.M. for all the slices tested. To control for variability of the slices, two values separated by 10 min were found during baseline recordings immediately preceding the first tetanus. These values were used to find a percentage change in baseline. Slices were not included when the baseline population spike amplitude or EPSP slope changed by more than 20%. The percentage changes following each stimulation were compared with changes during the baseline recording using paired, two-tailed Student's t tests to determine statistical significance.

For presentation of the time course of EPSP and population spike, data from some experiments were normalized. Average values of population spike amplitude and EPSP slope were determined during baseline recording and the population spike and EPSP slope are expressed as a percentage of the baseline values throughout the experiment.

RESULTS

Increases in population spike amplitude without accompanying EPSP change

The time course of a typical pairing experiment, along with sample waveforms, is shown in Fig. 2. In the time course figure (Fig. 2*B*), population spike amplitude and peak initial EPSP slope were normalized to the baseline values. Following 10 min of stable baseline recording, the orthodromic 5 Hz stimulation was given alone (Orthodromic stimulation); 15 min later, the antidromic patterned burst stimulation was given alone (Antidromic stimulation). Twenty minutes after these controls, antidromic and orthodromic stimuli were paired as in Fig. 1. The gaps in the time course indicate periods when a complete input–output curve was generated (Fig. 5). Throughout the experiment, the EPSP slope showed a slight upward drift, with no significant changes following either the control or paired stimuli. The population spike amplitude showed only slight changes following the orthodromic control stimuli, a temporary depression following the antidromic control stimuli (see Fig. 3), and a 48% increase relative to the amplitude preceding stimulation following paired stimulation. Population spike amplitude remained increased for the duration of the recording, approximately 45 min.

Sample population spike and EPSP responses at various points during the experiment are displayed in Fig. 2*A*.

Note that the population spike increases in amplitude following the paired stimulation, while the EPSP is unchanged throughout the experiment.

In many cases, application of the antidromic stimulation alone caused a temporary depression in the population spike without affecting the EPSP. Figure 3 shows an example of this in normal bathing medium (*A*) and in 20 μM picrotoxin + 20 μM bicuculline (*B*). Following antidromic stimulation, the population spike was depressed, but returned to baseline in 3–7 min.

In twenty-six slices tested, the values of population spike and EPSP slope during the 10 min baseline recording changed less than 20%. For eight of these slices, the control orthodromic stimulation caused an increase of

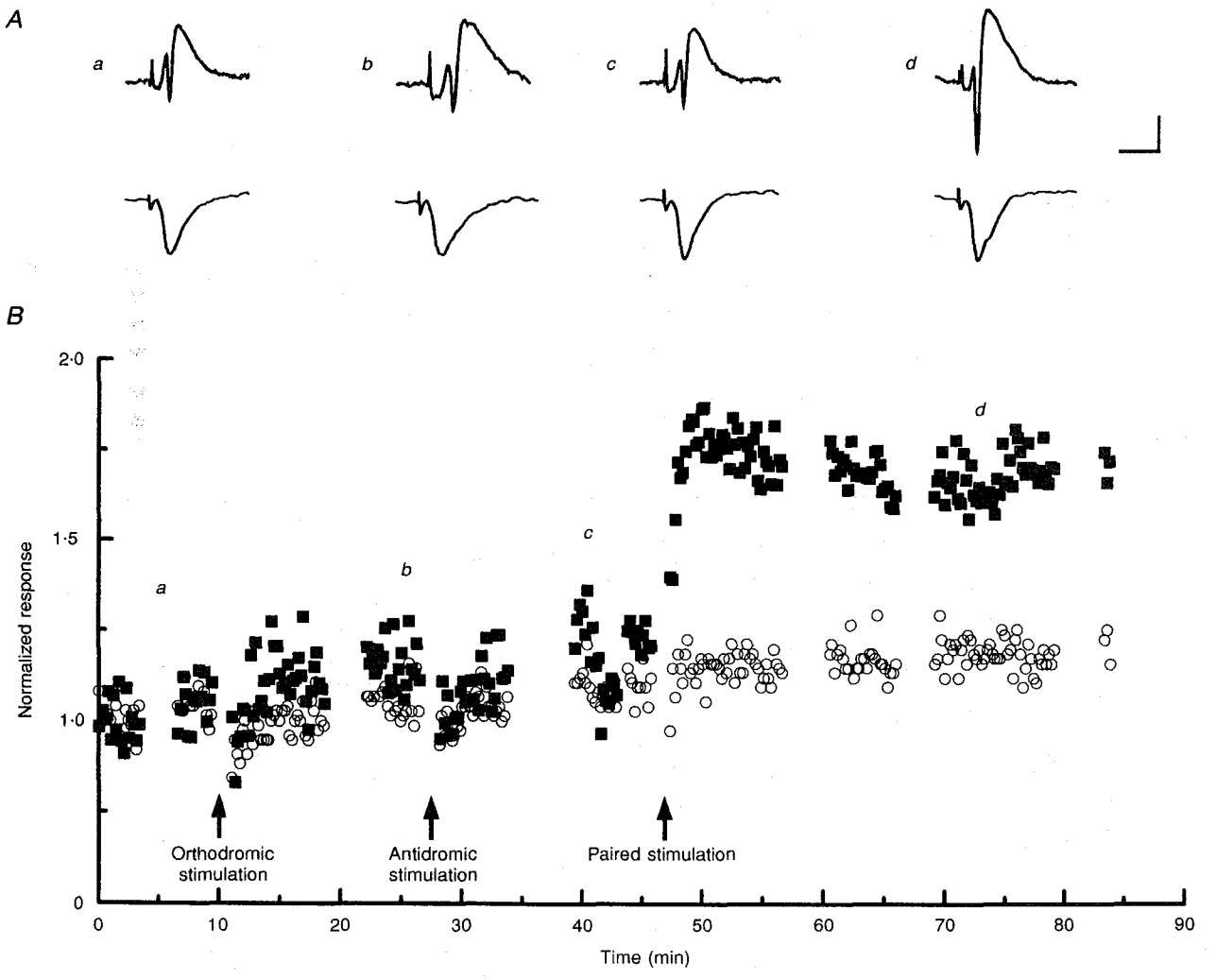

Figure 2. The time course of a typical pairing experiment and sample waveforms recorded throughout

A, sample population spike and EPSP recordings taken during the baseline recording period (*a*), following the control orthodromic (*b*) and antidromic stimulations (*c*), and following the paired stimulation (*d*). After pairing, the large increase in population spike without accompanying increase in the EPSP is evident. Calibration bar: 10 ms, 2 mV. *B*, time course of the experiment. The population spike amplitude (■) and EPSP slope (○) have been normalized by dividing each point by the average value found over the first 5 min so that the baseline value for each is 1. Following 10 min of stable baseline recording, the orthodromic 5 Hz stimulation was given alone. Fifteen minutes later, the antidromic patterned burst stimulation was given alone. Twenty minutes after these controls, the antidromic and orthodromic stimulation were paired (Paired stimulation). Throughout the experiment, the EPSP slope showed a slight increase, uncorrelated with any manipulation. The population spike amplitude showed a small increase following the orthodromic stimulation, an initial decrease following the antidromic stimulation and a very large increase following the paired stimulation. The gaps in the time course are points at which several different stimulus intensities were tested in order to measure EPSP–spike coupling. The positions of the waveforms in *A* are indicated above the trace in *B*.

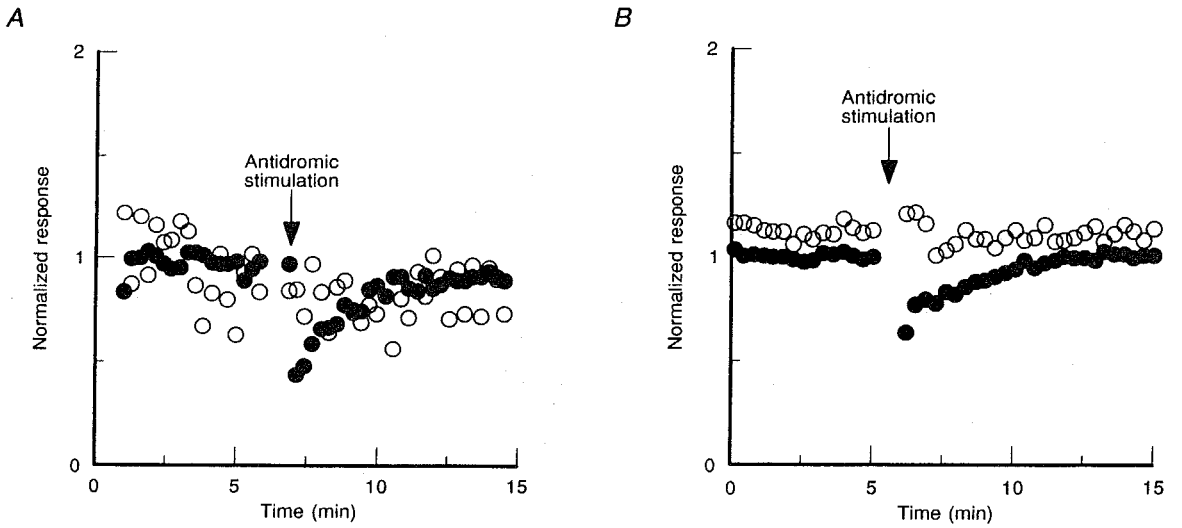

Figure 3. Population spike depression following unpaired antidromic conditioning
In some slices, unpaired antidromic conditioning caused a depression of the population spike amplitude (●) lasting from 3–7 min. The slope of the EPSP (○) was not usually affected by the temporary depression. The depression (arrow) occurs in normal CSF (*A*) and in CSF containing 20 μM bicuculline + 20 μM picrotoxin (*B*), and is not due to a temporary enhancement of $GABA_A$ inhibitory transmission. Figure 4 reflects this depression in the mean for the antidromic control.

more than 15%. In order to isolate the effect of the paired stimulation from that of the orthodromic stimulation alone, these slices were not included in the pairing data. This left eighteen slices which were analysed for the effect of the paired stimulus. The antidromic control stimulus never resulted in an increase in population spike or EPSP slope; hence, two slices were included which did not receive this control stimulus. Figure 4 summarizes the percentage changes observed in these twenty-six slices following the protocol described for Fig. 2. The changes following control and paired stimulations are compared with the baseline values, which show decreases of $3{\cdot}5 \pm 2{\cdot}2\%$ in population spike amplitude and $8{\cdot}3 \pm 2{\cdot}6\%$ in EPSP slope. Following the orthodromic control stimulation there was no change in population spike amplitude or EPSP slope. The antidromic control stimulation resulted in a decrease of the population spike by $11{\cdot}4 \pm 3{\cdot}8\%$ ($P = 0{\cdot}01$). Following the paired stimulus, there was a large increase in population spike amplitude ($33{\cdot}9 \pm 9{\cdot}6\%$, $P < 0{\cdot}003$). On average, the EPSP slope did not undergo changes significantly different from baseline variations. Of these eighteen slices, four had EPSP slope increases of greater than 10% whereas five had EPSP slope decreases greater than 10%.

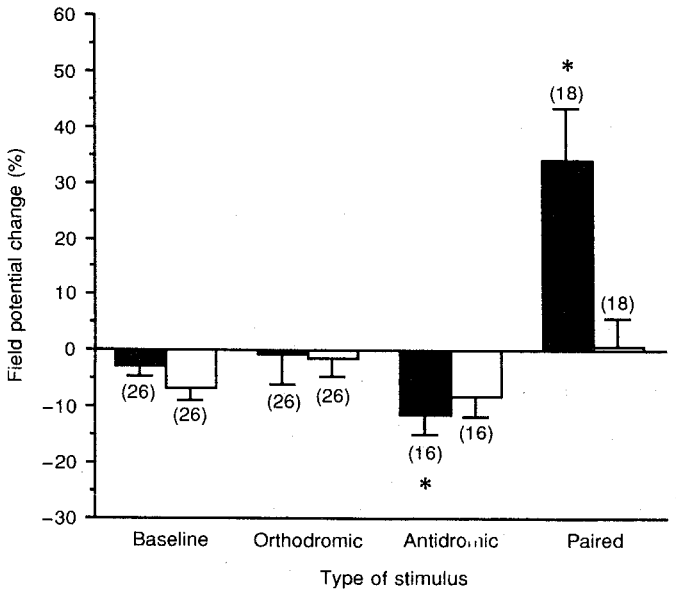

Figure 4. Summary of pairing experiments
■, population spike; □, EPSP. There are fewer slices in the paired category than in the others because experiments were discontinued and not included in analysis when there was an increase following the orthodromic control stimulus, so that any increases seen following paired stimulation would be specific to that type of stimulation. The antidromic stimulation never resulted in an increase, so two slices were included which did not receive this control stimulation. The antidromic stimulation alone resulted in a significant decrease ($11{\cdot}4 \pm 3{\cdot}8\%$, $P = 0{\cdot}01$) in the population spike. Paired stimulation caused an increase in population spike amplitude ($33{\cdot}9 \pm 9{\cdot}5\%$, $^*P = 0{\cdot}003$). The EPSP slope was not significantly different from baseline values ($P > 0{\cdot}05$) for any of the stimulations.

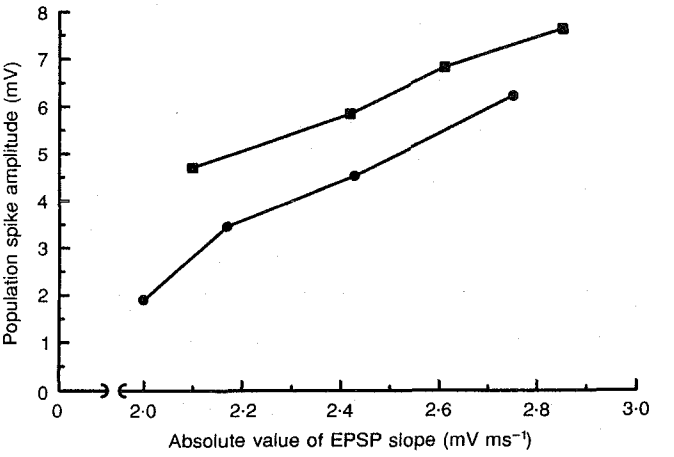

Figure 5. EPSP–spike coupling (E–S) potentiation in experiment of Fig. 2

Each point represents the absolute value of the EPSP slope plotted against the population spike amplitude for the same stimulus intensity. ●, values before the pairing; ■, values after the pairing. The curve shifted upward following the paired stimulation, indicating a larger population spike for a given EPSP or E–S potentiation of the slice.

In seven of the fifteen slices that displayed potentiation, the population spike remained increased for at least 40 min (the length of time that it was monitored). In three cases, the potentiation subsided during the recording period (by 16, 40 and 60 min). Overall, the potentiation lasted throughout the recording period, an average time of 38·1 ± 5·1 min. One slice was monitored for 2·5 h and the population spike potentiation endured throughout the recording period.

Input–output curves

If the effect of the paired stimulus is to increase E–S coupling, this will be apparent as in increase in population spike amplitude for a given EPSP size over a range of EPSP sizes. To test this, we used four different stimulus

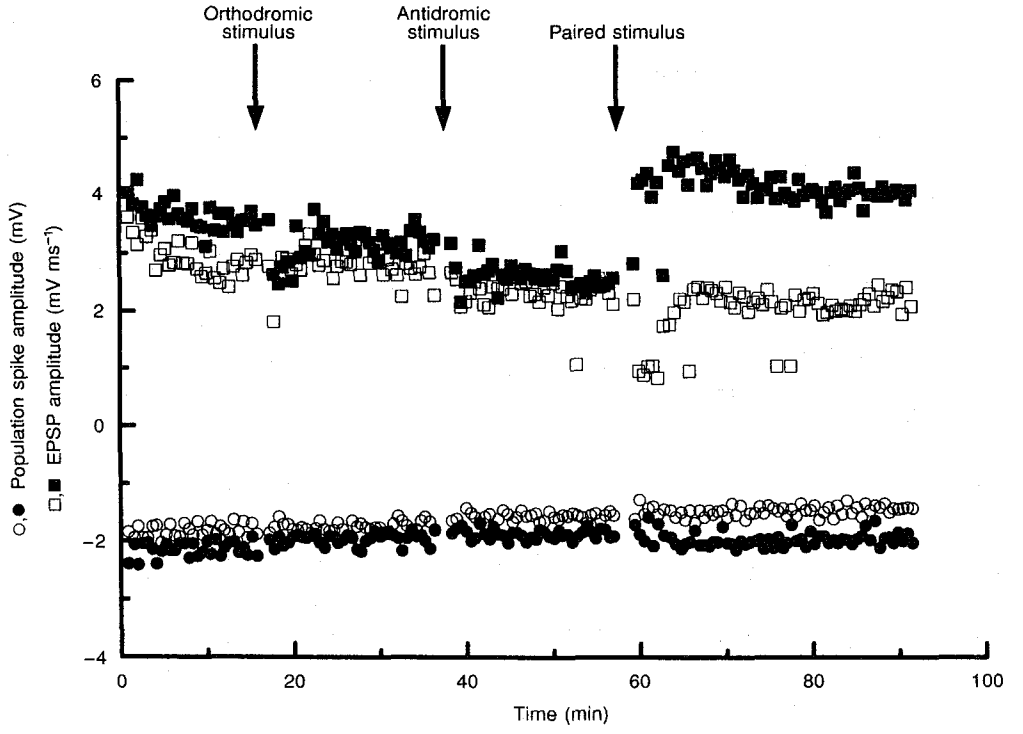

Figure 6. An example of an experiment in which the potentiation of the population spike was homosynaptic

The time course of the population spike amplitude (□,■) and EPSP slope (○,●) is plotted for two orthodromic inputs. The input that was paired with the antidromic conditioning is shown by filled symbols and the control input is shown by open symbols. In this experiment there was a slow decrease in the population spike of both orthodromic inputs up until the application of the paired antidromic and orthodromic stimulation. Following the paired stimulus, the population spike of the paired input showed an increase while the unpaired input's population spike did not change. Neither EPSP was affected by the paired stimulus. Control orthodromic and antidromic stimulations are marked.

intensities which resulted in a measurable population spike and EPSP at several time points throughout the experiment of Fig. 2. Each point in Fig. 5 represents the measurement, either before (●) or after (■) the paired stimulation, of the absolute value of the EPSP slope and population spike amplitude for a particular stimulus intensity. After the paired stimulation the curve shifted upward, indicating that an EPSP of a given size produced a larger population spike.

An input–output curve was generated for six experiments in which population spike potentiation was induced. Of these six, four showed an increase similar to the experiment depicted in Fig. 5, one did not change appreciably and one slice showed a decrease in the E–S curve (one of the few which did exhibit an increase in the EPSP slope).

Effect of pairing on inactive synapses

The changes seen with antidromic conditioning consisted of only a population spike increase without a change in the EPSP. A possible explanation is that the change underlying this phenomenon occurs on the dendrite between the synapse and the soma, causing more effective transfer of depolarization and increased probability of firing an action potential. If so, transfer of depolarization should also be enhanced for other synapses on the same dendrite, even if these synapses were not active during the pairing. We tested whether we could detect an increased response from inputs that had synapses on the same set of cells, if those synapses were inactive during the pairing. If enough of the synapses were on the same dendrites and were potentiated, then we should see an increase in the response to the unstimulated input. A control electrode was placed in a position to activate a second independent set of synapses (tested by lack of cross-facilitation) (McNaughton & Barnes, 1977) on the same group of postsynaptic neurons. This control electrode was placed in the stratum radiatum on the opposite side of the recording electrode from the paired stimulating electrode (Fig. 1, right, Orthodromic control input). The control input was used to test the response of the slice throughout the experiment, but did not receive any high-frequency stimulation and was not stimulated during the antidromic conditioning stimulation.

Figures 6 and 7 show two qualitatively different types of results obtained in these experiments. In both cases, as in the previous experiment (Fig. 2), we applied the orthodromic stimulation and the antidromic stimulation separately as controls after establishing a baseline and found no changes in either EPSP slope or population spike amplitude of either orthodromic input. After a 20–40 min period, the paired stimulation was given. Figure 6 shows a slice in which the pairing resulted in a purely

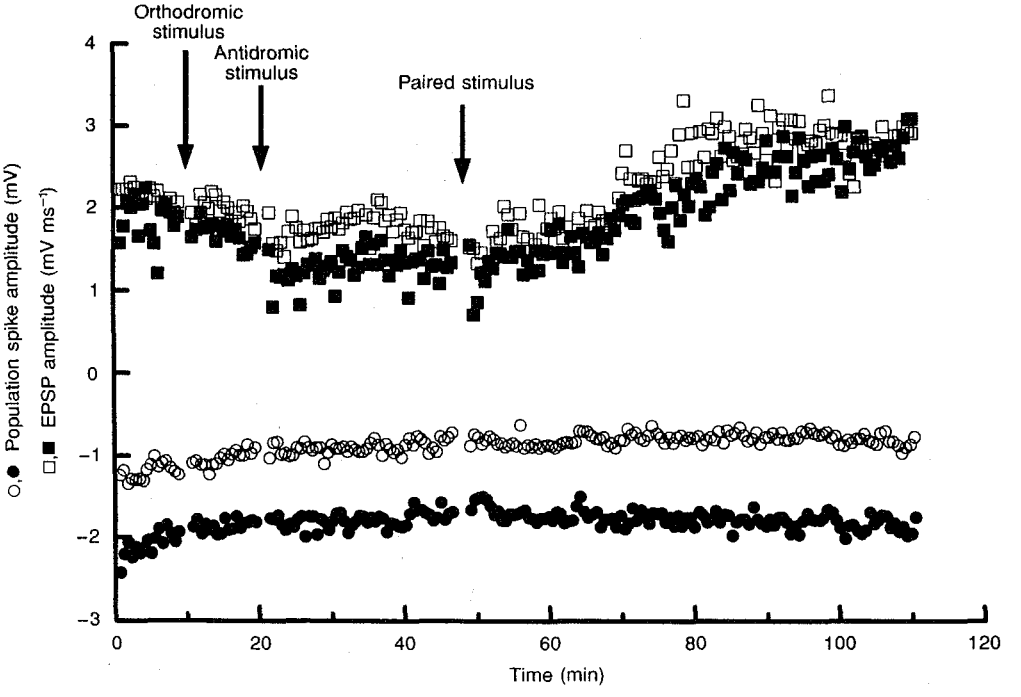

Figure 7. An example of an experiment in which the potentiation of population spike was heterosynaptic

Symbols are as in Fig. 6. In this case the population spikes of both paired and control electrodes showed a slow increase following the presentation of the paired stimulus. Again, the EPSP was unaffected for both electrodes.

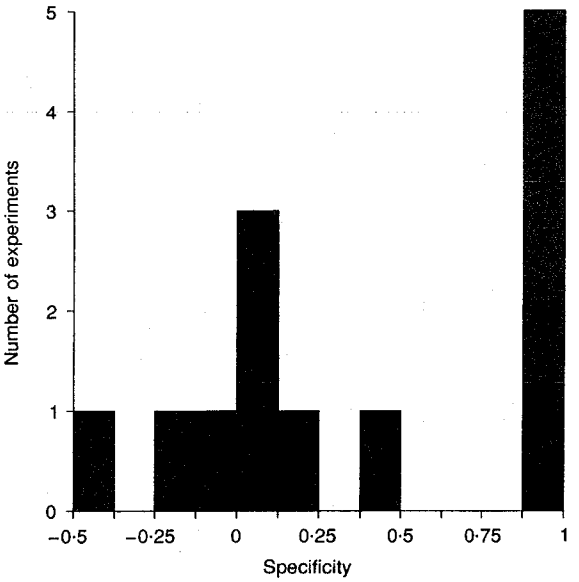

Figure 8. Histogram of specificity values
For each of the experiments in which a control orthodromic stimulating electrode was present and the paired stimulation caused E–S potentiation, the specificity was calculated as:

$$S = \frac{\Delta P - \Delta C}{|\Delta P| + |\Delta C|}.$$

Eight of the thirteen slices had specificity values less than 1, indicating heterosynaptic effects. (ΔP, percentage change of the population spike amplitude of the paired input, ΔC, percentage change of the control input).

homosynaptic effect. The population spike of the paired input showed an increase following the pairing, whereas the population spike of the control input did not change. The EPSP slope values of both the paired and control inputs were unchanged by the pairing, confirming the results of the first series of experiments.

By contrast, Fig. 7 shows the time course of the population spike amplitude and EPSP slope in a slice in which pairing increased the population spike of the control input as well as the paired input. Again, the EPSPs of both were unchanged throughout the recording. This experiment was one in which the time course of potentiation was slow, as discussed below. However, slower time to peak potentiation was not found to occur consistently with either heterosynaptic or homosynaptic effects.

In order to quantify the effect on the control input, the specificity, S, of the change in population spike amplitude was calculated as:

$$S = \frac{\Delta P - \Delta C}{|\Delta P| + |\Delta C|},$$

where ΔP is the percentage change in the paired population spike and ΔC is the percentage change in the control population spike. When there was no change or a decrease in the control, ($\Delta C \leqslant 0$) the specificity value was 1, indicating a perfectly homosynaptic effect. When the change in the control pathway was equal to that of the pathway receiving the paired stimulation, the specificity value was 0. Negative specificity values resulted when the control input increased more than the paired input ($\Delta C > \Delta P$). Figure 8 shows specificity values for the

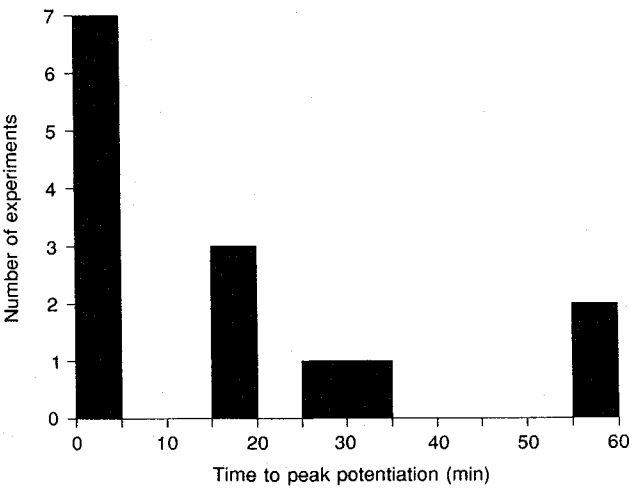

Figure 9. Time to peak potentiation
Data from each slice that showed population spike potentiation following the paired stimulation is depicted in the form of a histogram of the time to reach peak potentiation. Seven of these fourteen experiments showed peak potentiation occurring more than 5 min following the paired stimulation, with two reaching peak values 60 min after the stimulation.

thirteen experiments incorporating the orthodromic control input that showed potentiation of the paired population spike. Eight of thirteen slices had specificity values less than 1, indicating heterosynaptic effects resulting from the paired stimulation. Thus, potentiation of the population spike tended to be heterosynaptic when induced by orthodromic and antidromic pairing.

Slowly developing increases in population spike amplitude

In contrast with most reports of long-term potentiation, the pairing of antidromic and orthodromic stimulation used in these experiments often resulted in slowly developing changes in population spike amplitude. Figure 7 shows an example of an experiment in which the population spike amplitude increased over a period of approximately 30 min. In contrast, in the experiments shown in Figs 2 and 6, the peak population spike amplitude occurred within minutes of the paired stimulation.

The time to reach peak population spike amplitude following the paired stimulation was found for each experiment in which long-lasting potentiation occurred (14 of the experiments in Fig. 4). The times to peak ranged from 1 to 60 min. The distribution is plotted in Fig. 9. Of the fourteen experiments included in this figure, seven showed quickly developing potentiation, reaching peak amplitude in less than 5 min, and the other seven had slower time courses, including two which reached their maximum potentiation nearly an hour after the paired stimulation. This slow development of potentiation indicates that a process different from that which produces conventional high-frequency induced synaptic LTP may be involved.

As noted above, Fig. 7 shows a case where the population spike change was heterosynaptic and the time course of potentiation was quite slow. However, it was not found that these two properties co-varied consistently. This was quantified by separating slices into 'fast' (time to peak less than 5 min) and 'slow' (time to peak more than 5 min) and heterosynaptic ($S < 1$) and homosynaptic ($S = 1$) categories and constructing a χ^2 contingency table. With this analysis, there was no indication that the two variables were interacting ($P \geqslant 0{\cdot}1$).

Effects of strong orthodromic stimulation

In order to ensure that the failure to observe population EPSP changes was not due to an inability to obtain these changes in our experimental preparation, we did a second series of experiments in which we applied strong stimulation to the orthodromic test input. These experiments were done under the condition where the control orthodromic input was also present and therefore they also provide results on the specificity of

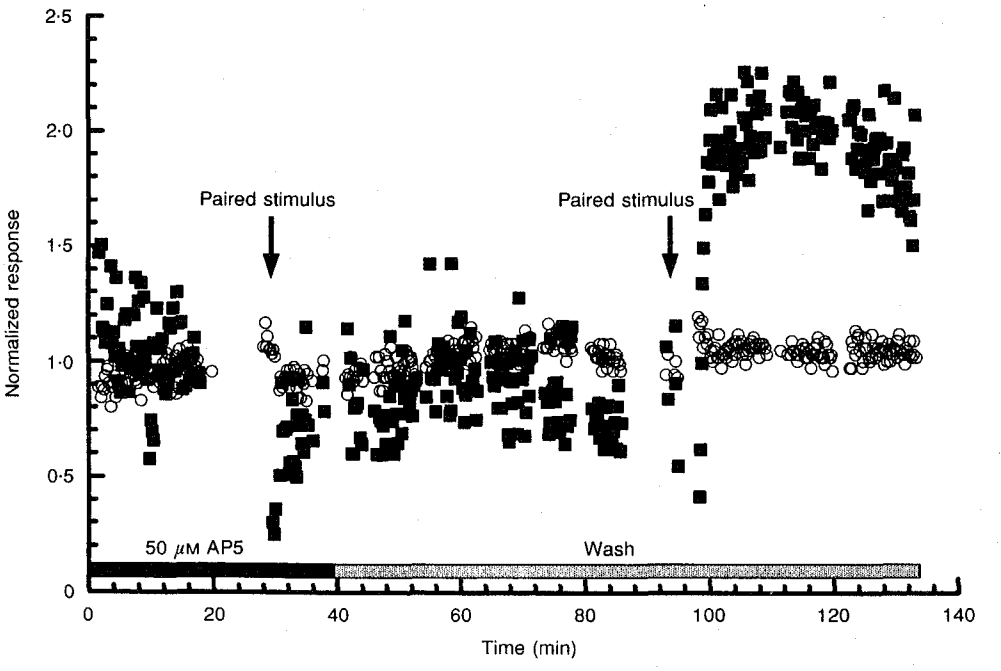

Figure 10. The normalized time course of an experiment depicting the ability of AP5 to block E–S potentiation induced by antidromic conditioning

The AP5 was in the bath at the beginning of the recording period. After a baseline recording of 23 min, a paired stimulus was given. No change was seen in either the population spike amplitude (■) or EPSP slope (○). After washing out the AP5, the paired stimulus was repeated. The population spike amplitude now increased by 155% over prestimulation values and the EPSP slope remained unchanged.

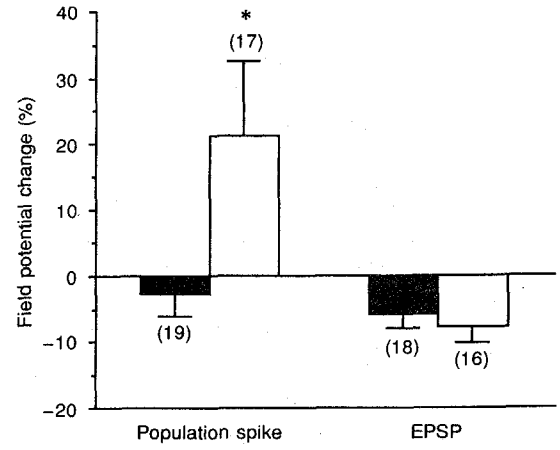

Figure 11. Summary of the experiments showing the effects of paired stimulation in the presence and absence of AP5 in the bathing medium

Two slices exhibited an increase in population spike following paired stimulation in the presence of AP5 (■). These were given a strong orthodromic stimulation to test whether AP5 was blocking LTP, but were not tested after washing out AP5. One slice did not have a measurable EPSP slope. In the presence of AP5, the population spike amplitude was unchanged by the paired stimulation. After washout of AP5 (□), the same stimulation caused an increase in the population spike amplitude of 21·4 ± 11·3% (* $P < 0·05$, Student's unpaired t test compared with effect of the paired stimulus in the presence of AP5). The EPSP slope was not significantly changed in either condition.

orthodromically induced LTP in this preparation. The stimulation used was the same patterned burst stimulation as used for antidromic conditioning in the pairing experiments but applied to the orthodromic test input. Of sixteen slices which were given the strong orthodromic stimulation, fifteen also incorporated the control stimulating electrode. The average population spike increase of the tetanized input was 58·9 ± 13·9% ($P < 0·001$) and the mean EPSP increase for this input was 22·1 ± 10·2% ($P < 0·05$). Neither the population spike nor the EPSP of the control input changed significantly. These experiments show stimulus-specific potentiation of both the population spike and EPSP with orthodromic stimulation at the test electrodes used in the antidromic conditioning experiments above. Therefore, the result of heterosynaptic E–S potentiation reported above for antidromic conditioning is specific to that type of conditioning and not explained by inability in our preparation to achieve homosynaptic LTP of the population EPSP as well as population spike.

Effect of AP5 on the induction of E–S potentiation

Associative LTP is believed to result from depolarization of the postsynaptic membrane relieving the Mg^{2+} block of the NMDA receptor channel (Wigström & Gustafsson, 1985). In order to test whether this mechanism could also account for the induction of antidromically conditioned E–S potentiation, we bath-applied 50 μM 2-amino-5-phosphonovalerate (AP5) to block the NMDA receptor. The response to the paired stimulation was tested in the presence of the AP5 and after washing the AP5 out.

Figure 10 shows the time course of the normalized population spike amplitude and EPSP slope of a typical AP5 experiment. The AP5 was added to the perfusing solution at the beginning of the experiment. After 30 min, the orthodromic stimulation was paired with antidromic conditioning. No significant change was seen in either the population spike amplitude or EPSP slope. The AP5 was then washed out and the paired stimulation repeated. This time, the population spike amplitude showed an increase of 155% from prestimulation values whereas the EPSP slope did not change significantly.

Figure 11 summarizes results from nineteen slices that were tested in the presence of AP5. In two of the nineteen experiments, population spike potentiation was seen while AP5 was in the bath. In these two cases, a strong tetanus was then applied to the orthodromic input to be sure that the AP5 was capable of blocking normal LTP. In both cases, the strong tetanus resulted in a large depression of the population spike amplitude, indicating that the AP5 was blocking LTP as expected, and the experiment was discontinued. In the rest of the slices, the AP5 was washed out and the paired stimulus repeated in the wash condition. In one slice, the EPSP was

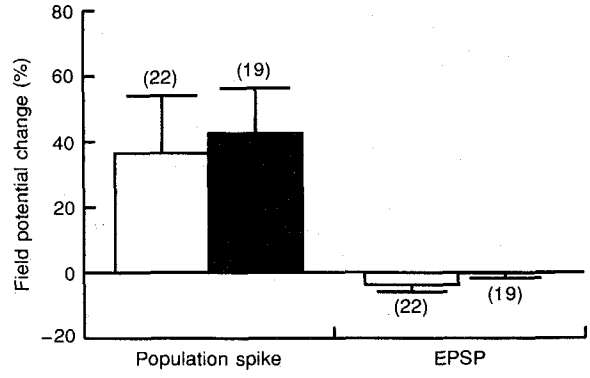

Figure 12. Summary of $GABA_A$-blocking experiments

Picrotoxin (10 μM) and/or bicuculline (10 μM) failed to block E–S potentiation. The mean population spike potentiation in untreated slices (□) was 36·5 ± 17·6%, and in bicuculline/picrotoxin-treated slices was 42 ± 13·8% (■). EPSP slope was virtually unaffected in both control and treated slices.

immeasurable. On average, in the presence of AP5, neither the population spike amplitude nor the EPSP slope showed significant changes after the pairing, with the values being $-2{\cdot}7 \pm 4{\cdot}7\%$ for population spike amplitude and $-3{\cdot}5 \pm 2{\cdot}6\%$ for EPSP slope. After the washout of AP5, however, the population spike amplitude showed an increase of $44{\cdot}5 \pm 14{\cdot}6\%$ ($P < 0{\cdot}01$); whereas the EPSP slope was unchanged.

Blockade of $GABA_A$-mediated inhibition

A change in the relative amount of synaptic inhibition and excitation following the induction of LTP has been suggested to explain E–S potentiation generated by orthodromic high-frequency stimulation (Wilson *et al.* 1981; Abraham *et al.* 1987). This has been tested by measuring the amount of E–S potentiation induced by high-frequency stimulation when the $GABA_A$ blocker picrotoxin is added to the superfusate. Several studies (Wilson *et al.* 1981; Abraham *et al.* 1987; Chavez-Noriega, Bliss & Halliwell, 1989) have shown that bath application of picrotoxin itself results in E–S potentiation. Two groups showed that high-frequency stimulation following the picrotoxin application did not result in further E–S potentiation (Abraham, Gustafsson & Wigström, 1987; Chavez-Noriega *et al.* 1989). However, others were able to achieve a substantial amount of high-frequency-induced E–S potentiation in the presence of 100 μM picrotoxin (Hess & Gustafsson, 1990).

In order to test whether a change in $GABA_A$ inhibition could account for the present results, we delivered paired orthodromic and antidromic stimulation in the presence of the $GABA_A$ antagonists picrotoxin (10 μM, $n = 5$), or bicuculline (10 μM, $n = 5$), or a combination of the two ($n = 9$). The application of $GABA_A$ blockers resulted in a large increase in the population spike amplitude without any tetanization. Single antidromic stimuli sometimes produced an orthodromic spike following the antidromic spike. Slices in which orthodromic population spikes contaminated the antidromic stimulation were excluded. Figure 12 shows that pairing resulted in an increase of the population spike of $36{\cdot}6 \pm 17{\cdot}6\%$ for twenty-two control and $42{\cdot}6 \pm 13{\cdot}8\%$ for nineteen $GABA_A$-blocked slices, with virtually no change in the EPSP slope ($-3{\cdot}8 \pm 2{\cdot}2\%$ and $-0{\cdot}2 \pm 1{\cdot}5\%$ respectively). Thus, it is unlikely that antidromically conditioned E–S potentiation is produced by a change in the relative amount of excitation and $GABA_A$ inhibition in these experiments.

Measuring EPSP amplitude at the soma

To address the possibility that E–S potentiation enhances conduction of synaptic potentials to the soma, EPSPs recorded by the extracellular electrode in the stratum pyramidale were compared before and after generation of E–S potentiation. The EPSP comparison was made using subthreshold stimulus intensities to avoid contamination of the EPSP waveform by the action potential. Slices that exhibited more than 10% potentiation of the population spike amplitude were included in this analysis. With suprathreshold stimulation, these slices had an average 62% potentiation of the population spike; however, with subthreshold stimulation no potentiation of the EPSP amplitude was observed: $0{\cdot}45 \pm 0{\cdot}11$ mV (mean ± S.E.M) before and $0{\cdot}49 \pm 0{\cdot}13$ mV after pairing; Student's paired t test; $n = 10$; $P \geqslant 0{\cdot}05$.

In some cells, the intracellular EPSP was quantified before and 15 min after paired tetanus. Paired stimulation had no effect on the intracellular EPSP amplitude (before, $5{\cdot}51 \pm 1{\cdot}59$ mV; 15 min after, $5{\cdot}48 \pm 2{\cdot}18$; Student's paired t test; $P \geqslant 0{\cdot}05$, $n = 4$).

Taken together, these data indicate that there is not an increased conduction of the EPSP to the soma, so the increase in firing of the cells must result from some other factor.

DISCUSSION

Comparison with synaptic LTP induced by orthodromic tetanic stimulation

We have shown that antidromic stimulation can serve as the conditioning input for an associative potentiation of the population spike amplitude. The potentiation demonstrated in these experiments differs from high-frequency-induced synaptic LTP in three ways. First, the antidromic conditioning led to an increase specifically in the population spike but not the EPSP. Second, in eight out of thirteen experiments in which potentiation was achieved, the population spike increase was heterosynaptic, in that it affected a second input that was not active during the pairing. Orthodromically induced LTP has been found to be homosynaptic (Andersen *et al.* 1980). Third, in seven of fourteen experiments, the potentiation took more than 5 min to develop, in contrast to the rapid onset of high-frequency-induced synaptic LTP in which the potentiation develops within tens of seconds of the tetanization and becomes stable after the decay of post-tetanic potentiation (Bliss & Lømo, 1973).

Specific E–S potentiation

In the first report of LTP in the hippocampus, Bliss & Lømo (1973) presented evidence for a change in the population spike that could not be accounted for by the change in the EPSP. They plotted population spike latency, which varies inversely with population spike amplitude, against population EPSP amplitude and found a downward shift following induction of LTP. In a later paper, these authors plotted population spike amplitude *vs.* EPSP amplitude and reported the leftward shift which we have observed (Bliss, Gardner-Medwin & Lømo, 1973). Andersen *et al.* (1980) plotted population spike amplitude *vs.* population EPSP slope and used the term EPSP-to-spike (E–S) potentiation to describe the upward shift following induction of LTP. They also reported finding in a few cells the intracellular correlate of this phenomenon,

an increase in the probability of firing in response to stimulation that was greater than expected from the increase in the amplitude of the intracellular EPSP.

In most of the studies that have examined E–S potentiation, the induction of LTP by high-frequency orthodromic stimulation has resulted in increases in EPSP slope as well as E–S potentiation. A few studies have, however, reported occurrences of E–S potentiation without EPSP changes. For instance, Bliss & Lømo (1973), as well as Abraham *et al.* (1985), reported cases in which the amplitude of the population spike was increased without an accompanying change in the population EPSP. In intracellular studies, Andersen *et al.* (1980) reported one cell in which there was increased firing probability with no detectable change in the EPSP. In experiments in which intradendritic as well as extracellular recordings were made, Taube & Schwartzkroin (1988) found five out of eighteen cases in which the population spike was increased by high-frequency stimulation with no change in the population EPSP, and in these cases the intradendritic EPSP was also unchanged, whereas the intradendritic EPSP was increased on average for the cases in which the population EPSP was also increased.

In our experiments, we consistently found an increase in the population spike only, indicating specific potentiation of the E–S coupling mechanism. The failure to observe EPSP changes is not due to our experimental preparation, since large and statistically significant population EPSP increases were seen following high-frequency orthodromic stimulation. Therefore, under these conditions a form of E–S potentiation can be induced independently of EPSP potentiation.

It is surprising that our experimental protocol does not result in EPSP potentiation. Associative LTP can be induced in two ways that are similar to our stimulation. One way is by using a high-frequency orthodromic conditioning input paired with single orthodromic shocks (Gustafsson & Wigström, 1986; Chattarji, Stanton & Sejnowski, 1989; Stanton & Sejnowski, 1989). Others have reported that antidromic conditioning is less effective at producing associative LTP than orthodromic conditioning (Lee, 1983; Gustafsson & Wigström, 1986). High-frequency orthodromic conditioning may result in more spatiotemporal summation than antidromic conditioning, and this could lead to more depolarization of the postsynaptic membrane and a greater chance of producing EPSP potentiation. There is also some evidence that dendritic input can cause calcium spiking in the dendrites and bursting in the soma, whereas an input to the soma will only elicit single spikes in the soma (Wong & Stewart, 1992). Therefore, the orthodromic conditioning input could cause calcium spiking in the dendrite whereas the antidromic conditioning input would not. The differences in calcium levels in the dendrites could be responsible for producing EPSP potentiation in the case of orthodromic conditioning and not in the case of antidromic conditioning.

Another type of conditioning that results in EPSP potentiation is pairing intracellular depolarization with low-frequency synaptic input (Sastry *et al.* 1986; Gustafsson *et al.* 1987; Bonhoeffer, Staiger & Aertsen, 1989). Intracellular depolarization differs from antidromic conditioning in two ways. First, the depolarization used was a step of current which would be transferred to the dendrite as a DC shift in potential, whereas firing in the soma will only result in brief potential variations. Second, the antidromic conditioning activates other pathways in the network through recurrent excitation and inhibition and these network properties could contribute to the differences that we found.

Heterosynaptic effects

Unlike E–S potentiation accompanying high-frequency-induced synaptic LTP, the effects of the pairing were heterosynaptic in eight of thirteen experiments showing population spike potentiation. Two types of experiments show that the control electrode was indeed stimulating a separate set of fibres. Double-pulse experiments showed that a shock to one pathway did not cause facilitation of a closely followed shock to the other pathway, indicating that non-overlapping fibres were being stimulated. Secondly, the specificity of LTP produced by strong orthodromic stimulation was tested with a control stimulating electrode in place, and in these experiments no heterosynaptic effects of either population spike or EPSP were seen.

Potentiation of the E–S coupling mechanism might be expected to have heterosynaptic effects. Wathey, Lytton, Jester & Sejnowski (1992) explored a model of E–S potentiation in high-frequency-induced LTP in which the population spike increase was due to the insertion of 'hot spots' of calcium channels in dendrites. The model showed that this mechanism resulted in variable specificity of the population spike changes, depending on the relative electrical distance of the tetanized and control inputs from the soma. If control synapses that were sampled were further from the soma than tetanized ones, they were also affected by the potentiation. Conversely, when the control inputs sampled were closer to the soma than the tetanized contacts, there was no evidence of potentiation. This is relevant in view of the results presented here with a control electrode to test the specificity of the potentiation. We found in some cases that the population spike increase was only seen in the paired input; however, in eight out of thirteen experiments both paired and control inputs were affected. The finding that some experiments produced heterosynaptic and others homosynaptic effects could be explained in terms of the Wathey *et al.* (1992) model if variation in placement of the stimulating electrodes between experiments led to stimulation of synapses at

different distances from the soma. We attempted to place the test and control stimulating electrodes equidistantly from the cell body layer; however, this procedure could not assure that the synapses being stimulated were equidistant.

The E–S potentiation reported by Andersen *et al.* (1980) in field CA1 was specific to the tetanized input; an untetanized pathway did not show the E–S potentiation. Furthermore, in intracellular experiments, they did not see any generalized increase in excitability in response to injected current, even when there was increased firing probability in response to a given EPSP amplitude. In the dentate gyrus, however, E–S potentiation has been found to be heterosynaptic. In a study of dentate gyrus LTP, Abraham *et al.* (1985) found E–S potentiation that was heterosynaptic but, since it was accompanied by a long-lasting depression of the EPSP heterosynaptically, the effect on the population spike was cancelled in the unstimulated pathway. Others (Hess & Gustafsson, 1990) found in CA1 that whereas using a tetanization strength equal to the test stimulation resulted in homosynaptic LTP and E–S potentiation following 50 Hz trains, the same tetanization at twice the stimulus intensity resulted in a heterosynaptic change in the shape of the field EPSP which resulted in a larger peak amplitude without a change in the peak initial slope. The shape change was accompanied by heterosynaptic E–S potentiation. All of these experiments were performed in the presence of 100 μM picrotoxin, to exclude the possibility of the changes resulting from plasticity in inhibitory responses. The change in EPSP shape was also blocked by the NMDA receptor antagonist AP5. Since the shape change was accompanied by E–S potentiation of an unstimulated pathway, they suggested that it might also underlie E–S potentiation. They attributed these heterosynaptic effects to using a higher stimulus strength than normal. This E–S potentiation is similar to ours in that it is heterosynaptic and blocked by AP5. Furthermore, it was a specific spike potentiation in many cases, although this was only after saturation of normal LTP by tetanization with a lower stimulus intensity. The difference was that their E–S potentiation was fully developed within 3–4 min of the high-intensity tetanization, unlike our slowly developing potentiation. Furthermore, we did not observe a shape change; in our experiments, neither the EPSP amplitude nor the EPSP slope was affected by the paired stimulus (data not shown).

Time course of potentiation

A third difference between high-frequency-induced synaptic LTP and our findings was the slow increase in the size of the population spike, which we found in seven of the fourteen slices which showed long-lasting potentiation. After the application of a high-frequency orthodromic stimulation there is normally an immediate large short-term potentiation which decays after a few minutes. At this time the amplitude of the LTP has already reached its maximum value. This is the case for both the EPSP and the population spike, indicating that the E–S potentiation also takes place within the first few minutes of tetanization (Bliss & Lømo, 1973; Andersen *et al.* 1980). In our experiments, we did not observe either post-tetanic potentiation, which has a time course of a minute or less, or short-term potentiation which lasts for several minutes. In many cases, there was a depression immediately following the paired stimulus which reversed within a few minutes and then often slowly developed into potentiation. Our findings confirm those of Ito *et al.* (1986). Their pairing of orthodromic and antidromic stimulation in a pattern similar to the one we were using also produced a population spike increase that developed over a period of approximately 30 min.

One case of high-frequency orthodromic stimulation causing a slow growth in response was reported by Grover & Teyler (1990), who found an AP5-resistant form of LTP in area CA1 when they applied a 200 Hz tetanus, and they reported that this potentiation developed over a period of 15–30 min. The time to maximum potentiation varied from immediate (Fig. 3*A*; Grover & Teyler, 1990) to 30 min. Our experiments showed similar variability in time course; however, our potentiation was sensitive to AP5.

Explanation of variable results

The observation of time courses that varied from an immediate change to one that developed over a period of more than an hour and the variability seen in heterosynaptic effects suggest the possibility that more than one mechanism may be involved in this potentiation. For instance, the slow growth in population spike seen in some cases could be the reflection of a depression that occurred immediately following the paired stimulation and decayed with a slow time course, revealing an underlying potentiation. It has been found that high-frequency antidromic stimulation of CA1 cells of hippocampus slices, given in the presence of high concentrations of Mg^{2+} to block synaptic transmission, resulted in a lasting depression of the population spike and EPSP which could be seen after the Mg^{2+} was returned to normal levels (Pockett & Lippold, 1986). In later experiments, depression of intracellular EPSPs when giving antidromic stimulation in raised-Mg^{2+} medium was demonstrated (Pockett, Brookes & Bindman, 1990). In normal medium, mixed results were reported, sometimes depression and sometimes potentiation (Pockett *et al.* 1990). Abraham & Wickens (1991) also found EPSP depression, without accompanying E–S depression, when giving alveal stimulation. The depression was greater and long-lasting in the presence of picrotoxin. In normal and in $GABA_A$-blocked medium, we also observed a depression of the population spike following the control stimulation of the antidromic input alone; however, we

did not see depression of the EPSP either in field recordings or intracellularly. An important difference in our experiments was that our stimulation was less intense than that used by Pockett *et al.* (1990) and Abraham & Wickens (1991): we used ten bursts with five pulses at 100 Hz in each burst whereas the other labs used six trains of fifty pulses each at 100 Hz (Abraham & Wickens used 15, 50 and 200 Hz). The antidromic stimulation could lead to varying degrees of depression which could account for the variability in the time course.

The potentiation reported by Grover & Teyler (1990) has an interesting similarity to that of Hess & Gustafsson (1990) in that Grover's observation of slowly developing potentiation was not seen when a lower frequency tetanus was applied whereas the change in the shape of the population EPSP was only seen by Hess following tetani with high stimulus intensities. This implies that there are other mechanisms of potentiation with slightly different properties and different thresholds from 'normal' LTP. We may be in a physiological range that is near the threshold for more than one of these types of potentiation, so that we observe different combinations of effects for the same stimulation.

Possible mechanisms

Associative LTP is induced under the condition that the postsynaptic membrane is depolarized while the excitatory neurotransmitter glutamate is bound to the NMDA-preferring glutamate receptor subtype (Wigström & Gustafsson, 1985). The NMDA receptor channel is blocked by Mg^{2+} at resting potential, but the block is relieved when the membrane is sufficiently depolarized (Mayer, Westbrook & Guthrie, 1984). When synaptic activation occurs simultaneously with postsynaptic depolarization, the unblocked NMDA receptor channel is opened. The activation of the NMDA receptor is necessary for the induction of LTP. It is widely believed that LTP is induced by calcium entry through the unblocked NMDA receptor channel, because buffering postsynaptic intracellular calcium to low levels blocks LTP (Lynch, Larson, Kelso, Barrionuevo & Schottler, 1983; Malenka, Kauer, Zucker & Nicoll, 1988) and an increase in calcium concentration can cause LTP without other stimulation (Turner, Baimbridge & Miller, 1982; Malenka *et al.* 1988).

We also found that the NMDA receptor was necessary for the induction of associative E–S potentiation. This would imply that the antidromic spike in the soma can invade the dendrites sufficiently to unblock the NMDA receptor channel. However, the differences between our results and associative LTP using orthodromic conditioning stimulation show that something must be different in the two protocols. One of the major differences is that the potentiation was heterosynaptic in many cases. In high-frequency-induced LTP, the depolarization of the NMDA receptor is believed to cause calcium influx through the receptor channel located in the dendritic spine. The calcium concentration in the small volume of the spine can be greatly changed by only a small influx of calcium (Gamble & Koch, 1987) which could then cause potentiation at the site of the synapse. However, we would not expect a change at the site of the synapse to result in heterosynaptic effects. In the case of antidromic conditioning, the NMDA receptor activation might be needed not to allow calcium entry but to boost the overall amount of depolarization of dendritic membrane so that the E–S potentiation could take place on dendritic membrane further from the synapse. This could also explain the two cases in which E–S potentiation was achieved in the presence of AP5, when the AP5 was subsequently shown to be successfully blocking the NMDA receptors. If the activation of the NMDA receptors was only necessary for additional depolarization, then it would be conceivable that in certain cases enough depolarization could be achieved without NMDA receptor activation by fortuitous placement of stimulating electrodes. Another of the differences was that we did not see potentiation of the EPSP, which we would expect if the NMDA receptor was activated and depolarized. The calcium influx through the NMDA receptor channel reflects the integral of its depolarization over the time during which it is activated. As discussed previously, antidromic conditioning may result in briefer and smaller depolarization so that the total calcium influx would be insufficient to induce EPSP potentiation.

Another possibility is that the NMDA activation necessary for the potentiation we saw is due to extrasynaptic or glial NMDA receptors (Pittaluga & Raiteri, 1992; Muller, Grosche, Ohlemeyer & Kettenmann, 1993) which may allow the induction of E–S potentiation but not EPSP potentiation.

Our data do not rule out changes in other parts of the hippocampal network that could result in an increase in the population spike without an EPSP change. Although we were concentrating on the spike activity of the stimulated cells as the important factor for the E–S potentiation, it must be kept in mind that other cells will be activated by our stimulation. Whenever a pyramidal cell spikes, it activates other cells to which it is connected, including feedback inhibitory connections and local excitatory connections. For instance, there is evidence for a local excitatory connection between CA1 pyramidal cells which is substantially NMDA receptor-mediated (Thomson & Radpour, 1991). There are also inhibitory feedback circuits that exist through oriens–alveus interneurons (Lacaille, Mueller, Kunkel & Schwartzkroin, 1987) as well as basket cells. Furthermore, stimulation of an electrode placed in the alveus will also directly stimulate these oriens–alveus neurons as well as any other types of neurons that are close enough to the stimulating electrode to be affected. When we used $GABA_A$ blockers in the perfusing medium, the alveal

stimulation often showed a late orthodromic component following the antidromic population spike. This could reflect the unmasking of recurrent circuitry which is also activated by the alveal stimulation. It is possible that our results are due to the high-frequency activation of one of these circuits, although the results with $GABA_A$ blockers suggest that fast inhibitory activity is not necessary for the change.

The measurement of the EPSP at the soma, with extracellular subthreshold stimulation and intracellular recording, showed that the EPSP is not changed at the soma. This suggests that antidromically conditioned E–S potentiation does not alter the dendritic input or the conduction of the dendritic depolarization to the soma, but that it modifies the soma or initial segment such that a given amount of depolarizing current from the dendrites generates more action potentials.

Conclusion

We have found conditions under which hippocampal neurons change their excitability when they are concurrently spiking at a high frequency and receiving low-frequency synaptic input. Since CA1 cells are capable of bursting and may even burst in a pattern similar to the one that we used for stimulation (Otto, Eichenbaum, Wiener & Wible, 1991), it is likely that similar conditions are seen *in vivo*. Our results suggest that synaptic input during such a burst should change the input–output relationship for many of the synapses on the cell, not just those active during the burst. This type of excitability change would therefore have an important effect on the overall output of the cell. Such a non-specific regulation of excitability may be useful in keeping the neuron in a desired state of readiness to fire.

It will be of interest to investigate whether this form of E–S potentiation occurs *in vivo* and to perform further experiments to resolve the underlying mechanisms.

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