28. T. W. Hansch and A. L. Schawlow, *Opt. Commun.* **13**, 68 (1975); U. S. Letokhow, *Comments At. Mol. Phys.* **6**, 119 (1977).
29. D. J. Wineland and W. M. Itano, *Phys. Today* **40** (no. 6), 34 (June 1987).
30. D. Kleppner, N. F. Ramsey, P. Fjelstadt, *Phys. Rev. Lett.* **1**, 232 (1958).
31. J. P. Gordon, H. Z. Geiger, C. H. Townes, *Phys. Rev.* **95**, 282 (1954); *ibid.* **99**, 1264 (1955).
32. H. M. Goldenberg, D. Kleppner, N. F. Ramsey, *Phys. Rev. Lett.* **5**, 361 (1960); *Phys. Rev.* **126**, 603 (1962).
33. D. Kleppner *et al.*, *ibid.* **138**, A972 (1965).
34. J. M. V. A. Koelman, S. B. Crampton, H. T. C. Luiten, B. J. Verhaar, *Phys. Rev. A* **38**, 3535 (1988). This paper contains an extended series of references to other papers on hydrogen maser limitations, principles, and practices.
35. R. R. Freeman, A. R. Jacobson, D. W. Johnson, N. F. Ramsey, *J. Chem. Phys.* **63**, 2597 (1975).
36. R. V. Reid and M. L. Vaida, *Phys. Rev. A* **7**, 1841 (1973).
37. K. K. Docken and R. R. Freeman, *J. Chem. Phys.* **61**, 4217 (1974).
38. G. L. Green *et al.*, *Phys. Rev. D* **20**, 2139 (1979).
39. E. R. Cohen and B. Taylor, *Rev. Mod. Phys.* **59**, 1121 (1987).
40. K. F. Smith *et al.*, *Phys. Lett. B* **136**, 327 (1984); *ibid.* **234**, 191 (1990).
41. H. Hellwig *et al.*, *IEEE Trans. Instrum. Meas.* **IM-19**, 200 (1970).
42. L. Essen, M. J. Donaldson, M. J. Bangham, E. G. Hope, *Nature* **229**, 110 (1971).
43. G. L. Baldwin and D. R. Yennie, *Phys. Rev. D* **37**, 498 (1988).
44. P. C. Gibbons and N. F. Ramsey, *Phys. Rev. A* **5**, 73 (1972).
45. P. F. Winkler, D. Kleppner, T. Myint, F. G. Walther, *ibid.* **5**, 83 (1972); E. Cohen and B. Taylor, *Phys. Lett. B* **204**, 1 (1988).
46. R. S. vanDyck, P. B. Schwinberg, H. Dehmelt, *At. Phys.* **9**, 53 (1984).
47. R. S. vanDyck, F. L. Moore, D. L. Farnum, P. B. Schwinberg, *Bull. Am. Phys. Soc.* **31**, 244 (1986); *At. Phys.* **9**, 75 (1984).
48. J. Taylor *et al.*, *Nature* **277**, 437 (1979); *ibid.* **315**, 547 (1985).
49. R. F. C. Vessot *et al.*, *Phys. Rev. Lett.* **45**, 2081 (1980).

Research Articles

Comparison of Two Forms of Long-Term Potentiation in Single Hippocampal Neurons

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In invertebrate nervous systems, some long-lasting increases in synaptic efficacy result from changes in the presynaptic cell. In the vertebrate nervous system, the best understood long-lasting change in synaptic strength is long-term potentiation (LTP) in the CA1 region of the hippocampus. Here the process is initiated postsynaptically, but the site of the persistent change is unresolved. Single CA3 hippocampal pyramidal cells receive excitatory inputs from associational-commissural fibers and from the mossy fibers of dentate granule cells and both pathways exhibit LTP. Although the induction of associational-commissural LTP requires in the postsynaptic cell *N*-methyl-D-aspartate (NMDA) receptor activation, membrane depolarization, and a rise in calcium, mossy fiber LTP does not. Paired-pulse facilitation, which is an index of increased transmitter release, is unaltered during associational-commissural LTP but is reduced during mossy fiber LTP. Thus, both the induction and the persistent change may be presynaptic in mossy fiber LTP but not in associational-commissural LTP.

REPETITIVE ACTIVATION OF SOME TYPES OF EXCITATORY synapses results in a long-lasting enhancement of synaptic transmission referred to as long-term potentiation (LTP). This use-dependent plasticity provides a useful cellular model for learning and memory. Our understanding of the cellular mechanisms of LTP comes largely from investigations of the CA1 and dentate regions of the hippocampus (*1, 2*) although a similar phenomenon occurs at several synapses in the brain (*3*). The current model for the induction of LTP requires simultaneous *N*-methyl-D-aspartate (NMDA) receptor activation and postsynaptic depolarization. The depolarization relieves a voltage dependent Mg^{2+} block of the NMDA receptor channel, and entry of Ca^{2+} through the NMDA receptor channel appears to serve as the trigger for LTP.

In the CA3 region of the hippocampus, pyramidal cells receive two anatomically distinct excitatory synaptic inputs (*4*), an associational-commissural (assoc-com) input and a mossy fiber input (Fig. 1A). The mossy fibers are the axons of dentate granule cells, and they form en passant synapses that are restricted to a narrow band (the stratum lucidum) on the proximal dendrites of CA3 pyramidal cells. The mossy fiber inputs are both anatomically and electrotonically closer to the soma than the assoc-com inputs, which are on more distal dendrites of the CA3 cells (*5*).

Both mossy fiber (*6, 7*) and assoc-com synapses (*7*) exhibit LTP, but unlike the assoc-com pathway very few NMDA receptors are associated with the mossy fiber synapses (*8*). Harris and Cotman (*7*) demonstrated that LTP, recorded extracellularly, was blocked by NMDA receptor antagonists in the assoc-com but not the mossy fiber pathway. We have taken advantage of this convergence of inputs to compare in single pyramidal cells the cellular mechanisms of LTP in the two pathways (*9–12*).

We used standard procedures to prepare and maintain guinea pig hippocampal slices (*13*). In most experiments one stimulating electrode was positioned in the granule cell layer of the dentate gyrus to activate mossy fibers, and another electrode was placed in the stratum radiatum to activate assoc-com fibers (Fig. 1A). Two recording electrodes were generally used: one for intracellular or whole cell recording from the cell body of a CA3 pyramidal cell, and the other for recording the extracellular assoc-com excitatory postsynaptic potentials (field EPSP's).

Brief high frequency stimulation (tetanus) of mossy fiber and assoc-com pathways produced long-lasting potentiation of the synaptic inputs. We determined the time course of this LTP in the assoc-com and mossy fiber pathways in the same group of cells (Fig. 1B). In both pathways potentiation generally declined for about 10

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to 15 minutes and then usually remained stable at 1 hour after the tetanus.

Mechanisms of LTP induction. We have confirmed the results of Harris and Cotman (7) by measuring the initial slope of the two types of EPSP's from the same cells with intracellular recordings and shown that assoc-com, but not mossy fiber LTP, is blocked by NMDA receptor antagonists. The mossy fiber LTP recorded in a group of cells in the presence of the NMDA antagonist 2-amino-5-phosphonovalerate (APV) was indistinguishable from that in a control group of cells without the antagonist (Fig. 1C). However, LTP in the assoc-com inputs in the same cells was completely blocked by APV (Fig. 1C). The assoc-com pathway in a control group of cells under the same conditions showed reliable LTP. (In eight of eight cells the increase was greater than 50 percent 10 minutes after tetanus). After removal of the antagonist, LTP was observed in four cells in the assoc-com pathway.

In CA1 neurons, chelating postsynaptic Ca^{2+} blocks LTP, and it has been proposed that it is Ca^{2+} entry through NMDA channels that triggers LTP (*14*). Although NMDA receptors are not required for mossy fiber LTP, postsynaptic Ca^{2+} might still play a critical role. To examine the role of postsynaptic Ca^{2+} concentration in assoc-com and mossy fiber LTP, we injected the CA3 neurons with high concentrations of the Ca^{2+} chelator 1, 2-bis(*o*-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA) (*15*) through low resistance intracellular electrodes. This chelator rapidly binds intracellular free Ca^{2+}. Cells were injected with BAPTA for 20 to 50 minutes before the LTP inducing stimuli were applied. As was expected from the results from CA1 cells (*14*), the NMDA receptor–dependent form of LTP was blocked by the Ca^{2+} chelator in the postsynaptic cell (Fig. 2A). To ensure that the assoc-com pathway in these experiments was, indeed, capable of generating LTP, we simultaneously recorded the extracellular field potential from neighboring neurons and these showed robust LTP (Fig. 2B). In contrast, BAPTA had no apparent effect on mossy fiber LTP in the same cells (Fig. 2A) even though the LTP inducing stimulus given to the mossy fiber pathway was only half that given to the assoc-com pathway and the mossy fiber synapses are morphologically and electrotonically closer to the recording and injection electrode. We obtained similar results in another group of cells in the presence of APV there was a greater than 50 percent increase in EPSP slope at 10 minutes after tetanus in all assoc-com inputs and seven out of eight mossy fiber inputs. In APV there was a greater than 20 percent increase in zero of seven of the assoc-com inputs and a greater than 50 percent increase in all mossy fiber inputs. In this and subsequent figures the upper part of the error bar on some points immediately after a tetanus is not shown.

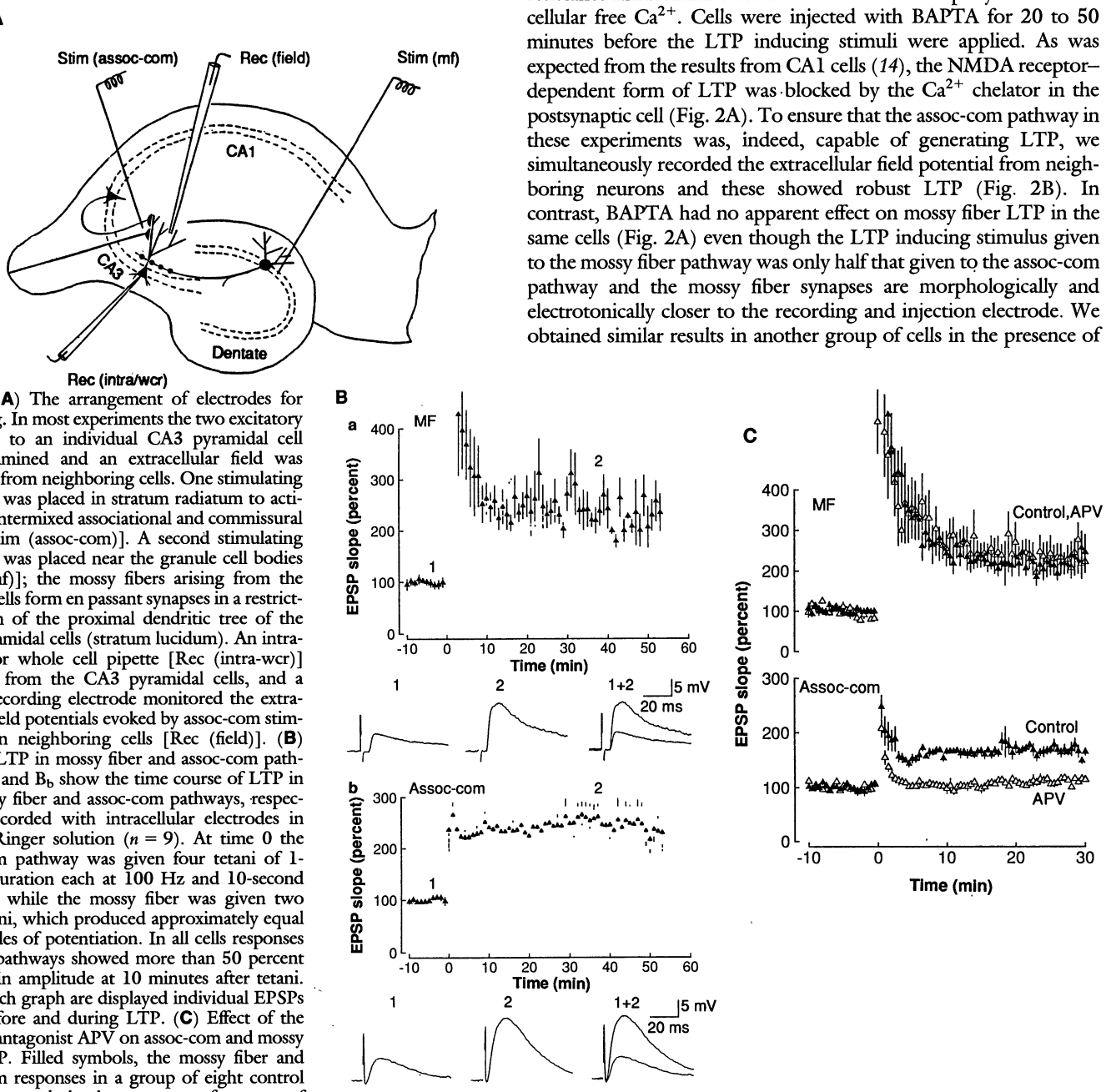

Fig. 1. (**A**) The arrangement of electrodes for recording. In most experiments the two excitatory pathways to an individual CA3 pyramidal cell were examined and an extracellular field was recorded from neighboring cells. One stimulating electrode was placed in stratum radiatum to activate the intermixed associational and commissural fibers [Stim (assoc-com)]. A second stimulating electrode was placed near the granule cell bodies [Stim (mf)]; the mossy fibers arising from the granule cells form en passant synapses in a restricted region of the proximal dendritic tree of the CA3 pyramidal cells (stratum lucidum). An intracellular or whole cell pipette [Rec (intra-wcr)] recorded from the CA3 pyramidal cells, and a second recording electrode monitored the extracellular field potentials evoked by assoc-com stimulation in neighboring cells [Rec (field)]. (**B**) Normal LTP in mossy fiber and assoc-com pathways. B_a and B_b show the time course of LTP in the mossy fiber and assoc-com pathways, respectively, recorded with intracellular electrodes in normal Ringer solution ($n = 9$). At time 0 the assoc-com pathway was given four tetani of 1-second duration each at 100 Hz and 10-second intervals, while the mossy fiber was given two such tetani, which produced approximately equal magnitudes of potentiation. In all cells responses of both pathways showed more than 50 percent increase in amplitude at 10 minutes after tetani. Below each graph are displayed individual EPSPs taken before and during LTP. (**C**) Effect of the NMDA antagonist APV on assoc-com and mossy fiber LTP. Filled symbols, the mossy fiber and assoc-com responses in a group of eight control cells; open symbols, the responses of a group of seven cells in the presence of APV. In this and all other experiments with APV, a concentration of 25 μM D-APV or 50 μM D,L-APV was applied for at least 6 minutes. All recordings in (C) were in the presence of the $GABA_A$ antagonist picrotoxin and 3 M CsCl in the recording electrodes; two 1-second duration, 100-Hz tetani were administered to induce LTP. The use of two tetani of stimuli instead of four accounts for the reduced magnitude of assoc-com LTP [compare to (B_b)] in control conditions. In the absence of

the γ-aminobutyric acid (GABA) antagonist picrotoxin (n = 9). When we combined experiments with and without picrotoxin, at 20 minutes after tetanus the potentiation in BAPTA-filled cells was 211 ± 24 percent and in control cells was 236 ± 23 percent. These values are not significantly different (*16*).

In LTP of CA1 cells the requirement for postsynaptic depolarization (*17, 18*) is functionally important; it provides the cellular basis for the cooperativity and associativity of LTP. Cooperativity is the requirement that a minimum number of afferent fibers be coactivated to induce LTP. When these fibers are activated at different sites, the phenomenon is referred to as associativity. Although our results with BAPTA suggest that postsynaptic Ca^{2+} does not affect mossy fiber LTP, they do not rule out a role for postsynaptic depolarization in the initiation of mossy fiber LTP. We have therefore examined the effects of manipulating postsynaptic membrane potential on the induction of LTP. In CA1, pairing of low frequency test stimuli with postsynaptic depolarization is sufficient to produce LTP (*17*). This procedure produced LTP in the assoc-com pathway, as expected, but not in the mossy fiber pathway in the same cells (n = 4) (Fig. 3A) (*19*). Pairing of test stimuli with dc currents, brief pulses, or Ca^{2+} spikes (in cesium-loaded cells) never produced LTP in the mossy fiber pathway. In addition, intense repetitive Ca^{2+} spiking (in cesium-loaded cells) in the postsynaptic cell had no effect on mossy fiber EPSP's.

Although pairing of stimuli with depolarization did produce LTP in the assoc-com pathway, the effect was not as strong as in CA1; in particular, more pairings were necessary and the procedure did not always produce strong potentiation. However, brief repetitive stimuli consistently revealed a voltage dependence in the assoc-com pathway. Groups of five stimuli (100 Hz) given without postsynaptic membrane depolarization did not induce LTP; the same stimuli presented with membrane depolarization did produce LTP in the assoc-com pathway (five of five cells) (Fig. 3B). No dependence on membrane potential was observed in the mossy fiber pathway (*20*). Finally, we attempted to reveal an effect of postsynaptic membrane potential on mossy fiber LTP by applying grouped stimuli in which the first presentation was accompanied by strong hyperpolarizing current (or voltage clamp at a hyperpolarized level) and the second presentation was accompanied by depolarizing current. Again, this procedure demonstrated the voltage dependence of assoc-com LTP (five of five cells), but no effect was seen on mossy fiber LTP (Fig. 3C). The number of stimuli necessary for producing LTP in the mossy fiber pathway was variable, but in no case did a stimulus

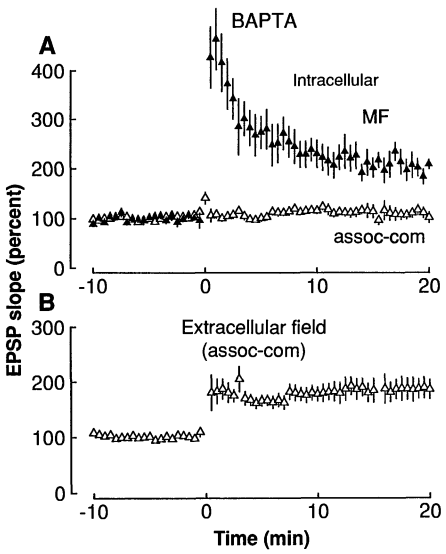

Fig. 2. Effect of chelation of postsynaptic Ca^{2+} in the assoc-com and mossy fiber (MF) pathway. Intracellular electrodes containing 200 mM BAPTA and 2 M potassium methyl sulfate were used to fill cells for from 20 to 50 minutes before attempting to evoke LTP in normal Ringer solution (n = 10). The mossy fiber synapse (filled symbols) showed normal LTP (ten of ten cells showed greater than 50 percent increase at 10 minutes after tetanus), while the assoc-com (open symbols) inputs to the same cells failed to show LTP [eight of ten cells showed less than 20 percent increase at 10 minutes after tetanus compared with zero of ten cells in controls (Fig. 1B)] despite receiving twice as many tetani as the mossy fiber pathway (four versus two as in Fig. 1B). (**B**) Extracellular field potentials from the assoc-com pathway recorded from neighboring cells demonstrated that the assoc-com pathway was capable of LTP in these slices (ten of ten cells showed greater than 40 percent increase 10 minutes after tetanus).

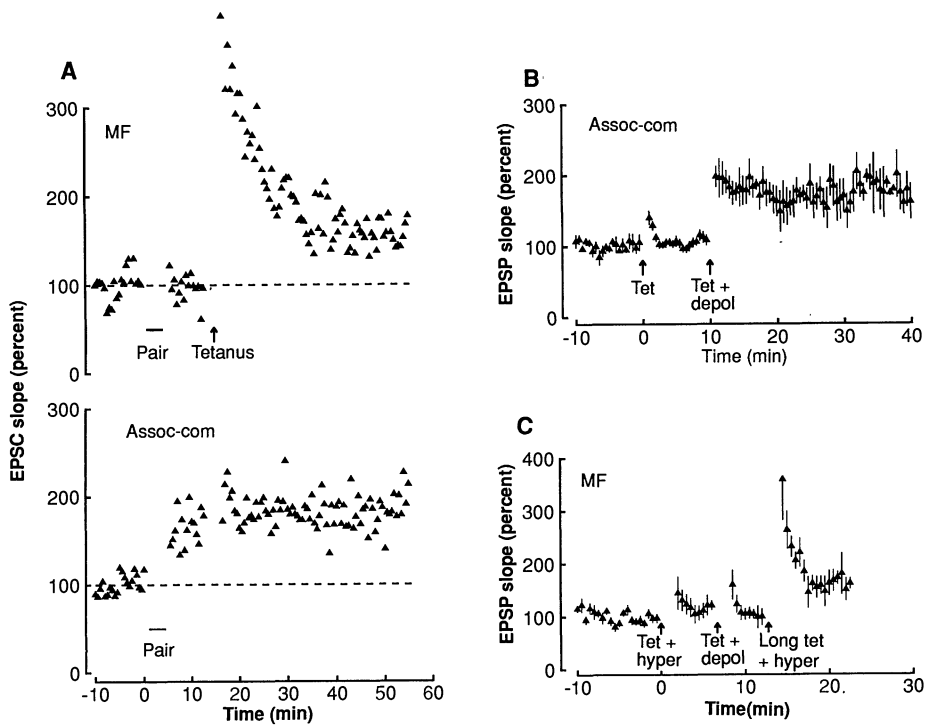

Fig. 3. Effect of postsynaptic membrane potential on the induction of LTP in the assoc-com and the mossy fiber (MF) pathways. (**A**) The result from a single cell in which pairing of depolarization (+1 nA, 40 pairings) with the normal 0.1-Hz test stimuli produced LTP in the assoc-com but not the mossy fiber pathway. Tetani to the mossy fiber pathway (two tetani each of 1-second duration and 100 Hz at 10-second intervals) in this cell (at arrow) subsequently produced normal LTP. (**B**) A series of brief tetani (four tetani each of 50-msec duration and 100 Hz, at 10-second interval) presented to the assoc-com pathway failed to evoke LTP, but the same stimuli accompanied by postsynaptic depolarization (0.5 to 3 nA injected current) reliably produced LTP (five of five cells as summarized in the graph). (**C**) An even more radical manipulation failed to reveal an influence of postsynaptic membrane potential in the mossy fiber pathway (which again worked reliably in the assoc-com pathway, n = 5); here two series of four to eight brief tetani were administered as in (B) but the first was accompanied by hyperpolarizing current (−2 to −4 nA) or voltage clamp at hyperpolarized potential (−80 to −100 mV) and the second by depolarization (+1 to 4 nA); in this group of cells (n = 7) the first two tetani did not produce LTP but a third tetanus that doubled the number of stimuli in each tetanus (to ten) accompanied by hyperpolarizing current showed LTP. Although there was substantial variability among cells in the number of brief tetani necessary to produce mossy fiber LTP, in no case was an influence of membrane potential on the induction of mossy fiber LTP evident (n = 15). The mossy fiber experiments were carried out in the presence of APV.

accompanied by depolarization produce LTP when the same stimuli presented with hyperpolarization did not. Figure 3C shows the results from seven cells in which no LTP was apparent with four to eight groups of five stimuli presented with hyperpolarization and then with depolarization; increasing the number of stimuli to ten accompanied by hyperpolarization did produce LTP. That is, the number of stimuli and not the postsynaptic membrane potential determined whether LTP was obtained (*21*).

Using these stimulus procedures that reliably demonstrate the voltage dependence of assoc-com LTP, we saw no effect on mossy fiber LTP (n = 15). The mossy fiber synapses are more electrically accessible than the assoc-com synapses to a recording electrode, and mossy fiber LTP is more easily induced by tetanus than assoc-com LTP. Thus the failure to induce LTP in the mossy fiber pathway by postsynaptic voltage manipulation implies that there is a qualitative rather than a quantitative difference between the role of postsynaptic voltage in the two pathways (*22*).

With intracellular recording experiments, the roles of postsynaptic Ca^{2+} and of postsynaptic membrane potential could be separately investigated. Whole-cell recording allowed more powerful, though less precisely delimited, alterations of the postsynaptic cell. The very low resistance whole-cell pipettes (2 to 3 megohms) allowed considerably greater access to the postsynaptic cell for voltage clamping and for altering the cell interior. High concentrations of BAPTA (10 to 25 mM) were included to buffer Ca^{2+}. In addition, fluoride (up to 120 mM, depending on the BAPTA concentration) was used as the major anion in the electrode solution. Fluoride itself binds Ca^{2+} strongly (*23*), reduces voltage dependent Ca^{2+} currents (*24*), and disrupts various intracellular enzymatic events that depend, for example, on G proteins and phosphastases (*25*). Finally, from 20 to 50 minutes were allowed for the dialysis of small molecules from the interior of the cell before LTP-inducing stimuli were administered (adenosine triphosphate was deliberately omitted from the recording solution).

Despite the postsynaptic insults enumerated above, LTP was routinely obtained in the mossy fiber pathway in whole-cell voltage clamp recordings (Fig. 4A). Monitoring LTP in the assoc-com pathway, in which LTP was blocked, provided an index of the stability of the recording and ruled out any spurious changes in the cells or the recording that might masquerade as LTP. Even when we used a stimulus protocol that was just sufficient to reliably elicit LTP (5 Hz for 60 seconds), whole-cell voltage clamping (which, under these conditions of low stimulus frequency, maintained cells well belowed the threshold for action potential initiation and limited apparent voltage changes to less than 5 mV during the tetanus) failed to block the potentiation (Fig. 4C).

Locus of increased synaptic strength. There is general agreement that the induction of LTP in CA1 hippocampal cells is postsynaptic, but whether the persistent change in synaptic strength is presynaptic or postsynaptic is still disputed. One early suggestion (*26*) that the increase in synaptic strength might be postsynaptic derives from experiments with paired-pulse facilitation. Paired-pulse facilitation has been studied at several synapses including those in the hippocampus (*26–28*) and is clearly due to an increase in transmitter release (*29*). Other manipulations that alter transmitter release, including post-tetanic potentiation (*26*), changes in Ca^{2+} and Mg^{2+} (*27*), and application of phorbol esters (*30*) affect paired-pulse facilitation. However, the magnitude of paired-pulse facilitation recorded extracellularly during NMDA-dependent LTP is not changed (*26, 27*). Therefore, either LTP expression in CA1 is presynaptic but affects a step in the release process that is independent of all of these, or, more simply, LTP expression is postsynaptic.

We have applied the same reasoning to the CA3 assoc-com pathways. Paired pulses at 50-msec intervals were delivered, and field EPSP's were recorded. We measured the LTP obtained in a group of field potential recordings (Fig. 5A) and normalized the ratio of pulse 2 to pulse 1 during the same time period (Fig. 5B) (the absolute magnitude of paired-pulse facilitation before LTP averaged 1.90 ± 0.20, n = 8). Paired-pulse facilitation was reduced during post-tetanic potentiation, the first minute or two after the tetanus, as expected, but showed no change during LTP (1.92 ± 0.15). This result is identical to that obtained for NMDA-dependent LTP elsewhere (*26, 27*). We also tested for possible interactions between paired-pulse facilitation and mossy fiber LTP, using whole-cell recording (*31*). In contrast to the results with the assoc-com pathway, during mossy fiber LTP (Fig. 5C), paired-pulse facilitation was significantly reduced (from 3.41 ± 0.54 to 1.48 ± 0.21, n = 8). This reduction implies that a presynaptic contribution to the increased synaptic strength occurs in the mossy fiber pathway. Similar results have recently been obtained by analyzing mossy fiber field potentials (*12*).

Contrasting properties of mossy fiber and assoc-com LTP.

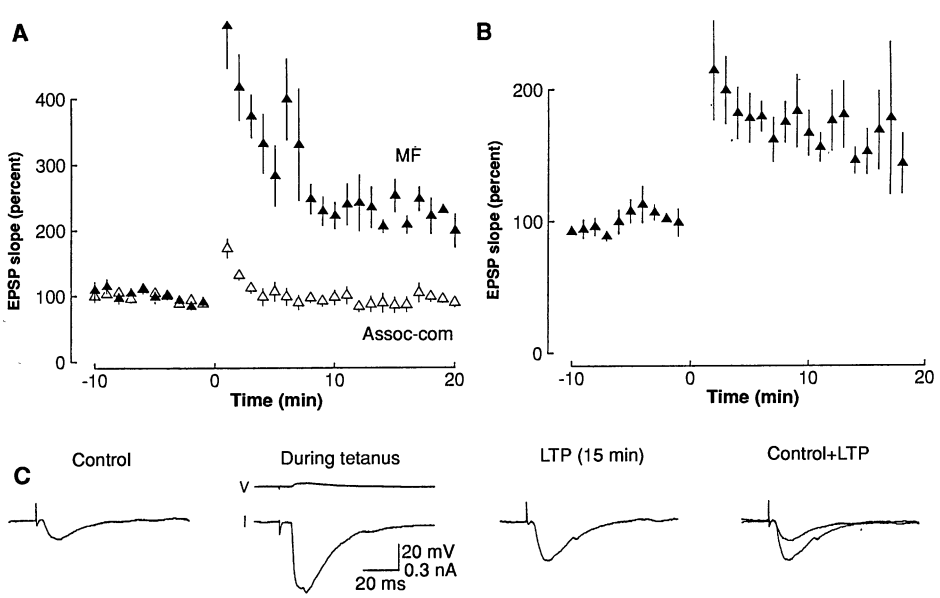

Fig. 4. Effect of BAPTA and fluoride on LTP measured with whole-cell voltage clamp recording in the assoc-comm and mossy fiber (MF) pathways. (**A**) LTP in the mossy fiber (filled symbols) pathway in whole-cell recording (n = 7; seven of seven cells showed greater than 50 percent increase 10 minutes after tetanus); the assoc-com pathway (open symbols) in these cells controlled for nonspecific changes that might mimic LTP(one of seven cells showed greater than 20 percent increase 10 minutes after tetanus). The field potential recorded from neighboring cells did show LTP in these slices. (**B**) A low-frequency LTP-inducing stimulus (5 Hz, 60 seconds) that produced a small amount of mossy fiber LTP in control slices was used; even with this stimulus the expected magnitude of LTP was obtained despite voltage clamp at a holding potential of approximately −100 mV during the tetanus, as shown in the single trace examples (n = 6; six of six cells showed greater than 20 percent increase 10 minutes after tetanus); LTP in the assoc-com pathway to normal high-frequency tetani was again blocked in these cells. APV was present in two of seven cells in (A) and four of six cells (B).

Although LTP of the assoc-com and the mossy fiber inputs to single CA3 pyramidal cells are phenomologically similar, the underlying cellular mechanisms are quite different. The mechanisms of LTP in the assoc-com pathway are qualitatively similar to those in the well-studied CA1 model system. In particular, both types of LTP require activation of the NMDA receptor, depend on postsynaptic Ca^{2+} concentrations and membrane potential, and are independent of the presynaptic phenomenon of paired-pulse facilitation. Therefore, the model for LTP in CA1 cells may be generally applicable to LTP at the several other synapses in the brain where a dependence on NMDA receptors is evident.

Intracellular manipulations of postsynaptic Ca^{2+} and membrane potential, and even the wholesale alterations of the postsynaptic biochemical machinery brought about by means of whole-cell recording with fluoride and BAPTA failed to reveal any postsynaptic dependence of LTP induction in the mossy fiber pathway despite the greater anatomical and electrotonic accessibility of these synapses. These findings favor a presynaptic locus for induction of mossy fiber LTP but do not rule out control by a postsynaptic mechanism that is independent of Ca^{2+} and membrane potential. The strong interaction of mossy fiber LTP with paired-pulse facilitation suggests that the persistent change during mossy fiber LTP is also presynaptic. Taken together, the results on induction and the persistent change strongly suggest that mossy fiber LTP is a presynaptic phenomenon.

Much of the interest in LTP has been fueled by its tantalizing phenomenological similarity to memory. LTP in the CA3 assoc-com pathway, as in other NMDA dependent forms of LTP, requires sufficient postsynaptic depolarization to relieve the Mg^{2+} block of the NMDA activated channel. This provides the basis for the cellular phenomena of cooperativity and associativity. The mossy fiber pathway shares neither the requirement for postsynaptic depolarization, nor, apparently, cooperativity itself with NMDA-dependent LTP. However, the same logical operations presumably govern mossy fiber LTP; they are simply one cell further back in the hippocampal circuitry. That is, although in the assoc-com pathway the CA3 pyramidal cell integrates its various inputs and controls the potentiation of active inputs, in mossy fiber LTP the presynaptic dentate granule cell would make the analogous computation, transform it into spiking frequency, and thus control the occurrence of LTP.

The different cellular mechanisms in mossy fiber and assoc-com LTP are likely to have different functional consequences in other ways as well. In particular, the set of strengthened synapses are probably selected differently in the two forms of LTP. In the assoc-com pathway the postsynaptic cell strongly influences that set; that is, the potentiated synapses are those that are active when a postsynaptic cell is sufficiently depolarized (*32*). In the mossy fiber pathway (which forms en passant synapses) all of the synapses of a particular dentate granule cell may be enhanced together, defining a very different set of potentiated synapses. An understanding of the consequences of increasing activity in these different sets of synapses must await a better understanding of the information processing functions of the hippocampal circuitry.

Finally it is reasonable that vertebrate central nervous system synapses can exhibit a presynaptic form of long-term synaptic

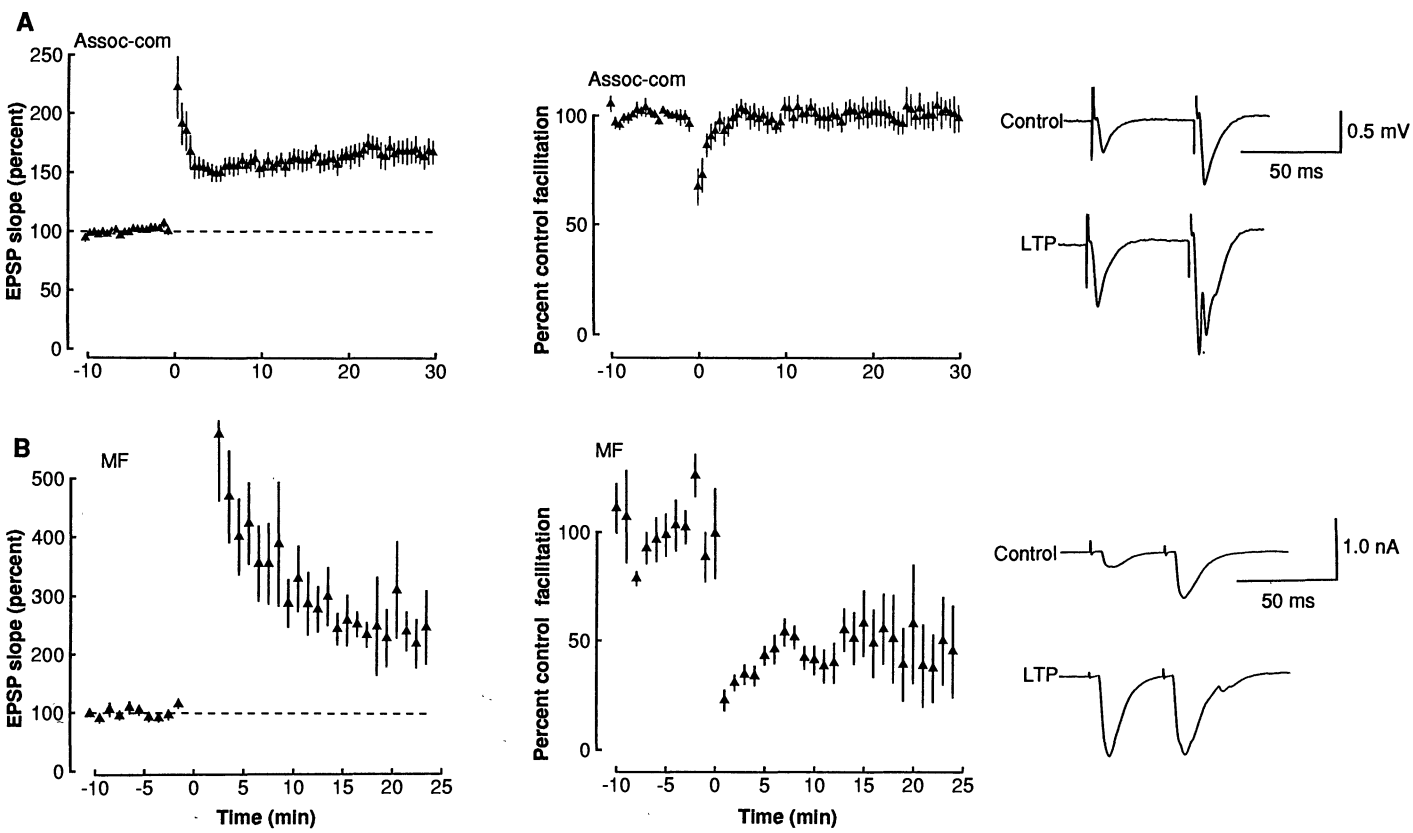

Fig. 5. LTP in the mossy fiber (MF) but not the assoc-com pathway interacts with the presynaptic phenomenon of paired-pulse facilitation. (**A**) Paired pulses at 50-msec intervals were given to the assoc-com pathway while field potentials were recorded (n = 8). (Upper panel) LTP (for the first pulse) and the lower paired pulse facilitation expressed as a ratio of the two pulses normalized to the control period before the tetanus. The magnitude of paired-pulse facilitation decreased transiently during posttetanic potentiation but was unchanged during LTP. (**B**) The interaction of paired-pulse facilitation and LTP in the mossy fiber pathway was examined with whole-cell recording (n = 8, six done in APV). Paired-pulse intervals from 50 to 80 msec were used. There was a clear reduction in paired-pulse facilitation during LTP in the mossy fiber pathway. Sample records are averages of five consecutive traces taken before and 15 minutes after LTP was induced.

plasticity. Presynaptic mechanisms for plasticity occur in peripheral vertebrate ganglia (*33*), and the best undestood models of plasticity are the long-term changes in transmitter release at invertebrate synapses (*34*). The general relevance of these models should become more apparent as the repertoire of plasticity in mammalian central nervous system is more fully explored.

REFERENCES AND NOTES

1. G. L. Collingridge and T. V. P. Bliss, *Trends Neurosci.* **10**, 288 (1987); B. Gustafsson and H. Wigstrom, *ibid.* **11**, 156 (1988).
2. R. A. Nicoll, J. A. Kauer, R. C. Malenka, *Neuron* **1**, 97 (1988).
3. A. Baranyi and M. B. Szente, *Brain Res.* **423** 378 (1987); A. Artola and W. Singer, *Nature* **330** 649 (1987); Y. Komatsu *et al., J. Neurophysiol.* **59**, 124 (1988); A. T. Perkins and T. J. Teyler, *Brain Res.* **439**, 222 (1988); P. Sah and R. A. Nicoll, *J. Physiol. (London)*, in press.
4. D. G. Amaral and M. P. Witter, *Neuroscience* **31**, 591 (1989).
5. T. H. Brown and D. Johnston, *J. Neurophysiol.* **50**, 487 (1983).
6. B. E. Alger and T. J. Teyler, *Brain Res.* **110**, 463 (1976); C. Yamamoto, K. Matsumoto, M. Takagi, *Exp. Brain Res.* **38**, 469 (1980).
7. E. W. Harris and C. W. Cotman, *Neurosci. Lett.* **70**, 132 (1986).
8. T. Monaghan and C. W. Cotman, *J. Neurosci.* **5**, 2909 (1985).
9. While these studies were in progress, several reports (*10–12*) have appeared that have direct bearing on some of our results. These reports are considered in the context of the specific results.
10. S. Williams and D. Johnson, *Neuron* **3**, 583 (1989).
11. D. B. Jaffe and D. Johnson, *Abstr. Soc. Neurosci* **15**, 399 (1989).
12. U. Staubli, J. Larson, G. Lynch, *Synapse* **5**, 333 (1990).
13. R. A. Nicoll and B. E. Alger, *J. Neurosci. Methods* **4**, 153 (1981). Transverse hippocampal slices (400 to 500 μm) from guinea pig were prepared in ice-cold Ringer on a vibratone. The slices were placed in a holding chamber for at least 1 hour and then transferred to the recording chamber. The superfusing medium contained 125 mM NaCl, 2.5 mM KCl, 1.3 mM $MgCl_2$, 2.5 mM $CaCl_2$, 26 mM $NaHCO_3$, 1 mM NaH_2PO_4, and 10 mM glucose and was equilibrated with 95 percent O_2 and 5 percent CO_2. In some experiments picrotoxin (100 μM) was added to the medium to block synaptic inhibition mediated by GABA acting on $GABA_A$ receptors. In this case, the concentrations of Ca^{2+} and Mg^{2+} were each increased to 4 mM or doubled to suppress bursting activity. A difficulty in studying the mossy fiber pathway is that either the pathway leaves the plane of the slice between the granule cell layer and site of recording or substantial contamination of axons other than those from mossy fibers contribute to the response evoked by the stimulating electrode (bipolar stainless steel). Unlike the mossy fibers, contaminating fibers are myelinated and therefore have a low threshold for activation and a faster conduction velocity. To maximize the presence of a mossy fiber input, each slice was screened by placing a stimulating electrode in stratum lucidum and a field electrode in the granule cell layer to record the antidromic field potential. Once the position in the granule cell layer that gave the largest field (at least 1 mV) was located, the stimulating electrode was placed at this site. Nevertheless, it was still often necessary to reposition the stimulating electrode during the intracellular recording to further improve the mossy fiber EPSP. Mossy fiber EPSP's were characterized by fast rise times, by discontinuous stimulus-response properties, and by their elicitation with small stimulus intensities. When these criteria were not met, particularly in slices with weak antidromic responses, clear APV-sensitive LTP could be elicited. The electrode in the assoc-com pathway was placed sufficiently far from stratum lucidum to ensure that mossy fibers were not inadvertently stimulated. Intracellular recording was performed with standard microelectrodes which, when filled with 3 M KCl, gave resistances in the range of 20 to 35 megohms. For chelating intracellular Ca^{2+} the electrodes contained 200 mM BAPTA in addition to either 3 M CsCl, 3 M KCl, or 2 M potassium methyl sulfate. In a pilot series of experiments, it was found that perfusing cells for 20 to 50 minutes with 10 mM BAPTA had no effect on assoc-com LTP. With BAPTA-containing electrodes that did not contain Cs^+, the Ca^{2+}-activated afterhyperpolarization was blocked within 10 to 15 minutes after penetrating the cell. When Cs^+-containing electrodes were used, loading of the cells was assessed by the spike broadening action of Cs^+. In a number of experiments the blind whole-cell recording technique was used [P. A. Coleman and R. F. Miller, *J. Neurophysiol.* **61**, 218 (1989); M. G. Blanton, J. J. Lo Turco, A. R. Kriegstein, *J. Neurosci. Methods* **30**, 203 (1989)]. These electrodes, which had a resistance of 2 to 3 megohm were filled with 110 mM CsF, 10 mM CsCl, 10 mM Hepes, and either 10 mM EGTA, 10 mM BAPTA, or 25 mM BAPTA. When BAPTA was used, the concentration of CsF was adjusted to maintain the osmolarity constant. An axoclamp 2a amplifier (Axon Instruments, Burlingame, CA) was used for current clamp, single-electrode voltage clamping with conventional intracellular electrodes (discontinuous mode, switching frequency between 3 and 8 kHz) and whole cell recording (continuous mode). The use of the initial slope of the EPSP to measure the strength of the synaptic input minimized problems with the appearance of polysynaptic input bursting. Nevertheless, due to the susceptibility of the CA3 regions to bursting, especially after tetanic electrical stimulation, approximately 20 percent of the preparations were discarded because bursting interfered with the measurement of the EPSP. Each pathway was stimulated at 0.1 Hz. The stimuli were alternated so that a response was recorded every 5 seconds. The standard stimulus for eliciting mossy fiber LTP was a 100-Hz tetanus for 1 second, repeated 10 seconds later. Since the magnitude of assoc-com LTP was less, more robust stimulation, consisting of 100 Hz, 1-second pulses, repeated four times was often used. Results are expressed as mean ± SEM.
14. G. Lynch *et al., Nature* **305**, 719 (1983); R. C. Malenka *et al., Science* **242**, 81 (1988).
15. R. Y. Tsien, *Biochemistry* **19**, 2396 (1980); E. Neher and A. Marty, *Biophys. J* **47**, 278a (1985).
16. Our results on the resistance of mossy fiber LTP to BAPTA differ from those recently reported (*10*). We have no obvious explanation for the difference.
17. B. Gustafsson *et al., J. Neurosci.* **7**, 774 (1987).
18. S. R. Kelso, A. H. Ganong, T. H. Brown, *Proc. Natl. Acad. Sci. U. S. A.* **83**, 5326 (1986); R. Malinow and J. P. Miller, *Nature* **320**, 529 (1986).
19. J. A. Kauer and R. A. Nicoll, unpublished data.
20. The advantage of using repetitive rather than single stimuli is presumably due to the considerably lower density of NMDA receptors in CA3 compared with CA1 cells (*8*), and the fact that repetitive stimulation results in marked summation of the NMDA synaptic response. [G. L. Collingridge, C. E. Herron, R.A.J. Lester, *J. Physiol. (London)* **399**, 301 (1988)].
21. Membrane potential has been reported to affect mossy fiber LTP (*11*). One possible source for this difference from our results is that a relatively low concentration of APV (20 μM) [E. J. Coan, A. J. Irving, G. L. Collingridge, *Neurosci. Lett.* **105**, 205 (1989)] was used and, since no verification of the effectiveness of APV was given in these experiments, it is conceivable that the reported voltage dependence resulted from a contribution of non-mossy fiber inputs.
22. Analogous experiments on extracellular fields also failed to demonstrate any postsynaptic voltage dependence of mossy fiber LTP. That is, a tetanus that was insufficient to produce LTP never produced any LTP if the stimulus strength was doubled during the tetanus, while increasing the number of stimuli at the lower stimulus strength did produce LTP. Thus we were unable to demonstrate any cooperativity in mossy fiber LTP with procedures that demonstrated cooperativity in NMDA dependent LTP, including assoc-com LTP in CA3 cells. Our result is in complete accord with the failure to demonstrate associativity in the mossy fiber pathway [J. A. Kauer and R. A. Nicoll, in *Synaptic Plasticity in the Hippocampus*, H. L. Haas and G. Buzsaki, Eds. (Springer, Berlin, 1988), p. 65; S. Chattarji, P. K. Stanton, T. J. Sejnowski, *Brain Res.* **495**, 145 (1989)].
23. See *CRC Handbook of Chemistry and Physics* (CRC Press, Boca Raton, FL, 1989), p. B-80.
24. P. G. Kostyuk and O. A. Kristal, *J. Physiol. (London)* **270**, 545 (1977); A. R. Kay, R. Miles, R. K. S. Wong, *J. Neurosci.* **6**, 2915 (1986).
25. H. R. Revel, *Methods Enzymol.* **6**, 211 (1963); P. C. Sternweis and A. G. Gilman, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4888 (1982); A. Wiseman, *Handb. Exp. Pharmacol.* **20**, Part 2, 48 (1970).
26. B. L. McNaughton, *J. Physiol. (London)*, **324**, 249 (1982).
27. D. Muller and G. Lynch, *Brain Res.* **479**, 290 (1989).
28. G. Hess, U. Kuhnt, L. L. Voronin, *Neurosci. Lett.* **77**, 187 (1987).
29. R. S. Zucker, *J. Physiol. (London)* **229**, 787 (1973); R. S. Zucker, *Annu. Rev. Neurosci.* **12**, 13 (1989).
30. D. Muller, J. Turnbull, M. Baudry, G. Lynch, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 6997 (1988).
31. The constancy of paired-pulse facilitation during LTP argues for the independence of the two processes. A change in paired-pulse facilitation during LTP could result from a number of factors other than genuine interdependence of the two phenomena. For instance, nonlinear summation of EPSP's [A. R. Martin, *J. Physiol. (London)* **130**, 114 (1955)] becomes a problem with the large magnitude of the potentiation of the second pulse. In addition, the intrusion of population spikes, which are highly nonlinear, into measurements of field EPSP's can be a problem especially with the close proximity of the mossy fiber input to the cell bodies. For these reasons we used whole cell recording and voltage clamping.
32. A recent paper raises the possibility that in cultured slices from very young rats, synapses that have not themselves been active during postsynaptic depolarization may become strengthened [T. Bonhoeffer, V. Staiger, A. Aertsen, *Proc. Natl. Acad. U.S.A.* **86**, 8113 (1989)]. This conclusion was based on the observation, with voltage sensitive dyes and paired intracellular recording, that the synapses onto neighboring cells were also enhanced when LTP was induced in a given cell by pairing synaptic activation with postsynaptic depolarization. However, in conventional slices, extracellular field recording shows no influence of such pairing on synaptic strength in neighboring cells (*17*; R. Nicoll *et al.*, unpublished observations).
33. C. A. Briggs, T. H. Brown, D. A. McAfee, *J. Physiol. (London)* **359**, 503 (1985); C. A. Briggs, D. A. McAfee, R. E. McCaman, *ibid.* **363**, 181 (1985); K. Kuba and E. Kumamoto, *ibid.* **374**, 515 (1986).
34. E. Kandel and J. H. Schwartz, *Science* **218** 433 (1982); P. Goelet, V. F. Castellucci, S. Schacher, E. R. Kandel, *Nature* **322**, 419 (1986); T. J. Carew and C. L. Sahley, *Annu. Rev. Neurosci.* **9**, 435 (1986); H. L. Atwood and J. M. Wostowicz, *Int. Rev. Neurobiol.* **28**, 275 (1986).

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