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Cooperative interactions in the induction of long-term potentiation and depression of synaptic excitation between hippocampal CA3–CA1 cell pairs *in vitro*

(learning/memory/hippocampus/synaptic plasticity)

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ABSTRACT The requirement for cooperative interactions between multiple synaptic inputs in the induction of long-term potentiation (LTP) and long-term depression (LTD) has been tested at Schaffer collateral synapses with paired recordings from monosynaptically coupled CA3–CA1 cell pairs in rat hippocampal slice cultures. Tetanization of single presynaptic neurons at 50 Hz (repeated 5–7 times for 300–500 ms each) induced only a transient potentiation (<3 min) of excitatory postsynaptic potentials (EPSPs). Persistent potentiation (>15 min) was induced only when single presynaptic action potentials were synchronously paired with directly induced postsynaptic depolarizing pulses (repeated 50–100 times). Tetanus-induced potentiation of extracellularly evoked EPSPs lasting >4 min could only be obtained if the EPSP was >4 mV. Because unitary EPSP amplitudes average ≈1 mV, we conclude that high-frequency discharge must occur synchronously in 4–5 CA3 cells for LTP to be induced in a common postsynaptic CA1 cell. Asynchronous pairing of presynaptic action potentials with postsynaptic depolarizing current pulses (preceding each EPSP by 800 ms) depressed both naive and previously potentiated unitary EPSPs. Likewise, homosynaptic LTD of unitary EPSPs was induced when the presynaptic cell was tetanized at 3 Hz for 3 min, regardless of their amplitude (0.3–3.2 mV). Homosynaptic LTD of extracellularly evoked Schaffer collateral EPSPs <4 mV could be induced if no inhibitory postsynaptic potential was apparent, but was prevented by eliciting a large inhibitory postsynaptic potential or by injection of hyperpolarizing current in the postsynaptic cell. We conclude that cooperative interactions among multiple excitatory inputs are not required for induction of homosynaptic LTD of unitary EPSPs.

Persistent changes in the strength of the synaptic connections between cells are likely to underlie learning and memory formation. Long-term potentiation (LTP) and long-term depression (LTD) of excitatory synaptic transmission are two alternative forms of synaptic plasticity, providing the best models of these cellular processes. The experimental protocols typically used to induce LTP and LTD, in particular the stimulation of large numbers of fibers to evoke compound synaptic responses, have proven invaluable for studying the fundamental mechanisms by which these phenomena are generated, but may have little relevance to the patterns of activity occurring in the brain during learning and memory.

The induction of LTP at Schaffer collateral synapses between hippocampal CA3 and CA1 cells requires *N*-methyl-D-aspartate (NMDA) receptor activation and elevation of the postsynaptic Ca^{2+} concentration (for review, see ref. 1). Associative and cooperative interactions among multiple synaptic inputs have been demonstrated for LTP induction (2–4), and are believed to reflect the requirement that the depolarization of the postsynaptic cell be sufficient to relieve the block of NMDA receptor-gated channels by Mg^{2+}. The number of presynaptic CA3 cells that must discharge synchronously to induce LTP of their synapses with a common postsynaptic CA1 cell is not known.

The associative induction of LTP at connections between pairs of hippocampal CA3 and CA1 neurons has been observed when low-frequency stimulation of the presynaptic neuron is paired with a maintained postsynaptic depolarization (5), but was not observed with high-frequency presynaptic tetani alone (6). The failure to observe tetanus-induced LTP in the latter study could result either because unitary inputs are simply too weak to engage the biochemical machinery responsible for potentiation or because of something intrinsic to the individual synapses studied, such as an inherent nonplasticity. To distinguish between these possibilities, the relative ability of tetanization and synchronous pairing to induce LTP must be compared on the same synaptic connections.

The induction of LTD in the CA1 region of the hippocampus also requires NMDA receptor activation and increases in postsynaptic Ca^{2+} (7–9). LTD can display associative properties (10), and it has recently been concluded that cooperative interactions are required for its induction (11). This conclusion is, however, difficult to reconcile with the successful induction of homosynaptic LTD when only one or a few synapses are stimulated extracellularly (12).

We have used dual intracellular recordings from pairs of monosynaptically connected neurons to address several questions. What patterns of activity in a single presynaptic neuron are able to induce changes in synaptic strength? When are cooperative interactions among afferent fibers required to induce LTP and LTD? How many cells must be conjunctively active to induce cooperative changes in synaptic strength? Organotypic hippocampal slice cultures are a favorable preparation with which to perform these experiments because monosynaptically coupled CA3–CA1 pyramidal cell pairs may be recorded with high probability (13) and the requirements for LTP and LTD induction in these cultures are identical to those in *ex vivo* hippocampal slices (9, 10). Furthermore, the amplitude of such unitary excitatory postsynaptic potentials (EPSPs) is more physiological than unitary EPSPs in dissociated hippocampal cell cultures (e.g., ref. 14).

MATERIALS AND METHODS

Hippocampal slice cultures were prepared and maintained as described (15). In brief, the hippocampi were dissected from 5- to

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Abbreviations: EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential; LTP, long-term potentiation; LTD, long-term depression; NMDA, *N*-methyl-D-aspartate.

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7-day-old rat pups that were decapitated. Hippocampi were then cut into 400-μm-thick slices that were then attached to glass coverslips in a chicken plasma clot. These coverslips were then placed in individual sealed test-tubes containing semisynthetic medium and maintained on a roller drum in an incubator for 2–4 weeks. After >14 days *in vitro*, cultures were transferred to a recording chamber mounted on an inverted microscope and were continuously superfused with a warmed (32°C) saline containing 149 mM Na^+, 149 mM Cl^-, 2.7 mM K^+, 2.8 mM Ca^{2+}, 2.0 mM Mg^{2+}, 11.6 mM HCO_3^-, 0.4 mM $H_2PO_4^-$, 5.6 mM glucose, and 10 mg/liter Phenol Red (pH = 7.4).

Pre- and postsynaptic cells were impaled in stratum pyramidale using 1 M potassium methylsulfate-filled sharp microelectrodes. EPSPs were recorded from postsynaptic CA1 pyramidal cells in response to single evoked action potentials in a presynaptic CA3 pyramidal cell. The criteria for establishing that EPSPs between cell pairs were monosynaptic included relatively short and invariant onset latencies, as described elsewhere (13). The input resistance of the postsynaptic cell was monitored continually during the experiment with short hyperpolarizing current pulses. Analog signals were digitized at 18 kHz and recorded on videotape. Off-line acquisition of 200–500 ms sequences was performed at a digitization rate of 8–10 kHz (ACQUIS1, DIPSI Industrie, Asnières, France). EPSP amplitudes were measured at fixed latency from the presynaptic action potential in each experiment. Monopolar stimuli (0.1 ms, −5 to −30 μA) were delivered from a patch pipette filled with extracellular saline.

Values of potentiation and depression were determined from averages of EPSPs over 2–3 min taken 10 min after the end of the pairing procedure or tetanus. Numerical values are given as mean ± SEM. Statistical comparisons were performed with the Mann–Whitney U test.

RESULTS

Induction of LTP Between Pairs of CA3 and CA1 Cells. The induction of LTP at unitary Schaffer collateral connections in hippocampal slice cultures was tested with two stimulation protocols that produce persistent LTP of extracellularly evoked EPSPs in hippocampal cultures (Fig. 1*A*). High-frequency tetanization of single presynaptic CA3 cells (50 Hz, repeated 3–7× at 0.3 Hz) (Fig. 1*B*), triggered by depolarizing current pulses (300–500 ms, 1–3 nA), was found to have no effect on the amplitude of the unitary EPSP recorded in a monosynaptically connected postsynaptic CA1 pyramidal cell, when measured >3 min after the tetanization (Fig. 1*C*). Pooled data (Fig. 1*E*) illustrate that only transient changes in unitary EPSP amplitude were observed after tetanization of single cells (102 ± 3% of the control amplitude 4 min after the tetanus, $n = 4$, $P > 0.5$). The mean membrane potential of the postsynaptic cells at the start of the tetani was −63 ± 1 mV. In the same pairs of neurons, however, 50–100 synchronous pairings between single presynaptic action potentials at low frequency (0.3 Hz) and a burst of 6–12 action potentials in the postsynaptic cell, triggered with current injection (240 ms duration, 0.5–2.5 nA), did induce persistent potentiation (Fig. 1*C*). Cooperative interactions among many synaptic inputs must therefore be required for induction of LTP by high-frequency discharge in single cells.

The number of presynaptic cells that must act cooperatively to induce LTP was estimated by examining the effects of 50 Hz tetanization on small compound EPSPs, evoked with extracellular stimulation within area CA3. If the compound EPSP in the postsynaptic CA1 cell was >4 mV, then either 50 Hz tetanization or synchronous pairing produced an increase in its amplitude lasting >10 min. High-frequency tetani (3–6 trains at 50 Hz, 300–400 ms duration repeated at 0.3 Hz) resulted in a potentiation of either the initial EPSP slope (to 164 ± 14% of control) or EPSP amplitude (to 149 ± 11% of control) that persisted for >20 min after induction ($n = 8$) (Fig. 1*A Left*). The mean membrane potential of the postsynaptic cells at the start of the tetani was −68 ± 2 mV. If the compound EPSP was <4 mV, however, then 50 Hz tetanization never produced an increase in its amplitude lasting >4 min (91 ± 10% of control amplitude, $n = 3$). Taken together with the mean amplitude of the unitary CA3–CA1 EPSPs that failed to show LTP in response to tetanization (0.8 ± 0.3 mV, $n = 4$), we estimate that 4–5 presynaptic CA3 cells must discharge in near synchrony to induce LTP of their synapses with a common postsynaptic CA1 cell.

The amount of potentiation of unitary EPSPs produced by the synchronous pairing procedure was to 269 ± 35% of the control amplitude ($n = 20$, Fig. 1*E*), and this potentiation persisted for as long as the impalements were maintained (Fig. 1*D*). Synchronous pairing of extracellular stimuli within area CA1 at 0.3 Hz and depolarizing current pulses in the postsynaptic cell also resulted in a potentiation of the amplitude of the compound EPSP that persisted for >20 min after induction (Fig. 1*A Right*). The amount of potentiation displayed by compound EPSPs (173 ± 20% of control, $n = 36$) was less than that expressed by unitary EPSPs, however.

Depression of Naive Unitary CA3–CA1 EPSPs. Associative LTD of extracellularly evoked EPSPs is induced when postsynaptic depolarization precedes presynaptic activity by 0.5–2 sec (9). We have tested whether associative LTD of unitary EPSPs could also be induced by asynchronous pairing of a unitary EPSP with a preceding (800 ms) burst of 6–12 action potentials in the postsynaptic cell (Fig. 2*A*). This asynchronous pairing protocol was found to result in a decrease in the amplitude of the unitary EPSP to 66 ± 13% of the control amplitude which persisted >15 min ($n = 5$) (Fig. 2*B*). No correlation was observed between the initial unitary EPSP amplitude and the magnitude of the depression ($r^2 = 0.19$; data not shown).

If homosynaptic LTD induction requires cooperative interactions (11), then LTD of unitary EPSPs should not be induced when the presynaptic cell is tetanized at low frequency. We found that a train of action potentials at low frequency (3 Hz for 3 min) in a single presynaptic CA3 pyramidal cell was, however, able to induce a persistent depression in the amplitude of the unitary EPSP in a monosynaptically connected postsynaptic CA1 cell (Fig. 2 *C* and *D*). The mean depression was to 60 ± 10% of the control amplitude ($n = 8$). LTD could also be induced with small unitary EPSPs (mean amplitude = 0.55 ± 0.07 mV) when single action potentials in the presynaptic cell were elicited at 1 Hz for 10 min (47 ± 15% of the control amplitude, $n = 3$). If cooperativity among multiple release sites was underlying the induction of LTD in these experiments, then the magnitude of the LTD should be greater for large unitary EPSPs, which have a larger quantal content than smaller EPSPs, probably because the axon forms more sites of synaptic contact with the postsynaptic cell (see ref. 16). No such correlation between the initial EPSP amplitude and the magnitude of the depression was found ($r^2 = 0.13$; Fig. 3*A*), however, suggesting that the induction of homosynaptic LTD is independent of the number of synaptic contacts formed by the axon of a cell with its postsynaptic target.

Tetanization at 3 Hz failed to elicit homosynaptic LTD of extracellularly evoked EPSPs of moderate amplitude (4–5 mV) when large IPSPs were also elicited by the stimulus (mean change = −7 ± 5%, $n = 3$), as illustrated in Fig. 3*B*. In the same experiments, however, a reduction in the stimulus intensity, so that EPSPs of ≈3 mV in amplitude that lacked a concomitant IPSP component were obtained, permitted another 3 Hz tetanus to successfully induce LTD (Fig. 3*B*). These results suggest that postsynaptic hyperpolarization during an IPSP can prevent the induction of LTD. We therefore tested whether hyperpolarizing current injection in the postsynaptic cell during the 3 Hz tetanus could prevent induction of LTD. Indeed, when the 3 Hz tetanus was delivered during postsynaptic hyperpolarization to −90 mV, only a weak depression of

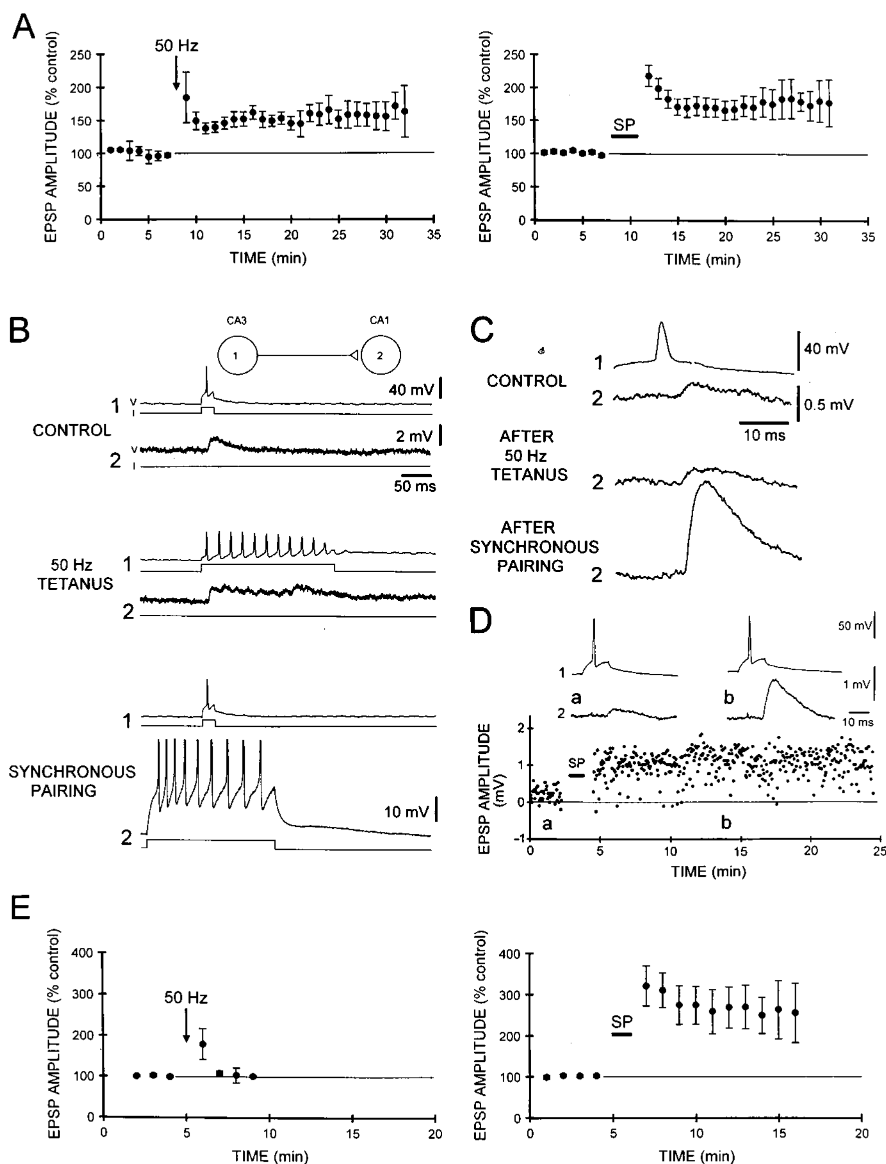

FIG. 1. Induction of LTP at CA3–CA1 cell synapses. (*A*) LTP of compound EPSPs was induced by 50 Hz tetanization (*Left*) or by synchronous pairing (*Right*). Normalized EPSP amplitudes in CA1 pyramidal cells in response to extracellular stimulation within area CA3 were averaged each minute. Stable potentiation of EPSPs lasting >20 min was induced either with a 50 Hz tetanus ($n = 8$), repeated 3–6 times, or with the synchronous pairing (SP) of postsynaptic depolarization and synaptic activity, repeated 50–100 times at 0.3 Hz ($n = 36$). (*B*) Protocols for inducing LTP of unitary Schaffer collateral EPSPs. The control unitary EPSP amplitude in an intracellularly recorded CA1 cell (2) was determined with single action potentials in a presynaptic CA3 cell (1), elicited with brief depolarizing pulses (10–30 ms duration) at 0.3 Hz. High-frequency tetanization of the presynaptic cell was produced with 3–6 depolarizing current pulses (240 ms duration) that triggered discharge at ≈50 Hz. Associative LTP induction was tested with a synchronous pairing of single presynaptic action potentials at 0.3 Hz and a depolarizing current pulse (240 ms) in the postsynaptic cell, repeated 50–100×. (*C*) A presynaptic action potential initially elicited a 0.2 mV EPSP in the postsynaptic cell. No significant change in EPSP amplitude was observed 4 min after five 50 Hz tetani in the presynaptic cell. The same synapse exhibit a 500% potentiation, however, following 30 synchronous pairings. (*D*) Stable potentiation of a unitary EPSP in another cell pair lasting >20 min after induction with the synchronous pairing protocol. (*E*) Pooled data illustrating the transient and persistent potentiation of unitary EPSPs induced with 50 Hz tetanization ($n = 4$ cell pairs) or the synchronous pairing procedure ($n = 20$ cell pairs).

unitary CA3–CA1 EPSPs occurred ($n = 3$; Fig. 3 *C*, *D a* and *b*, and *E*), as reported previously for compound EPSPs (8). Robust LTD was, however, induced in the same pairs when the 3 Hz tetanus was repeated without hyperpolarizing current injection ($n = 3$; Fig. 3 *C*, *D b* and *c*, *E*).

We conclude that naive synapses between single cells can be either homosynaptically or associatively depressed. Furthermore, cooperative interactions are not required for induction of homosynaptic LTD.

Depotentiation of Unitary CA3–CA1 EPSPs. We also investigated whether the cooperativity requirements for the induction of decreases in synaptic strength were different for previously potentiated synapses than for naive synapses. Potentiation of unitary EPSPs was first induced with synchronous pairing (as in Fig. 1*B*) and allowed to stabilize for 7–15 min. Associative or homosynaptic LTD induction protocols (as in Fig. 2) were then used in an attempt to reverse this potentiation. Asynchronous pairing (as in Fig. 2) produced a depotentiation of previously potentiated unitary EPSPs (Fig. 4*A*). The depotentiation of unitary EPSP amplitudes ($-32 \pm 9\%$, $n = 4$) was comparable to that observed with compound EPSPs using this protocol ($-41 \pm 6\%$, $n = 7$; data not shown). Tetanization (3 Hz) of individual presynaptic cells also resulted in a persistent decrease in the amplitude of the previously potentiated unitary

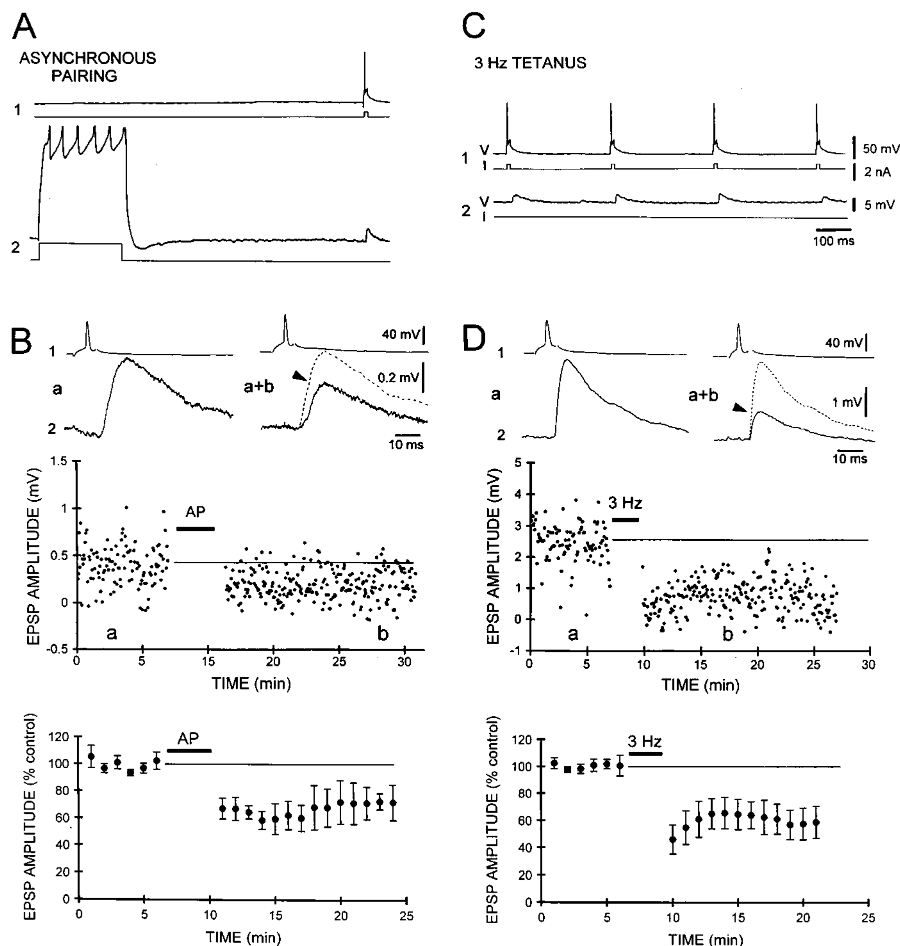

FIG. 2. LTD of naive CA3–CA1 unitary EPSPs. (*A*) Associative LTD of unitary CA3–CA1 EPSPs was induced with asynchronous pairing (AP) of single action potentials in a presynaptic cell (1) and a preceding (by 800 ms) depolarizing current pulse in the postsynaptic cell (2). (*B Upper*) Asynchronous pairings ($n = 100$) at 0.3 Hz induced a 45% depression of unitary EPSP amplitude persisting >15 min. Averaged traces from the times indicated are shown above. (*Lower*) Pooled data illustrating the effects of the asynchronous pairing protocol on unitary EPSP amplitude (n = 5 cell pairs). (*C*) Homosynaptic LTD of unitary EPSPs in a CA1 cell (2) was induced by tetanization of a single presynaptic CA3 neuron (1) at 3 Hz for 3 min. (*D Upper*) Tetanization at 3 Hz induced a 60% depression of unitary EPSP amplitude persisting >15 min. Averaged traces from the times indicated in the graph are shown above. (*Lower*) Pooled data illustrating the effects of 3 Hz tetanization on unitary EPSP amplitude (n = 8 cell pairs). (Scaling for A and C is the same.)

EPSPs (Fig. 4*B*). The depotentiation of unitary EPSP amplitudes ($-43 \pm 7\%, n = 3$) was comparable in size to that observed with compound EPSPs using this protocol ($-40 \pm 2\%, n = 4$; data not shown). We conclude that the strength of previously potentiated synapses can be reduced by either moderate frequencies of discharge in the presynaptic cell or through associative interactions (see ref. 10).

DISCUSSION

Cooperative Interactions Are Required for Induction of LTP. LTP of unitary EPSPs could not be induced with high-frequency tetanization of a single CA3 cell in our experiments, consistent with results in *ex vivo* hippocampal slices (6). The failure of this pattern of presynaptic activity to induce potentiation cannot be attributed to an inability of these synapses to express LTP, as suggested previously (6), because synchronous pairing of the same synapses reliably induced a persistent increase in the unitary EPSP amplitude. We conclude that tetanization of a single cell fails to induce LTP because unitary EPSPs are insufficient for the induction of LTP. Presumably, the depolarization produced by a unitary EPSP fails to relieve the Mg^{2+} block of the NMDA receptor-gated ion channel sufficiently. When the necessary postsynaptic depolarization was provided by current injection from the recording electrode, however, LTP of unitary EPSPs was readily induced, probably because the coincident backpropagation of action potentials into the dendritic tree directly relieves the Mg^{2+} block.

We can therefore infer that cooperative interactions between several presynaptic cells are required to produce a lasting potentiation of their synapses with a common postsynaptic cell, at least under the conditions of our experiments. We attempted to estimate the number of presynaptic cells that must be simultaneously active to induce LTP by examining the effects of 50 Hz tetanization on small, extracellularly evoked compound EPSPs. Potentiation of these compound EPSPs persisting >4 min was only obtained when the compound EPSP had an amplitude >4 mV. Previously reported data from *ex vivo* hippocampal slices are consistent with this conclusion (4, 6). Unitary Schaffer collateral EPSPs in hippocampal slice cultures have a mean amplitude of ≈1 mV (13). Assuming linear summation of unitary EPSPs, we can suggest that the nearly synchronous discharge of 4–5 cells is required to produce a persistent associative strengthening of their synapses.

This conclusion must be qualified in two potentially significant ways. First, the amount of NMDA receptor-mediated Ca^{2+} influx produced by a unitary EPSP will be a function of both the membrane potential of the postsynaptic cell and the extracellular Ca^{2+}/Mg^{2+} ratio. It is difficult to ascertain their

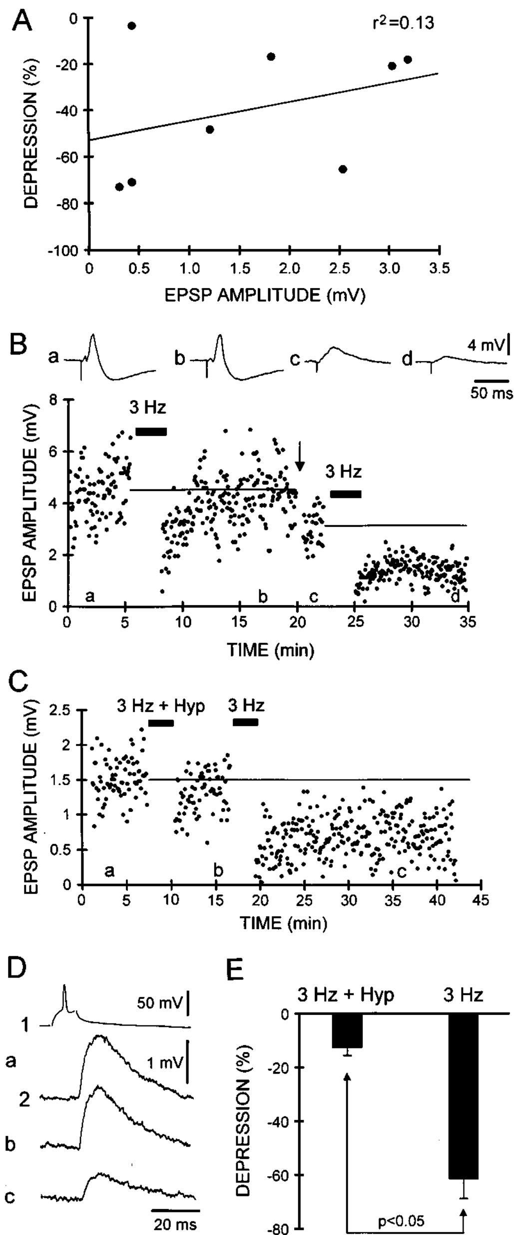

FIG. 3. Are cooperative interactions required for the induction of homosynaptic LTD? (*A*) The magnitude of LTD induced with 3 Hz tetanization was not correlated with the amplitude of the unitary CA3–CA1 EPSP (linear regression, $r^2 = 0.13$). (*B*) Large inhibitory postsynaptic potentials (IPSPs) prevented homosynaptic LTD induction. (*a*) A stimulating electrode was placed in stratum radiatum of area CA1, and the stimulus intensity was adjusted to elicit a small EPSP with a prominent IPSP. (*b*) This compound EPSP was only transiently depressed following a 3 Hz tetanus. (*c*) The stimulus intensity was then decreased (arrow) to evoke a smaller EPSP without an apparent IPSP. (*d*) Significant persistent depression was then induced following the 3 Hz tetanus. (*C* and *D*). No significant depression a unitary EPSP was induced when the postsynaptic cell was hyperpolarized to −90 mV during the 3 Hz tetanus (3 Hz + Hyp, *a* and *b*). (*c*) Significant depression was induced, however, when the tetanus was repeated without hyperpolarizing current injection (3 Hz). (*E*) Pooled data from three experiments as in *C* and *D*. The depression induced in the absence of postsynaptic hyperpolarization (3 Hz) was significantly greater than that induced during postsynaptic hyperpolarization (paired Student's *t* test, $P < 0.05$, $n = 3$).

exact values in the brain, but we believe the conditions of our experiments to be reasonably physiological (−62 mV, Ca^{2+}/Mg^{2+} ratio = 1.4). Second, learning and memory in the hippocampus *in vivo* are closely associated with the occurrence of theta rhythm oscillations (e.g., ref. 17), which are driven by cholinergic and extrinsic GABAergic inputs that are absent in our cultures. Activation of cholinergic receptors in the hippocampus affects presynaptic glutamate release, postsynaptic membrane potential, and the induction of LTP and LTD (18).

Cooperative Interactions Are Not Required for Induction of LTD. LTD of naive synapses could be induced reliably when either a single presynaptic cell was repetitively activated at 1–3 Hz or an asynchronous pairing procedure was used. Furthermore, the magnitude of LTD was not correlated with the initial EPSP amplitude. Depotentiation of previously potentiated synapses was also induced with both stimulation protocols. We therefore conclude that cooperative interactions are not required for the induction of LTD and depotentiation. In contrast, Kerr and Abraham (11) have recently suggested that induction of LTD in the hippocampus requires cooperative interactions among inputs. The discrepancy between their conclusion and ours could arise from the differences in the amount of synaptic inhibition recruited by extracellular and single cell stimulation. Kerr and Abraham (11) found that cooperativity was less critical when γ-aminobutyric acid type A receptors were blocked, probably because the IPSP hyperpolarizes the postsynaptic cell at the time when NMDA receptor-gated ion channel is most likely to be open, thus reducing or eliminating synaptic Ca^{2+} influx (19, 20). Consistent with this idea, we found that LTD of small EPSPs could not be induced when large IPSPs were elicited by the extracellular stimulus or when the postsynaptic cell was held at a hyperpolarized membrane potential during the tetanus. In contrast, unitary EPSPs and small compound EPSPs that lacked concomitant disynaptic IPSPs expressed robust homosynaptic LTD. An increase in the depolarization of the postsynaptic cell mediated by cooperative interactions between multiple inputs can apparently overcome the blocking effect of synaptic inhibition, and thus permit LTD induction under certain conditions.

The NMDA receptor-mediated component of EPSPs in CA1 pyramidal cells in response to single action potentials in CA3 cells appears to permit an influx of Ca^{2+} that is sufficient to trigger the cascade of biochemical events leading to induction of LTD, at least in hippocampal slice cultures (13). Because there is no tonic depolarization of the postsynaptic cell during 3 Hz tetanization of single presynaptic cells, a significant amount of NMDA receptor-mediated Ca^{2+} must occur near the resting membrane potential in the presence of a physiological extracellular Ca^{2+}/Mg^{2+} ratio, in contrast to common assumptions. The need for tetanization at frequencies of 1–5 Hz for the induction of LTD (5) therefore indicates that this small amount of Ca^{2+} influx must be temporally summated to engage the biochemical machinery producing the ultimate decrease in synaptic strength. The associative induction of LTD, in contrast, reflects the summation of voltage-dependent and NMDA receptor-mediated Ca^{2+} influx (9). Our observation that LTP is not induced by the even greater temporal summation of postsynaptic Ca^{2+} likely to be produced by high-frequency tetanization of a single CA3 cell provides additional evidence that a much larger elevation of the postsynaptic Ca^{2+} concentration must be achieved for the induction of LTP than for LTD.

The existence of bidirectional forms of plasticity permits synaptic gain to be reset after a synapse has been potentiated or depressed, thereby preventing saturation of the LTP mechanism (21), allowing further plasticity (22), and optimizing memory formation, at least in neural networks (23). The depotentiation of previously potentiated unitary EPSPs, in which the stimulation of a relatively small number of synaptic contacts can be well controlled, is consistent with previous observations of extracellularly evoked compound EPSPs (6, 7, 21) and offers additional persuasive evidence that a given synapse can undergo reversible changes in its synaptic strength.

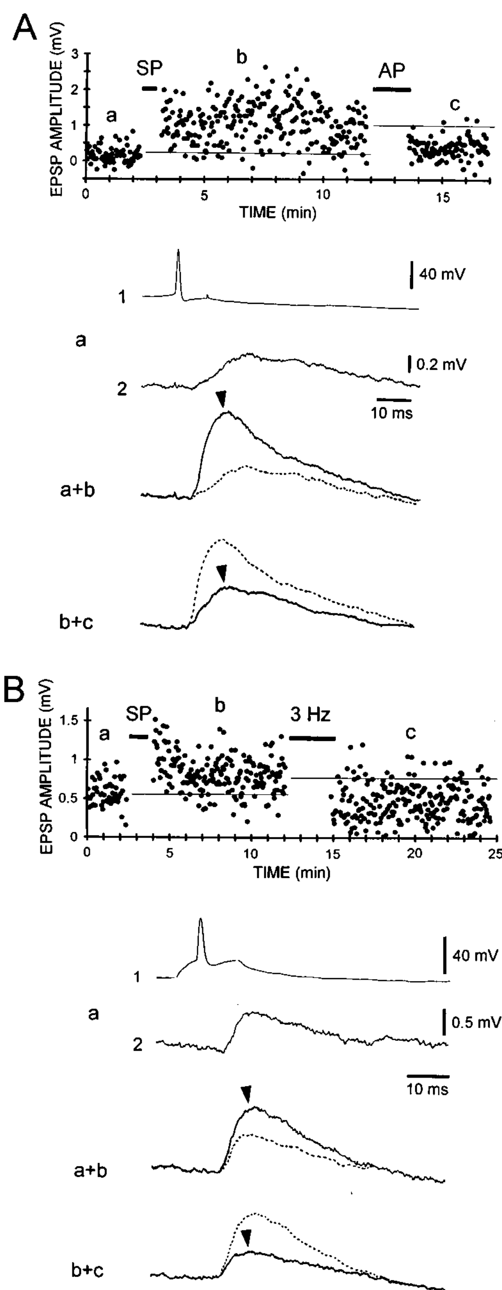

FIG. 4. Associative and homosynaptic depotentiation of unitary EPSPs. (*A*) Potentiation was induced by synchronous pairing (SP, *a* + *b*). After stabilization of the potentiation, asynchronous pairing (AP) between pre- and postsynaptic activity as in Fig. 2*A* (100 repetitions) resulted in a 50% depression of EPSP amplitude (*b* + *c*). (*B*) After potentiation with the SP procedure (a+b), single action potentials were elicited in the presynaptic cell at 3 Hz for 3 min, resulting in a 55% depression of EPSP amplitude (*b* +*c*).

Our results indicate that action potential discharge in a single cell is insufficient to produce a level of postsynaptic depolarization necessary for induction of LTP under normal conditions, but is capable of inducing LTD. This conclusion has two important implications. First, since hippocampal cells in behaving animals often discharge at frequencies that our results predict are sufficient for LTD induction, the strength of hippocampal synapses *in vivo* may normally be maintained in a relatively weak state. If so, then it can be expected that potentiation will be more difficult to induce, because weak synapses will be less effective at producing a sufficient postsynaptic depolarization. On the other hand, the signal-to-noise ratio of a synapse that has successfully become potentiated will be greater if other synapses are "depressed." Second, the persistence of LTP at Schaffer collateral synapses in freely behaving animals might be less than would be expected on the basis of typical *in vitro* LTP experiments. If 3 Hz discharge for 3 min is common, then previously potentiated synapses are likely to become depotentiated relatively rapidly.

The sign and magnitude of activity-dependent modifications of synaptic strength are a function of the activity of the postsynaptic cell, and this relationship has been described mathematically (24) and verified experimentally (7). We have found that the excitatory synapses between individual CA3 and CA1 cells are unable to produce a sufficiently large depolarization of the postsynaptic cell to exceed the threshold level of activity required for induction of LTP. This threshold level is not fixed, however, but is subject to important regulatory and developmental controls (25, 26). It will now be of interest to examine whether changes in the threshold for LTP induction produce changes in the amount of cooperativity required.

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