Neuron, Vol. 41, 445–454, February 5, 2004, Copyright ©2004 by Cell Press

Spike Train Timing-Dependent Associative Modification of Hippocampal CA3 Recurrent Synapses by Mossy Fibers

Katsunori Kobayashi and Mu-ming Poo*
Division of Neurobiology
Department of Molecular and Cell Biology
University of California, Berkeley
Berkeley, California 94720

Summary

In the CA3 region of the hippocampus, extensive recurrent associational/commissural (A/C) connections made by pyramidal cells may function as a network for associative memory storage and recall. We here report that long-term potentiation (LTP) at the A/C synapses can be induced by association of brief spike trains in mossy fibers (MFs) from the dentate gyrus and A/C fibers. This LTP not only required substantial overlap between spike trains in MFs and A/C fibers, but also depended on the temporal order of these spike trains in a manner not predicted by the well-known rule of spike timing-dependent plasticity and requiring activation of type 1 metabotropic glutamate receptors. Importantly, spike trains in a putative single MF input provided effective postsynaptic activity for the induction of LTP at A/C synapses. Thus, the timing of spike trains in individual MFs may code information that is crucial for the associative modification of CA3 recurrent synapses.

Introduction

The hippocampus is known to be essential for several forms of learning and memory (Jarrard, 1993; Kesner et al., 2000). Long-term modifications of synaptic transmission demonstrated in hippocampal excitatory synapses have been thought to be cellular mechanisms underlying learning and memory (Martin et al., 2000). The CA3 region of the hippocampus is characterized by extensive recurrent associational/commissural (A/C) connections made by axon collaterals of pyramidal cells as well as the convergence of different kinds of fibers, including mossy fibers (MFs) from the dentate gyrus. Synapses made by A/C fibers and MFs have several contrasting properties in both anatomical and physiological respects. The A/C fibers make small presynaptic terminals similar to Schaffer collateral-CA1 synapses, whereas MFs form unusually large presynaptic terminals with tens of synapses only on proximal apical dendrites of CA3 pyramidal cells (Amaral et al., 1990; Henze et al., 2000). Mainly based on these anatomical properties, theoretical studies have suggested that recurrent connections in the CA3 region can function as a network for associative memory storage and recall (Bennett et al., 1994), in which the MF input plays a key role during learning by reliably initiating postsynaptic spikes, therefore named "detonator" or "teacher" synapse (McNaughton and Morris, 1987; Rolls and Treves, 1998). Indeed, long-term potentiation (LTP) at A/C-CA3 pyramidal cells has been shown to be associative (Chattarji et al., 1989; Martinez et al., 2002), requiring postsynaptic depolarization (Zalutsky and Nicoll, 1990) and *N*-methyl-D-aspartate (NMDA) subtype of glutamate receptors (Harris and Cotman, 1986; Nakazawa et al., 2002; Zalutsky and Nicoll, 1990) for its induction. Mice deficient in NMDA receptors in CA3 pyramidal cells are impaired in associative memory recall (Nakazawa et al., 2002). Furthermore, a recent study has shown that burst activation of a single MF input can evoke spikes in postsynaptic CA3 pyramidal cells in vivo (Henze et al., 2002). Although results of these experimental studies support the theoretical model of the CA3 network, it remains largely unknown how MF inputs contribute to associative modification of A/C synapses (Chattarji et al., 1989). Studies of MF synapses have mainly dealt with modification of the MF synapse itself, although heterosynaptic effects of MF activation have been observed (Bradler and Barrionuevo, 1989; McMahon and Barrionuevo, 2002; Tsukamoto et al., 2003; Yamamoto and Chujo, 1978).

In the present study, in order to address the physiological role of MF inputs in the modification of A/C synapses, we examined the effects of associative activities of MF and A/C inputs on A/C synaptic transmission. Most previous studies of A/C LTP have been carried out using either strong tetanic stimulation of A/C fibers (Bradler and Barrionuevo, 1989; Harris and Cotman, 1986; Zalutsky and Nicoll, 1990) or by pairing A/C activation with postsynaptic depolarization (Nakazawa et al., 2002; Zalutsky and Nicoll, 1990). While these stimulating protocols can reliably induce A/C LTP, they are unlikely to reflect physiologically relevant activities of hippocampal neurons. Hippocampal neurons in vivo discharge in brief high-frequency bursts (Barnes et al., 1990; Jung and McNaughton, 1993; Henze et al., 2002), and such bursting activity has been proposed to carry functionally important neuronal signals (Lisman, 1997). Therefore, we have used protocols comprised of stimulation of both MFs and A/C fibers to fire brief trains of action potentials in order to obtain further insight into physiological aspects of associative modification of A/C synapses. Our results indicate that associative modification of A/C inputs by MFs depends on both the overlap and the temporal order of spike trains in the two pathways. This spike train timing-dependent plasticity may play an important role in the coding of temporal information in the hippocampus under physiological conditions.

Results

Associative A/C LTP Induced by MF and A/C Stimulation

First, we characterized the induction of A/C LTP by associative activation of A/C fibers and MFs by recording field excitatory postsynaptic potentials (fEPSPs). Mossy fiber fEPSPs were identified following the method previously described (Kobayashi et al., 1999) (see Experi-

*Correspondence: mpoo@uclink.berkeley.edu

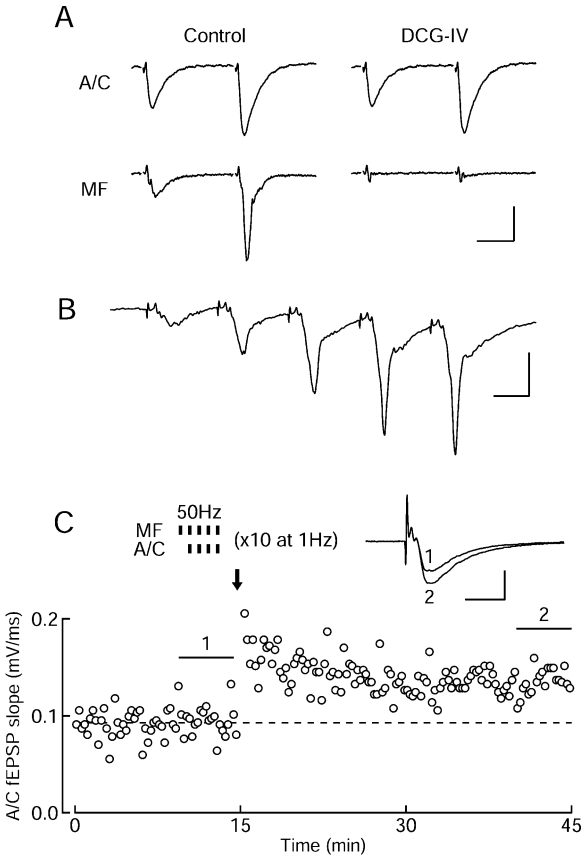

Figure 1. Associative Induction of A/C LTP by Pairing of MF and A/C Spike Trains

(A) Sample recordings of MF and A/C fiber fEPSPs. Field EPSPs evoked by paired-pulse stimulation (50 ms interval) of A/C fiber (top) and MF (bottom) in the absence (left) and presence (right) of DCG-IV. Traces show averages of ten consecutive fEPSPs. Stimulus artifacts are truncated. Scale: 0.2 mV, 20 ms.

(B) A typical example showing the prominent frequency facilitation of MF fEPSPs evoked by 50 Hz burst stimulation. Scale: 0.5 mV, 10 ms.

(C) A typical example of A/C LTP. Bursts (50 Hz, five pulses) of MF spikes were paired with those in A/C fibers (50 Hz, four pulses) as shown at the top. Each vertical bar represents a single stimulating pulse. The pairing was repeated ten times at 1 Hz at the time indicated by the arrow. The horizontal dashed line indicates the averaged value of the baseline A/C fEPSP slope. In all figures below, sample traces show averages of 20 consecutive EPSPs recorded just before and 25–30 min after the conditioning protocol (marked by 1 and 2 in this figure, respectively). Scale: 0.2 mV, 10 ms.

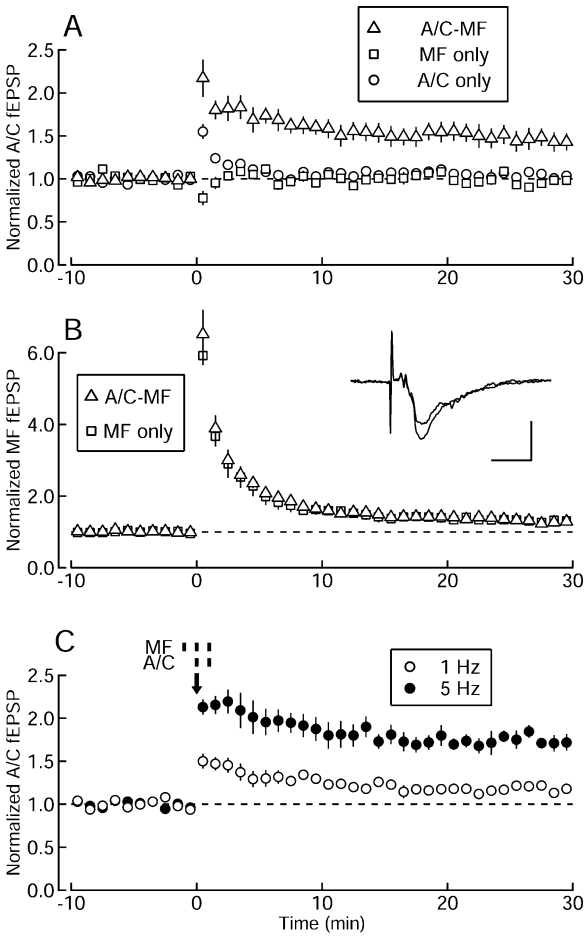

Figure 2. Characterization of A/C and MF LTP Induced by Burst Pairing

(A) Summary graphs showing the associative nature of A/C LTP. The slope of A/C fEPSP was normalized by its baseline value prior to paired stimulation. Triangles, burst pairing as in Figure 1B; squares, MF bursts only; circles, A/C bursts only.

(B) Nonassociative induction of MF LTP. The induction of MF LTP was done by pairing burst stimulation of MF and A/C inputs as in Figure 1B (triangles) or by MF burst stimulation alone (squares). The amplitudes of MF fEPSPs were measured and normalized. Scale: 0.1 mV, 10 ms.

(C) Induction of A/C LTP by burst pairing with the reduced number of pulses. Three MF spikes were paired ten times with two A/C fiber spikes at 1 Hz (open circles) or 5 Hz (filled circles).

mental Procedures) (Figure 1A). After recording stable baseline responses elicited by alternating stimulation of MF and A/C inputs at 0.067 Hz, repetitive burst stimulation was applied to MF and A/C inputs to produce spike trains that mimic high-frequency bursting activity observed in hippocampal neurons in vivo (Barnes et al., 1990; Jung and McNaughton, 1993; Henze et al., 2002) (Figures 1B and 1C). The MF burst evokes a train of postsynaptic action potentials (Henze et al., 2002), attributed in part to the prominent frequency facilitation of MF EPSPs (Kobayashi et al., 1996; Salin et al., 1996; Walker et al., 2001) (see Figures 1B and 6). The timing of stimuli applied to the MF and A/C inputs was set to induce four coincident spikes in the two pathways and one additional unpaired spike at the onset of the MF burst in order to enhance MF facilitation and resulting postsynaptic spike generation (Figure 1C). After pairing burst stimulation at 1 Hz, a persistent increase in the slope of A/C fEPSPs was induced (144.9% ± 11.9% of the baseline, n = 6) (Figures 1C and 2A). This A/C LTP was not induced by either A/C bursts alone (103.9% ± 4.1%, n = 5) or MF bursts alone (94.4% ± 2.4%, n = 4) (Figure 2A), indicating that the induction of A/C LTP requires associative A/C and MF activities. This result is consistent with previous results showing the associative nature of A/C LTP (Chattarji et al., 1989; Martinez et al., 2002).

The burst costimulation of MFs and A/C fibers also

potentiated MF fEPSPs. Mossy fiber fEPSPs showed large posttetanic potentation followed by slowly decaying potentiation (Figure 2B), which is characteristic of MF LTP (Kobayashi et al., 1996; Salin et al., 1996; Yeckel et al., 1999). In contrast to A/C LTP, the magnitude and the time course of this MF LTP were identical for MF bursts that were either paired (128.2% ± 5.2% of the baseline, n = 6) or not paired (131.0% ± 10.4%, n = 4) with the A/C bursts (Figure 2B), suggesting nonassociative nature of LTP at MF synapses. The magnitude of LTP is relatively small in the present condition, probably because the conditioning protocol used here is weaker than the tetanic stimulation commonly utilized for the induction of MF LTP (cf. 100 Hz, 1 s). Although we cannot exclude the possibility that the postsynaptic activity caused by the MF inputs overwhelmed that given by A/C fibers, our result is consistent with previous results that MF LTP does not require postsynaptic depolarization (Castillo et al., 1994; Zalutsky and Nicoll, 1990; but see Jaffe and Johnston, 1990; Urban and Barrionuevo, 1996; Yeckel et al., 1999).

We also examined effects of associative activity of shorter spike trains that are more frequently observed in hippocampal neurons in vivo (Harris et al., 2001). The number of spikes in each train in MF and A/C inputs was reduced to three and two, respectively. Pairing these shorter trains at 1 Hz induced only small LTP, but pairing at 5 Hz induced robust LTP (Figure 2C). The MF or A/C fiber bursts alone did not induce lasting changes in fEPSPs even at 5 Hz (data not shown). Thus, shorter trains are also highly effective in inducing associative A/C LTP.

Dependence on the Overlap of MF and A/C Spike Trains

To further examine the requirement for the associative spiking activities in MF and A/C inputs in burst pairing-induced modification of A/C synapses, we varied the relative onset time of the burst stimulation in the two pathways, using the same number of stimuli as in Figure 1C. As shown in Figure 3, the magnitude of A/C LTP decreased as the difference in onset times between bursts in the two inputs was increased, but no clear asymmetry in the induction of LTP was observed for bursts of opposite temporal order, in contrast to results of a similar analysis of synapses in the dentate gyrus (Levy and Steward, 1983). The magnitude of LTP was very small when there was no overlap in the spike trains in the two pathways. However, we did not observe clear long-term depression even at the interval of 500 ms (97.3 ± 2.4 of the baseline, n = 5), the longest possible interval using 1 Hz pairing. A change in the relative onset time by 10 ms in either direction, i.e., without precise coincidence of MF and A/C fiber spikes, did not cause any significant change in the magnitude of LTP (see middle three data points in Figure 3). Thus, the precise timing between individual MF and A/C fiber spikes is not important for the induction of A/C LTP. Rather, substantial overlap of MF and A/C spike trains is essential for LTP induction in the present condition.

Asymmetric Dependence on Spike Train Timing

At many excitatory synapses, the magnitude and direction of synaptic modification induced by correlated pre- and postsynaptic activity shows a striking asymmetry in the dependence on the temporal order of spiking in the pre- and postsynaptic cells: repetitive presynaptic spiking within tens of milliseconds before postsynaptic spiking results in LTP, whereas a reverse order of spiking leads to LTD (Bi and Poo, 1998; Debanne et al., 1998; Froemke and Dan, 2002; Markram et al., 1997; Nishiyama et al., 2000; Sjöström et al., 2001; Zhang et al., 1998). Although our above results suggest that the timing of individual spikes may not be relevant for the plasticity induced by spike trains, the temporal order of the entire spike trains in the MF and A/C inputs may still affect LTP, in addition to the extent of overlap in spike trains. To address this issue, we used a modified protocol in which all MF pulses are paired with A/C pulses to achieve identical spike coincidence and additional A/C pulses are added either before ("early") or after ("late") the paired pulses (Figure 4A). We found that the magnitude of LTP induced by these two protocols were significantly different ($p < 0.01$). The protocol with late unpaired A/C pulses induced larger LTP (172.8% ± 3.9%, n = 7) than that with early unpaired pulses (145.3% ± 7.1%, n = 7). A similar asymmetric timing dependence was observed at near physiological temperature (33°C −34°C) (Supplemental Figure S1 at http://www.neuron.org/cgi/content/full/41/3/445/DC1).

To examine whether this asymmetric timing dependence is specific to MF activation, we replaced MF stimulation with strong stimulation of A/C fibers. Two independent A/C pathways were activated, and one of these pathways was stimulated at the strength that gave about 10-fold larger fEPSP slope than usual (L A/C in Figure 4B) to generate postsynaptic spikes. This strong conditioning stimulation was given at the proximal region in the stratum radiatum to mimic MF activation, which is close to the soma. Pairing spike trains in these two pathways with early or late unpaired A/C pulses induced

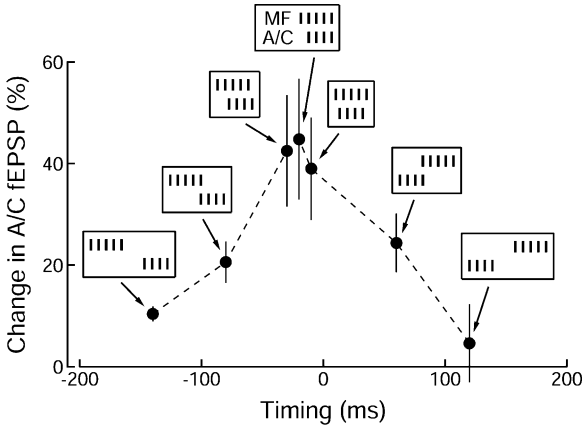

Figure 3. The Magnitude of A/C LTP Depends on the Extent of Overlap of Spike Trains

Pairing of the MF and A/C spike trains was carried out as in Figure 1B, but the timing of pairing was varied as shown in boxes. The magnitudes of changes in A/C fEPSPs were plotted against the time differences between first A/C pulse and first MF pulse (−140, −80, −30, −20, −10, 60, and 120 ms, n = 4, 7, 6, 6, 4, 6, and 5, respectively). Positive means A/C pulse first. The value at −20 ms was measured from the same data as shown in Figure 2A.

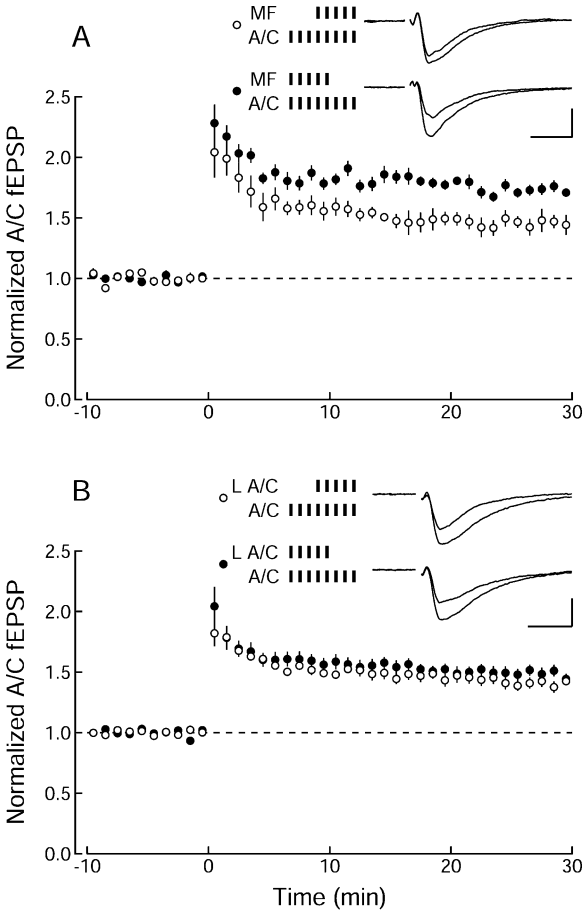

Figure 4. Timing of MF Spike Trains Controls Associative A/C LTP

(A) Dependence of A/C LTP on the relative timing of MF and A/C fiber spike trains. The number of pulses in each A/C burst was increased to eight and MF burst was paired ten times at 1 Hz with last five pulses (open circles) or first five pulses (filled circles). Larger LTP was induced by the latter protocol. The A/C bursts alone were insufficient for inducing significant potentiation (106.1% ± 2.6%, n = 5; $p > 0.05$, paired t test).

(B) Lack of timing dependence of A/C LTP induced by associative activation of two A/C pathways. The pairing was done as in (A), but the MF stimulation was replaced by strong stimulation of A/C fibers (L A/C). The stimulation of L A/C input was given only during the conditioning pairing. There was no difference in the slope of L A/C inputs between two groups: 0.87 ± 0.07 mV/ms (n = 7, open circle), 0.86 ± 0.06 mV/ms (n = 8, filled circle). Stimulus artifacts are truncated in sample traces. Scales: 0.2 mV, 10 ms.

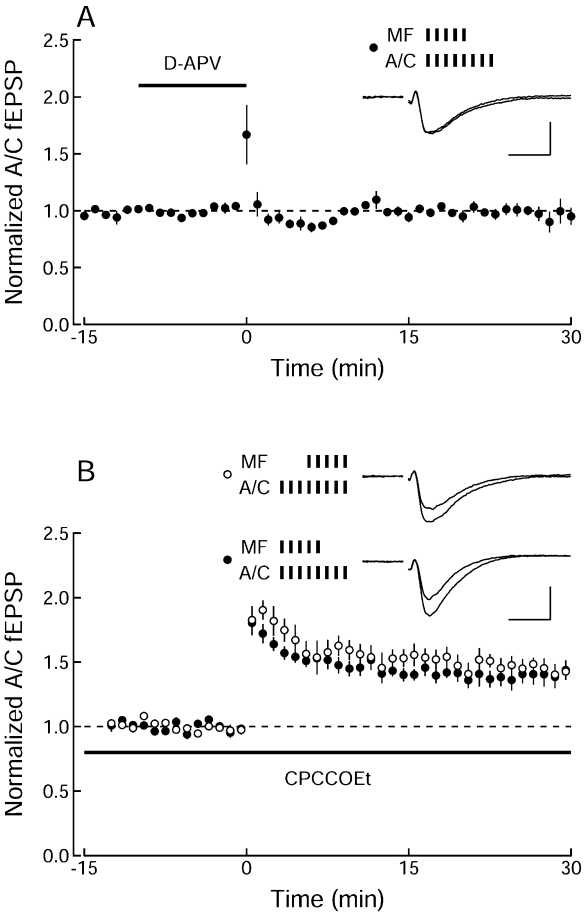

Figure 5. Pharmacological Properties of Spike Train Timing-Dependent A/C LTP

(A) Dependence of A/C LTP on NMDA receptor activation. The bar indicates bath application of D-APV (25 μM).

(B) Activation of mGluR1 is required for generation of the spike train timing dependence of A/C LTP. Bath application of CPCCOEt (50 μM) slightly potentiated both MF and A/C fEPSPs (by about 20%, data not shown). The drug was continuously applied throughout recordings, and the same protocols as in Figure 4A were applied after fEPSPs became stable. CPCCOEt was dissolved in DMSO, and the final concentration of DMSO was 0.05%. In control experiments using application of vehicle DMSO alone, the difference in the magnitude of LTP obtained by using the two protocols remained (data not shown). Stimulus artifacts are truncated in sample traces. Scales: 0.2 mV, 10 ms.

robust LTP, but the magnitude of LTP was similar between the two protocols (Figure 4B): 148.9% ± 4.6% (n = 8) for the protocol with late unpaired A/C, and 140.0% ± 4.1% (n = 7) for that with early unpaired pulses ($p > 0.1$). Thus, MF activation is essential for generating the dependence on spike train timing shown above. Our results showed that unpaired A/C pulses can affect the magnitude of A/C LTP, but they must act in association with MF activation.

Dependence on NMDA and Metabotropic Glutamate Receptors

The asymmetric timing dependence shown above cannot be attributed directly to the rule of spike timing-dependent plasticity (STDP) derived from spike pairing at low frequency, and suggests the existence of additional factors, e.g., history-dependent interactions among spikes in a train (Froemke and Dan, 2002) or regulation by calcium release from intracellular stores (Nishiyama et al., 2000). To investigate the mechanism underlying this spike train timing-dependent plasticity, we carried out the following pharmacological experiments. We found that A/C LTP induced by the protocol with late unpaired pulses was completely blocked by bath application of D-APV (97.7% ± 7.6%, n = 4, Figure 5A), confirming that NMDA receptor activation is essential for LTP induced by the protocol used here.

Previous studies have reported that group I metabotropic glutamate receptors (mGluRs), which are com-

prised of mGluR1 and mGluR5, contribute to LTP at the Schaffer collateral-CA1 synapse (Miura et al., 2002; Wilsch et al., 1998; but see Thomas and O'Dell, 1995). We examined the involvement of mGluR1, which is expressed in pyramidal cells in CA3 but not in CA1 (Luján et al., 1996). Two protocols the same as that in Figure 4A were applied in the presence of a specific antagonist of mGluR1, 7-(hydroxyimino) cyclopropa [b]chromen-1a-carboxylate ethyl ester (CPCCOEt) (Litschig et al., 1999). We found that the magnitudes of LTP induced by two protocols became similar (Figure 5B): 141.5% ± 8.3% (n = 5) for the protocol with late unpaired pulses and 146.5% ± 8.1% (n = 5) for that with early unpaired pulses ($p > 0.6$). Thus, the activation of mGluR1 is required for generating the dependence of LTP on the spike train timing. The timing dependence was also abolished by another mGluR1 antagonist, LY367385 (50 μM) (Clark et al., 1997): 169.9% ± 12.4% (n = 5) for the protocol with late unpaired pulses and 168.1% ± 8.8% (n = 5) for that with early unpaired pulses ($p > 0.9$). Although the latter results further supports the involvement of mGluR1 in the spike train timing-dependent regulation of LTP, this drug selectively depressed MF fEPSPs (to 33.1 ± 2.0 of the baseline, n = 10). Since this effect is similar to the effect of activation of group II mGluRs (Kamiya et al., 1996), LY367385 may have an agonistic side effect on these mGluRs. Even in the presence of LY367385, MF fEPSPs were strongly facilitated by the burst stimulation; thus, the robust LTP was induced by the burst pairing. Furthermore, the magnitude of LTP in LY367385 is apparently larger than that in CPCCOEt. We speculate that this result may also be explained by the possible side effect of LY367385 on group II mGluRs, because activation of group II mGluRs can reduce inhibitory synaptic transmission in the CA3 region (Poncer et al., 1995).

A/C LTP Induced by Single MF Input Stimulation

A recent study has shown that the burst activation of a single granule cell in vivo can evoke action potentials in postsynaptic CA3 pyramidal cells (Henze et al., 2002). Given that each pyramidal cell receives only tens of MFs (Amaral et al., 1990), we examined whether a single MF input can provide postsynaptic activity sufficient for the induction of associative A/C LTP, thus allowing single MF to code for associative signals for the modification of A/C inputs on a single pyramidal cell. Whole-cell recordings were made from CA3 pyramidal cells, and unitary MF responses were evoked using the minimal stimulation technique (Figure 6A). Unitary MF EPSPs did not evoke action potentials at low stimulus frequency (≤1 Hz), but higher-frequency stimulation in a burst (50 Hz) evoked a train of action potentials (Figure 6B). Stimulating a unitary MF input in the bursting pattern used in field recordings above evoked a variable number of action potentials (19.6 ± 2.4 spikes in response to 50 stimuli, n = 28). In most cases, the first pulse of each burst failed to evoke action potential, and the probability of firing increased for following pulses (Figure 6C), consistent with the previous result (Henze et al., 2002).

To demonstrate associative modification of A/C inputs by single MF input, we used burst pairing with late unpaired A/C pulses in the same manner as that used

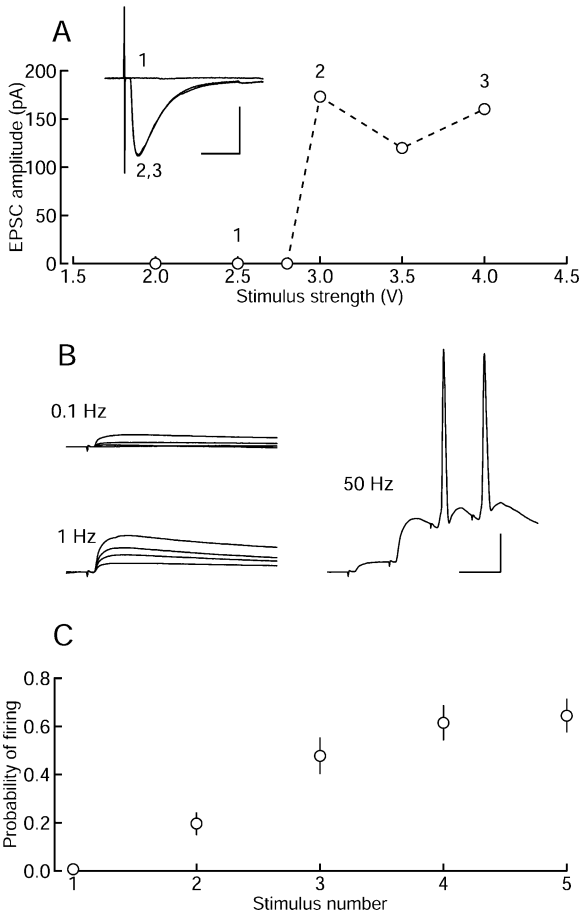

Figure 6. Spiking in CA3 Pyramidal Cells Evoked by Unitary MF EPSPs

(A) The minimal stimulation technique to activate a unitary MF input (see Experimental Procedures). Average amplitudes of excitatory postsynaptic currents (EPSCs, 20 trials) were plotted against the strength of stimulation applied. Inset shows EPSCs obtained at the stimulus strength indicated by the numbers. Scale: 100 pA, 20 ms.

(B) Action potential firing evoked by a burst of unitary MF EPSPs. In the same cell as shown in (A), MF was stimulated at the stimulus strength of 3.5 V and membrane potentials were recorded in the current-clamp mode. Scale: 20 mV, 20 ms.

(C) Probability of postsynaptic firing upon each MF stimulation during the 50 Hz trains.

earlier in the field recordings (Figure 7A). Consistent with the results from field recordings, this pairing protocol induced substantial LTP of A/C synapses (Figures 7A and 7B) (159.8% ± 12.4% of baseline, n = 5). Thus, a single MF input can indeed evoke sufficient postsynaptic activity for the induction of A/C LTP. In Figure 7A, LTP was not apparent at the MF input, due to a slow rundown of baseline EPSPs. In most cases, MF EPSPs showed gradual rundown and did not become stabilized during the limited baseline period allowed for whole-cell recordings before the pairing. Therefore, we did not analyze MF LTP in these experiments. This instability of MF EPSPs could be due to the intrinsic property of MF synapses, because MF fEPSPs tend to decrease during the initial phase of repetitive stimulation. Furthermore, relatively high-frequency stimulation (1 Hz), applied in order to identify the unitary MF input prior to baseline

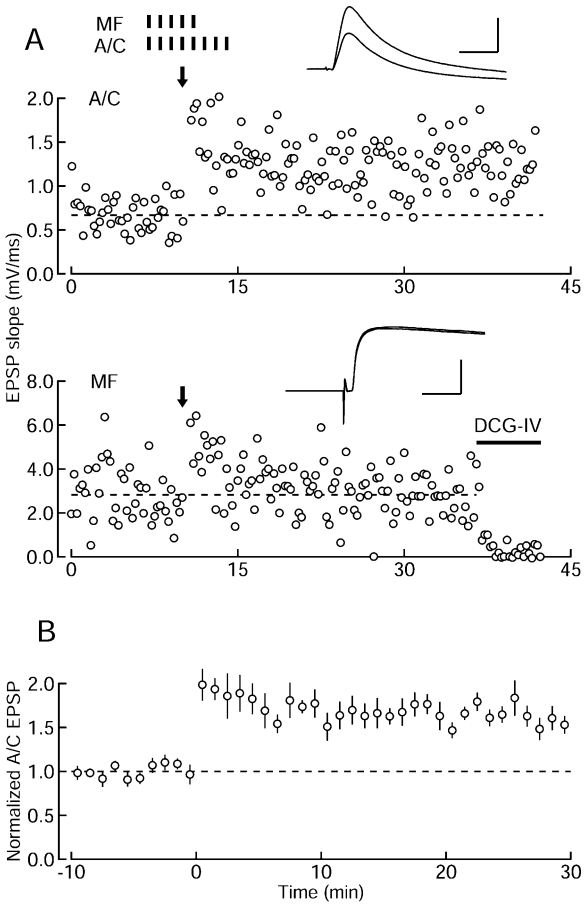

Figure 7. Induction of A/C LTP by Single-MF-Mediated Postsynaptic Activity

(A) A typical example of A/C LTP induced by pairing bursts in A/C inputs and a single MF input in a current-clamped CA3 pyramidal cell. The pairing protocol was the same as the one in Figure 4A that had late unpaired pulses. Recordings of A/C EPSPs (top) and MF EPSPs (bottom) were made from the same cell. The pairing was done at the time indicated by the arrow. In this cell, 35 spikes were evoked during the pairing. The bar indicates the time of DCG-IV application, which suppressed MF EPSPs to about 5% but had no effect on A/C EPSPs. Scales: 4 mV, 20 ms.

(B) Summary graph of A/C LTP induced by the burst pairing (n = 5).

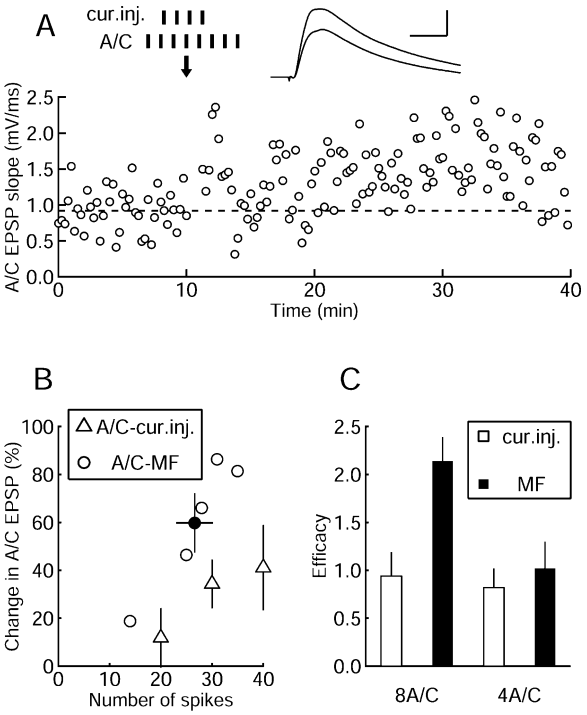

Figure 8. More Efficient Induction of A/C LTP by MF-Induced Postsynaptic Activity than by Current Injection-Induced Spiking

(A) Induction of A/C LTP by pairing of A/C bursts with postsynaptic spikes evoked by direct current injection. Each injection was delayed by 5 ms to roughly match the timing of action potential firing with that elicited by the MF stimulation. Scale: 4 mV, 20 ms.

(B) Summary graph showing that larger LTP was induced by MF stimulation than by current injection. Trains carrying eight A/C fiber spikes were paired with trains of MF spikes or current injection, and the magnitude of LTP was plotted against the number of spikes evoked in all trains. For A/C-MF pairing, the number of spikes evoked during the pairing was counted. Both individual data points (open circles) and the average (filled circle) are shown. For current injection, the number of somatic current injections was systematically changed from 20 to 40, and the data plotted are averages for each condition (triangles, n = 4–5 each).

(C) Unpaired A/C pulses are required for enhancement of A/C LTP by MF activity. The efficacy was calculated as the magnitude of LTP divided by the number of spikes. Cells in which MF bursts evoked less than ten spikes were excluded from analysis. (Left) (8A/C) The protocols with late unpaired A/C pulses were used. The efficacy was calculated from the same data as in (B). (Right) (4A/C) The protocols without late unpaired A/C pulses were used. For MF-A/C pairing, the protocol same as in Figure 1B was used. For current injection, the timing of current injection was delayed the same as in (A), and the number of current injection was systematically changed the same as in (B).

recordings or the exposure to the high-potassium pipette solution during the approach of the recording pipette to the cell, may also contribute to the instability.

Can the effect of single MF inputs be accounted for simply by their action in triggering spikes in the postsynaptic pyramidal cell? We have compared MF-induced A/C LTP with LTP induced by pairing A/C bursts with action potentials evoked by somatic current injections in the CA3 pyramidal cell (Figure 8A). Since the number of spikes evoked by MF stimulation was variable and unpredictable, we systematically changed the number of current injections. As shown in Figure 8B, when the magnitude of LTP was plotted against the number of spikes evoked during the burst stimulation, we found that LTP induced by the MF-A/C pairing is substantially larger than that induced by pairing A/C bursts with current injections. This difference of efficacy in inducing LTP was quantitatively analyzed by dividing the magnitude of LTP by the number of spikes evoked in each cell. The resulting value for the MF-A/C pairing (2.13 $\pm$ 0.25, n = 5) was significantly larger than that for the current injection (0.94 $\pm$ 0.25, n = 14, $p < 0.02$) (Figure 8C), indicating that MF activation is more effective in inducing A/C LTP than somatic current injection. The difference between MF stimulation and current injection was not observed when the protocol without late unpaired pulses (as in Figure 1C) was used: 1.02 $\pm$ 0.38 (n = 6) for the MF-A/C pairing and 0.82 $\pm$ 0.20 (n = 19) for current injection ($p > 0.1$) (Figure 8C). Thus, late unpaired pulses in A/C inputs appear to facilitate LTP,

consistent with results from field recordings described above. These results show that the MF input provides factors that facilitate LTP induction in addition to initiating spiking of the pyramidal cell.

Discussion

Our results showed that associative activation of MF and A/C spike trains produces LTP at the A/C synapses. The magnitude of A/C LTP depends on the overlap and also the temporal order of spike trains in MFs and A/C fibers, an effect requiring activation of mGluR1. Furthermore, the A/C LTP can be induced even when a single MF input is activated. These findings show that physiologically relevant activity is highly effective in inducing associative A/C LTP, and the timing of spike trains in individual MFs may carry information for associative modification in the hippocampus.

Distinct Delayed Action of MF Inputs in Associative A/C LTP

In whole-cell experiments, we have shown that MF activation is much more effective in inducing LTP of A/C inputs than somatic current injection. Because the facilitated induction of LTP by the MF activation was observed only when the protocol with late unpaired pulses was used, we suggest that MF activity may cause the release of specific factors or selective activation of receptors that exert a delayed effect and facilitate LTP induction at the A/C synapses. To explain the requirement for unpaired pulses for the LTP facilitation, MF effects must be mediated by a delayed signal, thus excluding the possibility that enhanced amplitude or duration of back-propagating action potentials mediates the effects. Similarly, differences in the timing of spikes in pyramidal cells between MF activation and direct current injection are unlikely to underlie the difference in the efficacy of LTP induction. Indeed, we showed by using field recordings that A/C LTP induced by pairing of MF and A/C fiber spike trains does not depend on the precise timing of individual spikes in the train.

Potential Mechanisms for the Delayed Effect of MF Stimulation

Delayed effects by MF activation might be due to enhanced dendritic depolarization mediated by polysynaptic recurrent A/C EPSPs, because MF stimulation can activate CA3 pyramidal cells other than the recorded cell but current injection cannot. However, this possibility is unlikely for the following reasons. Although pyramidal cells make extensive recurrent connections in vivo, they are not preserved completely in the slice preparation (Li et al., 1994), consistent with a very low probability (about 0.02) of successful recording from monosynaptically connected pairs of pyramidal cells (Miles and Wong, 1986). In addition, we used the minimal stimulation technique with special care to minimize the number of activated fibers (see Experimental Procedures) and kept the synaptic inhibition intact. Under this experimental condition, we typically observed a few small asynchronous EPSPs (about 1 mV in amplitude) following each train of the MF stimulation (see Supplemental Figure S2 at http://www.neuron.org/cgi/content/full/41/3/445/DC1). It is unlikely that these small delayed EPSPs caused the large difference in the efficacy of LTP induction between MF activation and current injection.

There are several possible candidate substances or receptors mediating the delayed effect of MF activation. Mossy fiber terminals are known to contain brain-derived neurotrophic factor (BDNF; Conner et al., 1997; Smith et al., 1997), and BDNF has been shown to exert strong facilitatory effects on LTP induction in the dentate gyrus through activation of TrkB receptors (Kovalchuk et al., 2002). Glutamates released from MF terminals can activate kainate receptors (Castillo et al., 1997; Vignes and Collingridge, 1997) and group I mGluRs (Heuss et al., 1999; Yeckel et al., 1999) in CA3 pyramidal cells. These glutamate receptors can also potentially facilitate LTP induction (Li et al., 2001; Miura et al., 2002; Wilsch et al., 1998, also see below). Substantial release of BDNF requires high-frequency neuronal activity (Balkowiec and Katz, 2000), and synaptic activation of mGluR and kainate receptors also requires high-frequency bursts of presynaptic fibers (Castillo et al., 1997; Heuss et al., 1999; Vignes and Collingridge, 1997), thereby causing a delay in signaling. Activation of all these receptors can cause membrane depolarization (Castillo et al., 1997; Heuss et al., 1999; Kovalchuk et al., 2002; Vignes and Collingridge, 1997). Indeed, we observed sustained membrane depolarization after MF bursts (see Supplemental Figure S2 at http://www.neuron.org/cgi/content/full/41/3/445/DC1), which was larger than that observed after spikes evoked by current injection (data not shown). The membrane depolarization will cause or help an elevation of intracellular Ca^{2+} concentrations and thus can facilitate the induction of A/C LTP. It is also possible that depolarization-independent Ca^{2+} elevation, such as by intracellular Ca^{2+} release (Yeckel et al., 1999), provokes propagating Ca^{2+} waves toward distal dendrites and facilitates the induction of LTP.

Spike Timing versus Spike Train Timing

At CA3 recurrent synapses in slice cultures (Debanne et al., 1998), STDP has been observed. However, we showed that the induction of A/C LTP does not depend on the precise timing of individual spikes in trains of MF and A/C inputs (see Figure 3). Rather, it depends on the timing of the entire spike train. We also showed that LTP is larger when A/C fibers are activated after associative activity of MF and A/C fibers than when they are activated before the associative activity. Since MF activation of postsynaptic firing occurs before late presynaptic A/C spiking, this timing dependence is not predicted by the rule of STDP. The rule of STDP might be affected when spikes are generated in bursts, as shown in the visual cortex (Froemke and Dan, 2002; Sjöström et al., 2001). Alternatively, the rule of STDP at the mature A/C synapse might be different from that at immature synapses in the slice culture and at many other synapses where STDP has been studied. However, this spike train timing dependence was dependent on mGluR1 activation and was specifically produced by MF stimulation but not by A/C fiber stimulation. Thus, factors other than simple interaction among spikes may contribute to the generation of the asymmetric spike train timing dependence. The specific involvement of MF activation argues

against the possibility that polysynaptic recurrent A/C EPSPs evoked by MF bursts generate this asymmetric timing dependence, because the strong stimulation of A/C fibers would also cause similar activation of polysynaptic EPSPs. Indeed, MF and A/C fiber stimulation evoked asynchronous EPSPs in a comparable manner (see Supplemental Figure S2 at http://www.neuron.org/cgi/content/full/41/3/445/DC1).

Models for the Spike Train Timing Dependence

Based on the results from both field and whole-cell recordings, we favor the following model for the asymmetric spike train timing dependence in associative A/C LTP: the MF bursts activate mGluR1, and the resulting delayed signal facilitates LTP induction synergistically with late unpaired A/C pulses. The mGluR1 is known to distribute over the entire dendritic field of CA3 pyramidal cells (Luján et al., 1996), and MF stimulation can actually activate mGluR1 (Heuss et al., 1999; Yeckel et al., 1999). An alternative model to explain the asymmetry in the spike train timing dependence is a suppression of LTP induction by early unpaired A/C fiber activation. This latter model cannot explain the results of whole-cell recordings (Figure 8) and requires a more complicated underlying mechanism: the A/C fiber bursts activate mGluR1, which specifically suppresses postsynaptic activity evoked by MF bursts but not by A/C fiber bursts. Postsynaptic activity mediated by MF bursts might be indirectly suppressed by presynaptic inhibition of MF synaptic transmission. Although mGluR1 has not been demonstrated in mossy fiber terminals (Luján et al., 1996), it can activate interneurons (Mori and Gerber, 2002), and then the interneuron activation can suppress MF synaptic transmission through presynaptic GABA receptors (Ruiz et al., 2003; Vogt and Nicoll, 1999). Finally, we note that interneurons are also major targets of MFs (Acsády et al., 1998). However, interneuron activation by MFs is unlikely to mediate the spike train timing dependence, because interneuron activation by MFs must facilitate LTP induction at A/C synapses in order to explain the timing dependence.

Functional Roles of MF Inputs in Synaptic Plasticity in CA3 Network

Hippocampal neurons in vivo typically fire in short bursts. We have shown that short burst firing of MFs can provide strong postsynaptic activity for the associative induction of A/C LTP. Due to extensive recurrent associational connections of CA3 pyramidal cells, the CA3 network may maintain excitation after strong activation of MF inputs. Spike train timing dependence shown in our study predicts that A/C LTP is most efficiently induced in such a situation. Thus, strong activation of MFs can generate an optimal condition for the induction of A/C LTP in concert with the network property of CA3. The protocol used to demonstrate the timing dependence in the present study was comprised of rather long spike trains (eight spikes) compared with typically observed neuronal activity in the hippocampus (Harris et al., 2001). It remains to be seen what kind of pattern is actually utilized in vivo during functionally relevant synaptic plasticity in the CA3 neuronal network.

An individual MF makes up to 35 synapses onto a single pyramidal cell, while a single CA3 pyramidal cell is estimated to receive only about 50 MF inputs (Amaral et al., 1990). One may expect that an individual MF input would have substantial effects on postsynaptic pyramidal cells. Indeed, it has recently been shown that the repetitive activation of a single granule cell in vivo evokes action potentials in postsynaptic CA3 pyramidal cells (Henze et al., 2002). Our results are consistent with this previous study and further show that the single MF-mediated activity is sufficient for associative induction of LTP at A/C synapses. These results provide direct physiological evidence for theoretical models of associative modifications of the CA3 recurrent network, in which the MF input was assumed to primarily contribute to initiation of postsynaptic spiking, therefore being the teacher or detonator input (Henze et al., 2000; McNaughton and Morris, 1987; Rolls and Treves, 1998). Since MF activation is much more effective in inducing LTP of A/C inputs than somatic current injection, the MF input is more specialized for efficient LTP induction at converging A/C inputs than was expected. The efficient postsynaptic firing required burst activation of MFs due to very low transmitter release probability at MF synapses. Burst activation of a single MF can provide suprathreshold depolarization through strong frequency facilitation of synaptic transmission and temporal summation of EPSPs, which is facilitated by a slow decay of MF EPSPs (see Figure 7). A functional consequence of the requirement for the burst activity is an increase in the signal-to-noise ratio: occasional spontaneous firing of granule cells cannot activate CA3 pyramidal cells, but burst firing of granule cells driven by external signals does. This view is consistent with the result that the timing of spike trains, but not of individual spikes, is important for the associative induction of LTP at A/C synapses.

Experimental Procedures

Hippocampal slices (350–450 μm) were cut from Sprague Dawley rats (17–26 days old). Rats were deeply anesthetized with halothane and decapitated. Transverse slices were cut from isolated hippocampi and all recordings were made in a submersion-type recording chamber superfused with a saline composed of (in mM) NaCl 119, $NaHCO_3$ 26.2, glucose 11, KCl 2.5, NaH_2PO_4 1, $CaCl_2$ 2.5, and $MgCl_2$ 1.3 and saturated with 95% O_2 and 5% CO_2. Experiments were carried out at 24°C–26°C unless otherwise specified. Field potentials were recorded in the CA3 region with a glass electrode that was filled with 1 M NaCl and placed in the stratum lucidum for recording MF fEPSPs and in the stratum radiatum for A/C fEPSPs (Supplemental Figure S3A at http://www.neuron.org/cgi/content/full/41/3/445/DC1). Bipolar tungsten electrodes were placed in the dentate granule cell layer to stimulate MFs and in the CA3 stratum radiatum to stimulate A/C fibers. Initial slopes of A/C fEPSPs were measured on analysis. The baseline slope was kept as small as about 0.1 mV/ms (see Figures 1, 4, and 5) unless otherwise specified to avoid induction of plastic changes by A/C fiber activation alone. Mossy fiber fEPSPs were identified and analyzed following a previously described method (Kobayashi et al., 1999). In the present study, large paired-pulse facilitation (>2.5-fold) and a reversal of the fEPSP polarity in stratum radiatum were routinely used as criteria for the MF input. These criteria were verified by a more than 90% block of fEPSPs by a group II mGluR agonist, (2S,2′R,3′R)-2-(2′,3′-Dicarboxycyclopropyl) glycine (DCG-IV, 1 μM, Tocris, USA) (Kamiya et al., 1996) (see Figure 1A). The baseline amplitude of MF fEPSP was kept around 0.15 mV.

In some experiments (Figure 4B), another A/C fiber pathway, instead of MFs, was stimulated at a higher intensity, and this second

A/C stimulating electrode was placed at the proximal region in the stratum radiatum (Supplemental Figure S3B at http://www.neuron.org/cgi/content/full/41/3/445/DC1). The independence of two A/C pathways was verified by the lack of synaptic facilitation when two pathways were stimulated successively with a short interval of 50 ms. In these experiments, field potentials were also monitored by the electrode in the stratum lucidum (Supplemental Figure S3B) to verify the lack of MF stimulation by the proximal stimulating electrode.

Whole-cell recordings were made from CA3 pyramidal cells by the blind whole-cell patch-clamp technique with a pipette (tip resistance 7–13 MΩ) filled with a solution composed of (in mM) potassium gluconate 118, HEPES 20, KCl 8, MgATP 4, Na_2GTP 0.4, EGTA 0.05, and phosphocreatine 10 (pH adjusted to 7.2 with KOH). In current-clamp recordings, the membrane potential was adjusted at −70 mV by constant current injection. The input resistance was monitored by injection of negative currents, and data were discarded if the input resistance changed more than 5% during recordings. Mossy fiber EPSPs were evoked by a glass electrode filled with the saline and placed in the dentate granule cell layer. Unitary MF EPSPs were evoked following a method of minimal stimulation (Jonas et al., 1993; Walker et al., 2001). Briefly, the stimulus intensity was increased gradually until abrupt appearance of synaptic responses in an all-or-none manner (see Figure 6). A position of the stimulating pipette was adjusted to minimize the threshold strength of the stimulation, thereby minimizing the number of activated fibers. After confirming the plateau in response sizes upon a further increase in the stimulus intensity, the intensity was kept about 20% above the threshold. We cannot exclude the possibility that input fibers making synapses on the recorded cell were simultaneously activated with a similar threshold by this method. However, since a single CA3 pyramidal cell receives only tens of MF inputs (Amaral et al., 1990), it is relatively hard to find intact MF input in slices. Therefore, the MF input evoked by the minimal stimulation technique is most probably a single input. The identity of MF EPSPs was verified by a more than 90% block of EPSPs by DCG-IV (see Figure 7A). Cells with clear polysynaptic EPSPs after single stimulation of MFs were excluded. At the end of each recording, EPSPs were blocked by CNQX (10–20 μM), and data were discarded if the initial slope of A/C EPSPs were contaminated by inhibitory synaptic potentials. The conditioning protocols were applied within 25 min after break-in in order to minimize the washout of LTP. Initial slopes of EPSPs were measured on analysis. The baseline amplitude of A/C EPSPs was about 4 mV and was much smaller than the depolarization required for firing action potentials in the present condition (about 30 mV).

In all experiments, the magnitude of LTP was evaluated by averaging values measured 25–30 min after the conditioning stimulation. All values are shown as mean ± SEM. The statistical significance was evaluated by two-tailed unpaired t test unless otherwise specified. All glutamate receptor antagonists were purchased from Tocris.

Acknowledgments

We thank T. Takahashi and T. Manabe for their helpful comments on the manuscript. This work was supported by grants from NIH (NS 36999). K.K. was supported by a Human Frontier Long Term Fellowship.

Received: June 2, 2003
Revised: September 26, 2003
Accepted: December 5, 2003
Published: February 4, 2004

References

Acsády, L., Kamondi, A., Sík, A., Freund, T., and Buzsáki, G. (1998). GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. J. Neurosci. *18*, 3386–3403.

Amaral, D.G., Ishizuka, N., and Claiborne, B. (1990). Neurons, numbers and the hippocampal network. Prog. Brain Res. *83*, 1–11.

Balkowiec, A., and Katz, D.M. (2000). Activity-dependent release of endogenous brain-derived neurotrophic factor from primary sensory neurons detected by ELISA *in situ*. J. Neurosci. *20*, 7417–7423.

Barnes, C.A., McNaughton, B.L., Mizumori, S.J.Y., Leonard, B.W., and Lin, L.-H. (1990). Comparison of spatial and temporal characteristics of neuronal activity in sequential stages of hippocampal processing. Prog. Brain Res. *83*, 287–300.

Bennett, M.R., Gibson, W.G., and Robinson, J. (1994). Dynamics of the CA3 pyramidal neuron autoassociative memory network in the hippocampus. Philos. Trans. R. Soc. Lond. B Biol. Sci. *343*, 167–187.

Bi, G.-q., and Poo, M.-m. (1998). Synaptic modifications in cultured hippocampal neurons: Dependence on spike timing, synaptic strength, and postsynaptic cell type. J. Neurosci. *18*, 10464–10472.

Bradler, J.E., and Barrionuevo, G. (1989). Long-term potentiation in hippocampal CA3 neurons: Tetanized input regulates heterosynaptic efficacy. Synapse *4*, 132–142.

Castillo, P.E., Weisskopf, M.G., and Nicoll, R.A. (1994). The role of Ca^{2+} channels in hippocampal mossy fiber synaptic transmission and long-term potentiation. Neuron *12*, 261–269.

Castillo, P.E., Malenka, R.C., and Nicoll, R.A. (1997). Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons. Nature *388*, 182–186.

Chattarji, S., Stanton, P.K., and Sejnowski, T.J. (1989). Commissural synapses, but not mossy fiber synapses, in hippocampal field CA3 exhibit associative long-term potentiation and depression. Brain Res. *495*, 145–150.

Clark, B.P., Baker, S.R., Goldsworthy, J., Harris, J.R., and Kingston, A.E. (1997). (+)-2-methyl-4-carboxyphenylglycine (LY367385) selectively antagonises metabotropic glutamate mGluR1 receptors. Bioorg. Med. Chem. Lett. *7*, 2777–2780.

Conner, J.M., Lauterborn, J.C., Yan, Q., Gall, C.M., and Waron, S. (1997). Distribution of brain-derived neurotrophic factor (BDNF) protein and mRNA in the normal adult rat CNS: Evidence for anterograde axonal transport. J. Neurosci. *17*, 2295–2313.

Debanne, D., Gähwiler, B.H., and Thompson, S.M. (1998). Long-term synaptic plasticity between pairs of individual CA3 pyramidal cells in rat hippocampal slice cultures. J. Physiol. *507*, 237–247.

Froemke, R.C., and Dan, Y. (2002). Spike-timing-dependent synaptic modification induced by natural spike trains. Nature *416*, 433–438.

Harris, E.W., and Cotman, C.W. (1986). Long-term potentiation of guinea pig mossy fiber responses is not blocked by N-methyl D-aspartate antagonists. Neurosci. Lett. *70*, 132–137.

Harris, K.D., Hirase, H., Leinekugel, X., Henze, D.A., and Buzsáki, G. (2001). Temporal interaction between single spikes and complex spike bursts in hippocampal pyramidal cells. Neuron *32*, 141–149.

Henze, D.A., Urban, N.N., and Barrionuevo, G. (2000). The multifarious hippocampal mossy fiber pathway: A review. Neuroscience *98*, 407–427.

Henze, D.A., Wittner, L., and Buzsáki, G. (2002). Single granule cells reliably discharge targets in the hippocampal CA3 network in vivo. Nat. Neurosci. *5*, 790–795.

Heuss, C., Scanziani, M., Gähwiler, B.H., and Gerber, U. (1999). G-protein-independent signaling mediated by metabotropic glutamate receptors. Nat. Neurosci. *2*, 1070–1077.

Jaffe, D., and Johnston, D. (1990). Induction of long-term potentiation at hippocampal mossy-fiber synapses follows a Hebbian rule. J. Neurophysiol. *64*, 948–960.

Jarrard, L.E. (1993). On the role of the hippocampus in learning and memory in the rat. Behav. Neural Biol. *60*, 9–26.

Jonas, P., Major, G., and Sakmann, B. (1993). Quantal components of unitary EPSCs at the mossy fibre synapse on CA3 pyramidal cells of rat hippocampus. J. Physiol. *472*, 615–663.

Jung, M.W., and McNaughton, B.L. (1993). Spatial selectivity of unit activity in the hippocampal granular layer. Hippocampus *3*, 165–182.

Kamiya, H., Shinozaki, H., and Yamamoto, C. (1996). Activation of metabotropic glutamate receptor type 2/3 suppresses transmission at rat hippocampal mossy fibre synapses. J. Physiol. *493*, 447–455.

Kesner, R.P., Gilbert, P.E., and Wallenstein, G.V. (2000). Testing neural network models of memory with behavioral experiments. Curr. Opin. Neurobiol. *10*, 260–265.

Kobayashi, K., Manabe, T., and Takahashi, T. (1996). Presynaptic

long-term depression at the hippocampal mossy fiber-CA3 synapse. Science *273*, 648–650.

Kobayashi, K., Manabe, T., and Takahashi, T. (1999). Calcium-dependent mechanisms involved in presynaptic long-term depression at the hippocampal mossy fibre-CA3 synapse. Eur. J. Neurosci. *11*, 1633–1638.

Kovalchuk, Y., Hanse, E., Kafitz, K.W., and Konnerth, A. (2002). Postsynaptic induction of BDNF-mediated long-term potentiation. Science *295*, 1729–1734.

Levy, W.B., and Steward, O. (1983). Temporal contiguity requirements for long-term associative potentiation/depression in the hippocampus. Neuroscience *8*, 791–797.

Li, X.G., Somogyi, P., Ylinen, A., and Buzsáki, G. (1994). The hippocampal CA3 network: An in vivo intracellular labeling study. J. Comp. Neurol. *339*, 181–208.

Li, H., Chen, A., Xing, G., Wei, M.L., and Rogawski, M.A. (2001). Kainate receptor-mediated heterosynaptic facilitation in the amygdale. Nat. Neurosci. *4*, 612–620.

Lisman, J.E. (1997). Bursts as a unit of neural information: making unreliable synapses reliable. Trends Neurosci. *20*, 38–43.

Litschig, S., Gasparini, F., Rueegg, D., Stoehr, N., Flor, P.J., Vranesic, I., Prezeau, L., Pin, J.-P., Thomsen, C., and Kuhn, R. (1999). CPCCOEt, a noncompetitive metabotropic glutamate receptor 1 antagonist, inhibits receptor signaling without affecting glutamate binding. Mol. Pharmacol. *55*, 453–461.

Luján, R., Nusser, Z., Roberts, J.D.B., Shigemoto, R., and Somogyi, P. (1996). Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. Eur. J. Neurosci. *8*, 1488–1500.

Markram, H., Lübke, J., Frotscher, M., and Sakmann, B. (1997). Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. Science *275*, 213–215.

Martin, S.J., Grimwood, P.D., and Morris, R.G.M. (2000). Synaptic plasticity and memory: An evaluation of the hypothesis. Annu. Rev. Neurosci. *23*, 649–711.

Martinez, C.O., Do, V.H., Martinez, J.L., Jr., and Derrick, B.E. (2002). Associative long-term potentiation (LTP) among extrinsic afferents of the hippocampal CA3 region in vivo. Brain Res. *940*, 86–94.

McMahon, D.B.T., and Barrionuevo, G. (2002). Short- and long-term plasticity of the perforant path synapse in hippocampal area CA3. J. Neurophysiol. *88*, 528–533.

McNaughton, B.L., and Morris, R.G.M. (1987). Hippocampal synaptic enhancement and information storage within a distributed memory system. Trends Neurosci. *10*, 408–415.

Miles, R., and Wong, R.K.S. (1986). Excitatory synaptic interactions between CA3 neurones in the guinea-pig hippocampus. J. Physiol. *373*, 397–418.

Miura, M., Watanabe, M., Offermanns, S., Simon, M.I., and Kano, M. (2002). Group I metabotropic glutamate receptor signaling via $G\alpha q/G\alpha 11$ secures the induction of long-term potentiation in the hippocampal area CA1. J. Neurosci. *22*, 8379–8390.

Mori, M., and Gerber, U. (2002). Slow feedback inhibition in the CA3 area of the rat hippocampus by synergistic synaptic activation of mGluR1 and mGluR5. J. Physiol. *544*, 793–799.

Nakazawa, K., Quirk, M.C., Chitwood, R.A., Watanabe, M., Yeckel, M.F., Sun, L.D., Kato, A., Carr, C.A., Johnston, D., Wilson, M.A., and Tonegawa, S. (2002). Requirement for hippocampal CA3 NMDA receptors in associative memory recall. Science *297*, 211–218.

Nishiyama, M., Hong, K., Mikoshiba, K., Poo, M.-m., and Kato, K. (2000). Calcium stores regulate the polarity and input specificity of synaptic modification. Nature *408*, 584–588.

Poncer, J.-C., Shinozaki, H., and Miles, R. (1995). Dual modulation of synaptic inhibition by distinct metabotropic glutamate receptors in the rat hippocampus. J. Physiol. *485*, 121–134.

Rolls, E.T., and Treves, A. (1998). The hippocampus and memory. In Neural Networks and Brain Function (New York: Oxford University Press), pp. 95–135.

Ruiz, A., Fabian-Fine, R., Scott, R., Walker, M.C., Rusakov, D.A., and Kullmann, D.M. (2003). $GABA_A$ receptors at hippocampal mossy fibers. Neuron *39*, 961–973.

Salin, P.A., Scanziani, M., Malenka, R.C., and Nicoll, R.A. (1996). Distinct short-term plasticity at two excitatory synapses in the hippocampus. Proc. Natl. Acad. Sci. USA *93*, 13304–13309.

Sjöström, P.J., Turrigiano, G.G., and Nelson, S.B. (2001). Rate, timing, and cooperativity jointly determine cortical synaptic plasticity. Neuron *32*, 1149–1164.

Smith, M.A., Zhang, L.-X., Lyons, W.E., and Mamounas, L.A. (1997). Anterograde transport of endogenous brain-derived neurotrophic factor in hippocampal mossy fibers. Neuroreport *8*, 1829–1834.

Thomas, M.J., and O'Dell, T.J. (1995). The molecular switch hypothesis fails to explain the inconsistent effects of the metabotropic glutamate receptor antagonist MCPG on long-term potentiation. Brain Res. *695*, 45–52.

Tsukamoto, M., Yasui, T., Yamada, M.K., Nishiyama, N., Matsuki, N., and Ikegaya, Y. (2003). Mossy fibre synaptic NMDA receptors trigger non-Hebbian long-term potentiation at entorhino-CA3 synapses in the rat. J. Physiol. *546*, 665–675.

Urban, N.N., and Barrionuevo, G. (1996). Induction of Hebbian and non-Hebbian mossy fiber long-term potentiation by distinct patterns of high-frequency stimulation. J. Neurosci. *16*, 4293–4299.

Vignes, M., and Collingridge, G.L. (1997). The synaptic activation of kainate receptors. Nature *388*, 179–182.

Vogt, K.E., and Nicoll, R.A. (1999). Glutamate and γ-aminobutyric acid mediate a heterosynaptic depression at mossy fiber synapses in the hippocampus. Proc. Natl. Acad. Sci. USA *96*, 1118–1122.

Walker, M.C., Ruiz, A., and Kullmann, D.M. (2001). Monosynaptic GABAergic signaling from dentate to CA3 with a pharmacological and physiological profile typical of mossy fiber synapses. Neuron *29*, 703–715.

Wilsch, V.W., Behnisch, T., Jäger, T., Reymann, K.G., and Balschun, D. (1998). When are class I metabotropic glutamate receptors necessary for long-term potentiation? J. Neurosci. *18*, 6071–6080.

Yamamoto, C., and Chujo, T. (1978). Long-term potentiation in thin hippocampal sections studied by intracellular and extracellular recordings. Exp. Neurol. *58*, 242–250.

Yeckel, M.F., Kapur, A., and Johnston, D. (1999). Multiple forms of LTP in hippocampal CA3 neurons use a common postsynaptic mechanism. Nat. Neurosci. *2*, 625–633.

Zalutsky, R.A., and Nicoll, R.A. (1990). Comparison of two forms of long-term potentiation in single hippocampal neurons. Science *248*, 1619–1624.

Zhang, L.I., Tao, H.W., Holt, C.E., Harris, W.A., and Poo, M.-m. (1998). A critical window for cooperation and competition among developing retinotectal synapses. Nature *395*, 37–44.