Multiple forms of LTP in hippocampal CA3 neurons use a common postsynaptic mechanism

Mark F. Yeckel, Ajay Kapur and Daniel Johnston

Division of Neuroscience, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, USA
Correspondence should be addressed to D.J. (dan@mossy.bcm.tmc.edu)

We investigated long-term potentiation (LTP) at mossy fiber synapses on CA3 pyramidal neurons in the hippocampus. Using Ca^{2+} imaging techniques, we show here that when postsynaptic Ca^{2+} was sufficiently buffered so that $[Ca^{2+}]_i$ did not rise during synaptic stimulation, the induction of mossy fiber LTP was prevented. In addition, induction of mossy fiber LTP was suppressed by postsynaptic injection of a peptide inhibitor of cAMP-dependent protein kinase. Finally, when ionotropic glutamate receptors were blocked, LTP depended on the postsynaptic release of Ca^{2+} from internal stores triggered by activation of metabotropic glutamate receptors. These results support the conclusion that mossy fiber LTP and LTP at other hippocampal synapses share a common induction mechanism involving an initial rise in postsynaptic $[Ca^{2+}]$.

Dynamic changes in synaptic strength are thought to provide a cellular basis for information storage in the nervous system. For example, long-term potentiation (LTP) of synaptic transmission is a long-lasting, activity-dependent form of synaptic plasticity that is expressed by all principal neurons in the hippocampus—a brain structure implicated in certain forms of long-term memory[1]. In the hippocampus (and elsewhere), multiple forms of LTP have been described: those that require activation of the *N*-methyl-D-aspartate (NMDA) subclass of glutamate receptors for induction, and those that are independent of NMDA receptor activation[2]. At nearly all glutamatergic synapses in the hippocampus, including commissural/associational (C/A) synapses in CA3, LTP induction has been firmly established to depend on an initial postsynaptic rise in cytosolic Ca^{2+} concentration[1,3,4]. This rise can occur via NMDA receptors, voltage-gated Ca^{2+} channels or release from internal stores[5,6], and in turn, triggers a cascade of biochemical events resulting in the expression of an increase in synaptic strength. Although the site of induction of NMDA-receptor-dependent LTP is clearly postsynaptic, the site of expression is more controversial, with reports supporting both pre-[7,8] and postsynaptic mechanisms[9].

The one hippocampal synapse that seems to be an exception to this rule is the mossy fiber input to CA3 pyramidal neurons from dentate granule cells. This synapse has several unusual structural features, including large terminals, multiple release sites and a proximal termination zone along the apical dendrites of CA3 neurons[10,11]. At this synapse, LTP does not require NMDA receptor activation[2] and reportedly can be induced independently of postsynaptic activity; thus, it is thought to be triggered entirely within the presynaptic terminal[3,12]. Other reports, however, have suggested that similar, to NMDA receptor-dependent LTP, the induction of mossy fiber LTP depends on an increase in postsynaptic $[Ca^{2+}]$[13] and is regulated by the postsynaptic membrane potential[14]. Apart from several methodological differences, these contrasting results and conclusions have been difficult to reconcile. More recently, it has been suggested that NMDA-receptor-independent LTP at mossy fiber synapses should be subdivided into two forms, depending on the duration of the stimulus train used for induction: brief trains of high-frequency stimulation applied to the mossy fibers (B-HFS) induce a form of LTP that depends on an initial postsynaptic step, whereas long trains (L-HFS) elicit presynaptically induced LTP[15]. Although the induction mechanisms are still unresolved, there is general agreement that the maintenance or expression of mossy fiber LTP is due to presynaptic changes[16–18].

We have used high-speed fluorescence imaging and improved methods for stimulating and recording from the CA3 region to re-examine the possible contribution of Ca^{2+} to the induction of these hypothesized multiple forms of LTP, and to determine the specific conditions for their induction. LTP induced with either stimulation protocol similarly required a rise in postsynaptic $[Ca^{2+}]$. LTP induced with B-HFS or L-HFS was prevented by chelation of postsynaptic $[Ca^{2+}]$, although the concentration of Ca^{2+} buffer required for blocking LTP was substantially different for the two protocols. Additionally, a rise in postsynaptic $[Ca^{2+}]$, sufficient to induce mossy fiber LTP, occurred even when fast synaptic transmission was blocked by the ionotropic glutamate receptor antagonist kynurenate. These Ca^{2+} rises resulted from release by internal Ca^{2+} stores and depended on metabotropic glutamate receptor (mGluR) activation. Finally, LTP was significantly suppressed by postsynaptic inhibition of cAMP-dependent protein kinase. Taken together, these results suggest that mossy fiber LTP shares with other hippocampal synapses the common induction mechanism of an initial rise in postsynaptic $[Ca^{2+}]$.

Results

Mossy fiber responses to stratum lucidum stimulation

Whole-cell patch-clamp recordings were obtained from visu-

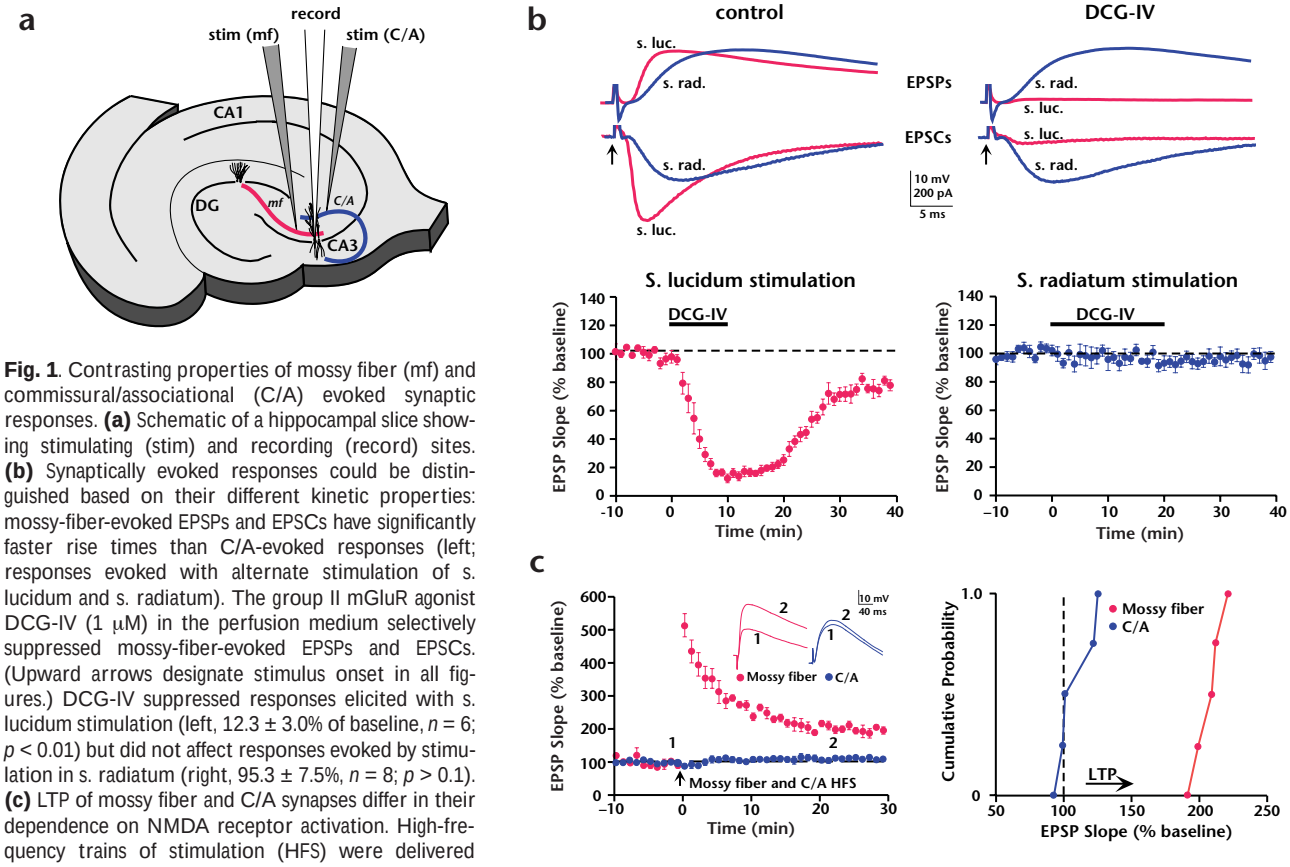

Fig. 1. Contrasting properties of mossy fiber (mf) and commissural/associational (C/A) evoked synaptic responses. **(a)** Schematic of a hippocampal slice showing stimulating (stim) and recording (record) sites. **(b)** Synaptically evoked responses could be distinguished based on their different kinetic properties: mossy-fiber-evoked EPSPs and EPSCs have significantly faster rise times than C/A-evoked responses (left; responses evoked with alternate stimulation of s. lucidum and s. radiatum). The group II mGluR agonist DCG-IV (1 μM) in the perfusion medium selectively suppressed mossy-fiber-evoked EPSPs and EPSCs. (Upward arrows designate stimulus onset in all figures.) DCG-IV suppressed responses elicited with s. lucidum stimulation (left, 12.3 ± 3.0% of baseline, $n = 6$; $p < 0.01$) but did not affect responses evoked by stimulation in s. radiatum (right, 95.3 ± 7.5%, $n = 8$; $p > 0.1$). **(c)** LTP of mossy fiber and C/A synapses differ in their dependence on NMDA receptor activation. High-frequency trains of stimulation (HFS) were delivered simultaneously to mossy fibers and C/A fibers in the presence of the NMDA antagonists APV (50 μM) and MK-801 (20 μM; $n = 5$). Left, following HFS, LTP of mossy-fiber-evoked responses (206.5 ± 5.2%) differed significantly from C/A-evoked responses (108.0 ± 6.5%; $p < 0.001$). Right, cumulative probability plots graphically summarize the data; each point represents the magnitude of change relative to baseline for a given experiment 20–25 min (average) after HFS. The start of the horizontal arrow corresponds to our operational definition for LTP (>20% above baseline). In all figures, responses shown are averages of 3–6 consecutive trials recorded at 0.05–0.1 Hz.

ally identified CA3 pyramidal neurons in hippocampal slices (Fig. 1a). To insure that the responses were evoked monosynaptically by mossy fiber input, we used previously developed biophysical criteria[19] and did additional experiments to distinguish mossy fiber-evoked responses from those of other afferents. We identified mossy fiber responses based on rapid rise of the EPSP and EPSC and short onset latency (<3 ms; Methods; Fig. 1b). The validity of these criteria was tested with the group II mGluR agonist DCG-IV, which selectively blocks mossy fiber responses[20]. DCG-IV significantly reduced the short-latency, fast-rising responses evoked by s. lucidum stimulation, consistent with activation of mGluR2 receptors located on mossy fiber terminals, but had little effect on the slower-rising responses evoked by stimulating the C/A fibers in s. radiatum (Fig. 1b), a region lacking mGluR2 receptors[21].

Despite rejection of data that did not conform to these criteria, it is still possible that C/A fibers traversing s. lucidum could contribute to an apparent mossy fiber LTP. Although the NMDA receptor antagonists APV and MK-801 were present in all experiments (unless otherwise specified), NMDA-receptor-independent LTP is observed in CA1 neurons[5] and might be induced in CA3 neurons by C/A afferents. To test this possibility, high-frequency stimulation was applied simultaneously to s. lucidum and to s. radiatum. The stimulation consisted of three trains of 100 pulses (100 Hz) every 10 seconds, or four of these trains given in conjunction with postsynaptic depolarization (1 nA). Following high-frequency stimulation, the relative change in EPSP slope was significantly greater for mossy fiber stimulation in s. lucidum than for stimulation of C/A afferents in s. radiatum (Fig. 1c), suggesting that NMDA receptor-independent LTP at C/A synapses is unlikely to have contributed to our results.

Mossy fiber LTP depends on a rise in postsynaptic $[Ca^{2+}]$

Previous investigations of mossy fiber LTP differed in both stimulation pattern and total number of pulses (B-HFS, 105 pulses; L-HFS, 300 pulses)[3,14,15]. Therefore, we induced LTP by using the following stimulation protocols: B-HFS, 15 trains of 7 stimuli (100 Hz) repeated every 5 seconds, while depolarizing the CA3 neuron (300–500 pA); L-HFS-3, three trains of long, high-frequency stimulation (100 pulses; 100 Hz; one train every 10 s). We also included a protocol with approximately the same number of total pulses as B-HFS but the sustained stimulation pattern of L-HFS, termed L-HFS-1, the same as L-HFS-3 but with one train (Fig. 2a). The magnitude of LTP induced with L-HFS-3 was significantly greater than that induced by either B-HFS or L-HFS-1. In addition, L-HFS-3 had a significantly higher probability of inducing mossy fiber LTP than either B-HFS or L HFS-1 ($p < 0.01$; Fig. 2b and c).

The previous data suggest that the number of stimulation

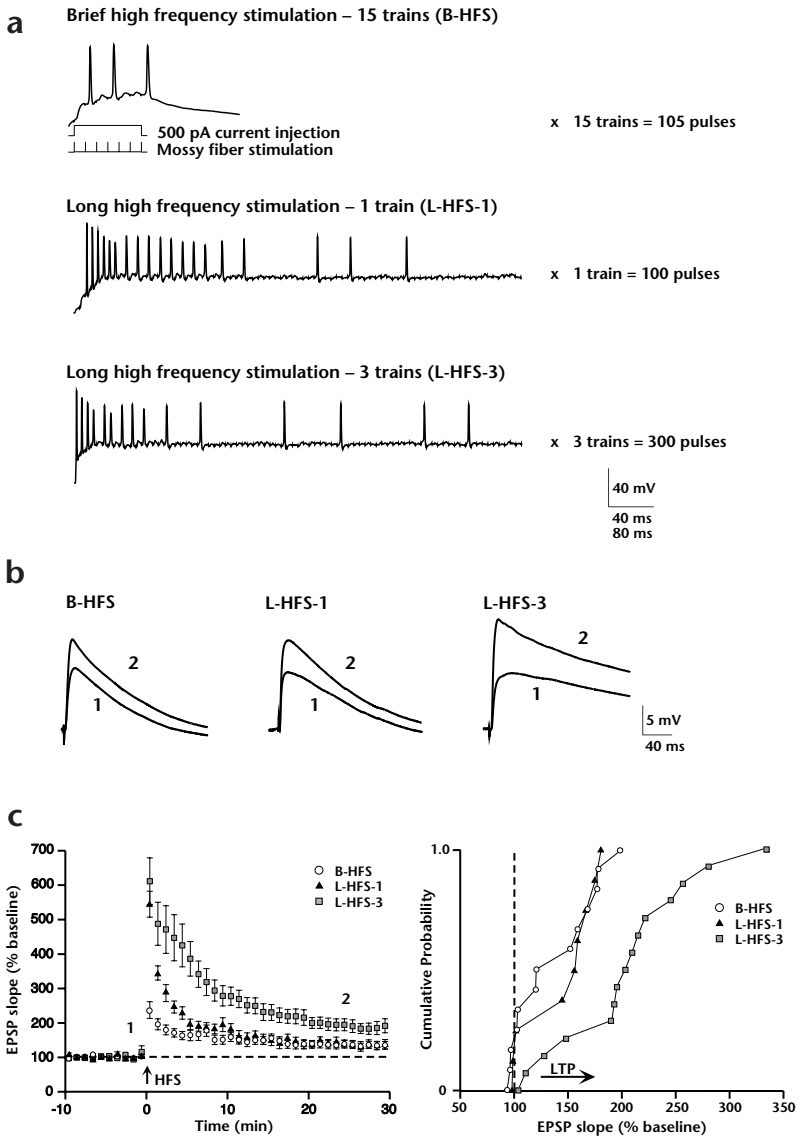

Fig. 2. Mossy fiber LTP induced by three different stimulation protocols. **(a)** Top, a representative response evoked by one train of B-HFS (15 trains, given every 5 seconds, each consisting of 7 stimuli at 100 Hz with simultaneous postsynaptic depolarization; scale bar, 40 ms). Middle and bottom, responses evoked by L-HFS (100 pulses at 100 Hz); L-HFS-1 and L-HFS-3 differ only by the total number of trains (one and three, respectively, given every 10 seconds for L-HFS-3). These responses were evoked by the first and third stimulation trains using L-HFS-3 (scale bar, 80 ms) **(b)** Representative responses before (1) and 25 min after HFS (2). **(c)** Left, time course and magnitude of potentiation evoked by the different stimulation protocols. The magnitude of LTP induced with B-HFS and L-HFS-1 did not differ significantly from each other, but both differed significantly from LTP induced with L-HFS-3 (L-HFS-3, 202.0 ± 16%, n = 15; B-HFS, 140 ± 11%, n = 13; L-HFS-1, 142 ± 11%, n = 9, $p < 0.01$). Right, cumulative probability graph shows the probability and magnitude of LTP induced for each stimulation protocol 20–25 min after HFS (probability: B-HFS, 54%; L-HFS-1, 67%; L-HFS-3, 87%).

pulses used for induction is an important determinant of the magnitude and induction probability of mossy fiber LTP. We next examined whether the protocols were differentially sensitive to the Ca^{2+}-chelator BAPTA. BAPTA (1 mM) was included in the recording pipet to suppress a rise in postsynaptic $[Ca^{2+}]$ evoked by mossy fiber stimulation. At this concentration, postsynaptic BAPTA blocked B-HFS-induced LTP but had little effect on LTP induced with L-HFS-3 (Fig. 3a and c). Higher BAPTA concentrations (5–10 mM), however, blocked L-HFS-1 LTP and significantly suppressed L-HFS-3 LTP (Fig. 3b and c), suggesting a postsynaptic induction mechanism for all three protocols. The probability of inducing LTP was substantially different for the three protocols: with 5–10 mM BAPTA in the pipet, LTP was induced in 5 of 18 cases by L-HFS-3, whereas B-HFS and L-HFS-1 never induced LTP (Fig. 3b and c). Moreover, if the data analysis were limited to only those cases in which L-HFS-3 caused LTP, then it might seem that BAPTA did not significantly affect the magnitude of mossy fiber LTP (5–10 mM BAPTA, 190 ± 23%, n = 5; control, 217 ± 15%, n = 13).

The hypothesis that a rise in postsynaptic $[Ca^{2+}]$ is necessary for the induction of mossy fiber LTP was further explored by quantifying the relative change in $[Ca^{2+}]_i$ during B-HFS and L-HFS with the Ca^{2+}-indicator dye fura-2 (80–200 μM) in the recording pipet. Under control conditions (Fig. 4a), B-HFS evoked a relatively brief increase in $[Ca^{2+}]_i$ (peak, 30 ± 3.1% $\Delta F/F$; half-width, 1.0 ± 0.1 s, n = 6); L-HFS-3 elicited a persistent rise in $[Ca^{2+}]_i$ (peak, 43 ± 4.2% $\Delta F/F$; half-width, 3.1 ± 0.2 s, n = 10). Cumulative probability analysis showed that the peak amplitude of Ca^{2+} fluorescence was not significantly different for the two patterns of stimulation (Fig. 4b), but the duration of the Ca^{2+} response was significantly longer for L-HFS than for B-HFS.

Consistent with the finding that BAPTA blocked B-HFS-induced LTP, BAPTA significantly suppressed a B-HFS-evoked rise in postsynaptic $[Ca^{2+}]$. As predicted from the concentration-dependent effect of BAPTA on LTP, 1 mM BAPTA had little effect on postsynaptic $[Ca^{2+}]$ during L-HFS-3, whereas 5–10 mM BAPTA significantly suppressed this rise in $[Ca^{2+}]_i$. The efficacy of BAPTA depended, at least in part, on the pattern of stimulation used to increase $[Ca^{2+}]_i$: BAPTA was more effective at suppressing transient increases in $[Ca^{2+}]_i$ evoked with B-HFS (1 mM BAPTA, 8.4 ± 1.6% $\Delta F/F$, n = 8; 5–10 mM BAPTA, 2.2 ± 0.5% ΔF/F, n = 6) than sustained increases evoked with L-HFS-3. There was a dose-dependent effect of BAPTA on L-HFS-3: 1 mM BAPTA had little effect on $[Ca^{2+}]_i$ (39.9 ± 3.5% ΔF/F, n = 8), whereas 5–10 mM BAPTA had a significant effect on Ca^{2+} (13.7 ± 3.7% $\Delta F/F$, n = 8) in 63% of the cases (that is, in 47% of the cases Ca^{2+} was not blocked; Fig. 4a and b).

We also examined the rise in $[Ca^{2+}]_i$ evoked by high-frequency stimulation of C/A afferents and the ability of BAPTA (5–10 mM) to suppress $[Ca^{2+}]_i$ during stimulation. (NMDA antagonists were not included in these experiments.) Long stimulation trains applied to C/A afferents elicited a smaller-amplitude and shorter-duration Ca^{2+} signal than that observed during mossy fiber stimulation. BAPTA was significantly more

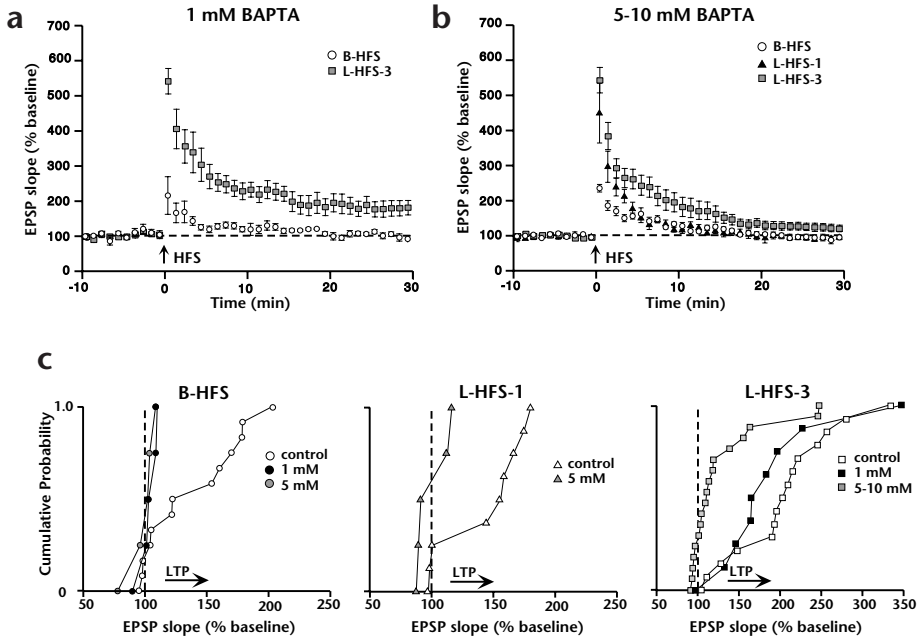

Fig. 3. Effects of postsynaptic BAPTA on the induction of mossy fiber LTP with B-HFS and L-HFS. **(a)** 1 mM BAPTA blocked the induction of LTP by B-HFS (102 ± 3%, n = 5) but had little effect on LTP induced by L-HFS-3 (187 ± 24%, n = 9, $p < 0.01$). **(b)** Higher concentrations of BAPTA (5–10 mM) blocked the induction of mossy fiber LTP induced with B-HFS (99 ± 5%, n = 6) and L-HFS-1 (99 ± 6%, n = 5) and significantly suppressed L-HFS-3-induced LTP (128 ± 11%, n = 18) compared to control (202 ± 16.3%, n = 15, $p < 0.001$). **(c)** Cumulative probability plots summarize the effects of 1 mM and 5–10 mM BAPTA on the three different stimulation protocols. Although 5–10 mM BAPTA blocked the induction of LTP with B-HFS and L-HFS-1 in all cases, LTP was induced in at least 30% of the cases when L-HFS-3 was used.

effective at suppressing the Ca^{2+} signal evoked by C/A afferents (Fig. 4c) than at suppressing the sustained rise in $[Ca^{2+}]_i$ evoked by mossy fibers. These findings are consistent with a previous report that some concentrations of BAPTA can prevent C/A LTP but not mossy fiber LTP[3].

Because 10 mM BAPTA did not block induction of mossy fiber LTP with L-HFS-3 in all CA3 neurons tested, and based on evidence that 30–40 mM BAPTA was required to block certain forms of synaptic plasticity in cerebellar Purkinje neurons[22], we tested whether similarly high concentrations of postsynaptic BAPTA might also be required to completely block mossy fiber LTP. Including 30 or 50 mM BAPTA in the recording pipet prevented a rise in postsynaptic $[Ca^{2+}]$ during the L-HFS-3 stimulus trains and blocked LTP induction in 10 of 10 neurons (Fig. 5). To examine whether high postsynaptic BAPTA might be affecting extracellular $[Ca^{2+}]$ and thereby reducing transmitter release[23], we analyzed the responses observed during the stimulus trains with and without BAPTA in the pipet. There was no significant difference in peak depolarization (control, 45.2 ± 1.5 mV, n = 24; BAPTA, 45.2 ± 3.8 mV, n = 13; $p > 0.05$). In addition, facilitation was not affected by BAPTA, based on comparing the ratio of the amplitude of the response to the second stimulation pulse versus the first pulse (R2/R1; control, 2.3 ± 0.21, n = 13; BAPTA, 2.2 ± 0.18, n = 14; $p > 0.1$) or R10/R3 (control, 1.1 ± 0.02, n = 13; BAPTA, 1.1 ± 0.02, n = 14; $p > 0.1$).

LTP in the absence of fast synaptic transmission

Although the data presented thus far support the hypothesis that postsynaptic $[Ca^{2+}]$ is necessary for the induction of mossy fiber LTP, these findings do not address previous results showing that mossy fiber LTP can be induced in the apparent absence of synaptic transmission[15,24]. In those studies, an ionotropic glutamate receptor antagonist (kynurenate or CNQX) was present during high-frequency stimulation, and despite suppression of fast synaptic transmission, mossy fiber LTP was still observed, leading to the conclusion that induction occurs presynaptically. Based, however, on evidence that high-frequency stimulation of mossy fibers in the presence of glutamate antagonists elicits a rise in postsynaptic $[Ca^{2+}]$[25], we tested whether L-HFS-3 would also evoke a rise in $[Ca^{2+}]_i$ in the presence of kynurenate, and if so, whether it would correlate with the induction of mossy fiber LTP. Addition of kynurenate to the perfusion media resulted in the gradual loss of mossy fiber-evoked responses; when synaptic transmission seemed to be totally blocked (10–20 min), L-HFS-3 stimuli were given, and the rise in $[Ca^{2+}]_i$ was recorded. After high-frequency stimulation, kynurenate was washed out, and mossy fiber-evoked responses were evaluated for at least 60 minutes (Fig. 6a). In 5 of 8 cases, L-HFS elicited a delayed, yet significant rise in $[Ca^{2+}]_i$ localized to the dendrite in s. lucidum (Fig. 6a and c). In each of these five cells, LTP was induced. LTP was not induced in the three cells in which a Ca^{2+} signal was not observed.

We further tested whether the $[Ca^{2+}]_i$ rise merely correlated with LTP induction or whether $[Ca^{2+}]_i$ was required for induction. A concentration of BAPTA (1 mM) shown previously to be insufficient to prevent LTP with the L-HFS-3 protocol was included in the patch-recording pipet and kynurenate added to the perfusion media. Combining kynurenate and low BAPTA prevented a rise in $[Ca^{2+}]_i$ and completely blocked the induction of mossy fiber LTP (Fig. 6b and c). This result indicates that even when fast synaptic transmission is blocked, mossy fiber LTP depends on postsynaptic $[Ca^{2+}]$.

Induction of mGluR-dependent mossy fiber LTP

Although fast synaptic transmission, as measured by the amplitude of single EPSPs, was blocked in the kynurenate experiments, a sustained depolarization of between 5 and 30 mV was still observed in some cells during long trains of stimulation. Because kynurenate is a relatively low-affinity, competitive antagonist, the large amount of glutamate released during the trains might partially displace kynurenate from glutamate binding sites, resulting in the small, sustained depolarization. To test whether the rise in $[Ca^{2+}]_i$ that we observed in the presence of kynurenate depended on this sustained depolarization, we held cells at –75 mV under voltage clamp during the trains. We

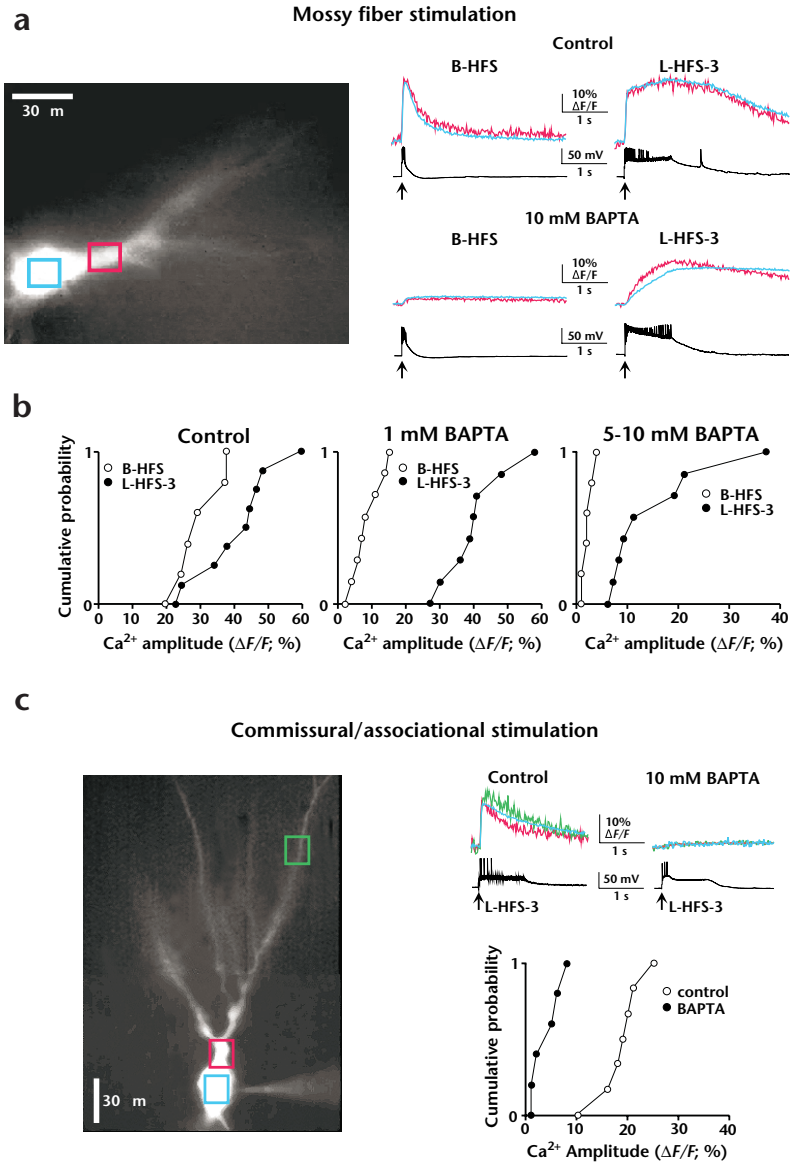

Fig. 4. Ca^{2+} fluorescence imaging during B-HFS and L-HFS. **(a)** Example fura 2-filled CA3 pyramidal neuron. Colored boxes show where postsynaptic Ca^{2+} transients were measured during high-frequency stimulation. Right, data from individual experiments, including one of the three cells in which BAPTA did not appear to block the Ca^{2+} transient evoked by L-HFS-3. (BAPTA data are from the neuron on the left.) **(b)** Left, under control conditions, peak $[Ca^{2+}]_i$ did not differ significantly for B-HFS and L-HFS-3; however, the half-width was significantly different for the two patterns of stimulation (Kolmogrov-Smirnov, $p < 0.001$; data not shown). Middle, 1 mM BAPTA significantly suppressed a rise in $[Ca^{2+}]_i$ evoked by B-HFS compared to control (Kolmogrov-Smirnov, $p < 0.001$) but did not significantly affect L-HFS-3-evoked $[Ca^{2+}]_i$. Right, 5–10 mM BAPTA significantly suppressed peak $[Ca^{2+}]_i$ for both B-HFS and L HFS-3 (Kolmogrov-Smirnov, $p < 0.001$). **(c)** Ca^{2+} transients evoked by L-HFS-3 of C/A. (NMDA antagonists were not present.) $[Ca^{2+}]_i$ was evaluated at several dendritic branches and at several locations on an individual dendritic branch. The site with the largest Ca^{2+} response was used for quantitation (control peak, 18.4 ± 1.8% ΔF/F; control half-width, 2.0 ± 0.3 s, $n = 7$). 5–10 mM BAPTA effectively blocked a rise in $[Ca^{2+}]_i$ (3.8 ± 1.2% ΔF/F, $n = 6$; Kolmogrov-Smirnov, $p < 0.05$). Ca^{2+} traces and electrical traces are from individual experiments and are averages of three trains of either B-HFS or L-HFS.

found that the rise in $[Ca^{2+}]_i$ evoked by L-HFS with the cells in voltage clamp was not significantly different from the rise evoked with cells in current clamp (50.3 ± 6.6% versus 50.9 ± 5.8% ΔF/F, $p > 0.05$, $n = 6$). These results suggest that the rise in postsynaptic $[Ca^{2+}]$ in the presence of kynurenate was not due solely to the activation of voltage-gated Ca^{2+} channels.

To identify the source of this Ca^{2+}, we tested the hypothesis that when fast synaptic transmission is blocked (and voltage-dependent Ca^{2+} channels are not activated), the delayed rise in $[Ca^{2+}]_i$ revealed during L-HFS-3 results from Ca^{2+} release from internal stores. We included either ryanodine (20 μM) or thapsigargin (5 μM) in the media to deplete internal Ca^{2+} stores (for review, see ref. 26). In all cases, ryanodine ($n = 3$) or thapsigargin ($n = 2$) irreversibly blocked a rise in Ca^{2+} during L-HFS-3 (Fig. 7b). To determine whether internal Ca^{2+} release was triggered by the presumed second messenger inositol 1,4,5-trisphosphate via activation of group I mGluR receptors[6,27], we included one of three different antagonists for group I mGluRs in the bath. Addition of either of the competitive antagonists MCPG (500 μM) or AIDA (500 μM) reversibly blocked the rise in Ca^{2+} during L-HFS-3 ($n = 3$ and $n = 6$, respectively; Fig. 7a and b). Similarly, the noncompetitive antagonist CPCCOEt (100 μM) reversibly blocked the rise in Ca^{2+} during L-HFS-3 ($n = 3$; Fig. 7b).

Finally, we determined whether antagonists of group I mGluRs, in conjunction with kynurenate (10 mM), blocked the induction of mossy fiber LTP. In all experiments, AIDA ($n = 3$) or CPCCOEt ($n = 3$) blocked the rise in $[Ca^{2+}]_i$, and correspondingly prevented mossy fiber LTP (Fig. 7c). Although group I mGluRs are found primarily at postsynaptic sites on CA3 pyramidal neurons[21], it is possible that the group I antagonists used in these experiments were also acting presynaptically to reduce transmitter release. However, there was no significant difference in sustained depolarizations during the trains with and without mGluR antagonists in the bath (control, 16.7 ± 2.2 mV, $n = 24$; antagonist, 14.6 ± 4.5 mV, $n = 8$; $p > 0.05$). Taken together, these results support the conclusion that when fast synaptic transmission is blocked, the rise in postsynaptic $[Ca^{2+}]$ observed during long trains of mossy fiber stimulation is mediated, at least in part, by mGluR-stimulated release of Ca^{2+} from internal stores, and that this rise in $[Ca^{2+}]_i$ is required to induce mossy fiber LTP.

Mossy fiber LTP depends on postsynaptic PKA

LTP of mossy fiber synapses can be blocked by extracellular PKA inhibitors[28,29]. Thus, it was suggested that the inhibitors were acting on the presynaptic mossy fiber terminal where an increase in cAMP is thought to be triggered by Ca^{2+} influx during stimulation trains. Based on our findings that mossy fiber LTP induction requires postsynaptic $[Ca^{2+}]$ elevation, and previous findings

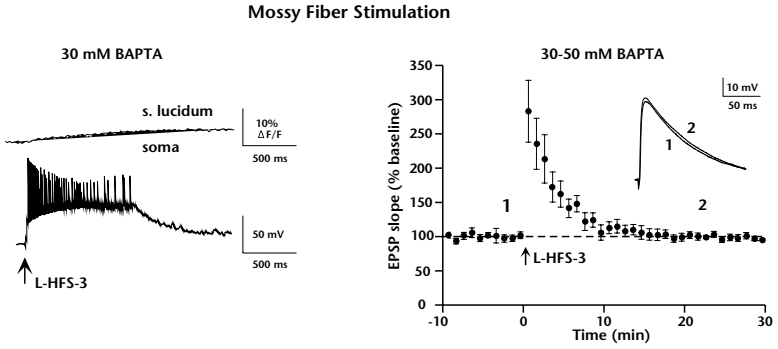

Fig. 5. High BAPTA blocks Ca^{2+} signals and LTP. High concentrations of BAPTA (30–50 mM) in the postsynaptic patch pipet blocked Ca^{2+} transients during L-HFS-3 (left, Ca^{2+} responses from the soma and s.lucidum; 2.7 ± 0.3% ΔF/F) and correspondingly prevented LTP induction (101.2 ± 4.3% of baseline, $n = 10$, $p < 0.001$ versus control).

Discussion

These results strongly support the conclusion that, as with other hippocampal synapses, LTP induction at the mossy fiber synapse depends on an initial rise in postsynaptic $[Ca^{2+}]$: manipulations that conclusively blocked a rise in postsynaptic $[Ca^{2+}]$ prevented the induction of LTP. In further support that the induction of mossy fiber LTP occurs postsynaptically, PKA inhibitors injected postsynaptically significantly reduced the magnitude of LTP. Given that the induction of LTP at other mammalian synapses, as well as some invertebrate synapses, also depends on an increase in postsynaptic $[Ca^{2+}]$[31,32], it is reasonable to speculate that transient changes in postsynaptic $[Ca^{2+}]$ may be a ubiquitous and highly conserved feature of neurons for the regulation of synaptic input strength.

Despite its similarity with other synapses in requiring a rise in postsynaptic $[Ca^{2+}]_i$ for LTP, it is clear that this is an unusual synapse for which different stimulus protocols invoke separate mechanisms for increasing intracellular $[Ca^{2+}]$. Each mossy fiber bouton terminates on the proximal portion of the apical dendrites and has up to 35 release sites[10]. This region of the dendrites has a high density of voltage-gated Ca^{2+} channels[33], and because of the local surface-to-volume ratio, undergoes a larger rise in $[Ca^{2+}]_i$ than any other site in the neuron during somatic and proximal dendritic depolariza-

that postsynaptic injection of the cAMP analog 8-bromo-cAMP facilitates LTP induction[30], we examined the possibility that a postsynaptic $[Ca^{2+}]$ rise might also trigger a cAMP cascade and consequently activate PKA. When the PKA inhibitor peptide 5–24 (30–100 μM) was included in our whole-cell recording pipets, L-HFS-3 induced a decremental form of potentiation significantly smaller than potentiation under control conditions (Fig. 8). Mean potentiation 26–30 minutes after tetanus was 133 ± 9% ($n = 12$) with the inhibitor peptide and 221 ± 22% ($n = 5$) without it ($p < 0.05$). The peptide had no apparent effect on the intrinsic properties of CA3 pyramidal cells (in peptide, $n = 12$, versus controls, $n = 5$, respectively, resting membrane potential, –71 ± 1 mV versus –69 ± 1 mV; input resistance, 129 ± 12 MΩ versus 132 ± 18 MΩ; membrane time constant, 55 ± 4 ms 55 ± 5 ms). In addition, we tested whether the peptide caused a generalized depression of responses by extending our baseline period to 30 minutes before L-HFS-3 ($n = 3$). In these experiments, we never observed a decrement in the slope or amplitude of mossy fiber-evoked responses during the baseline period, and LTP was still significantly reduced following L-HFS-3 (data not shown).

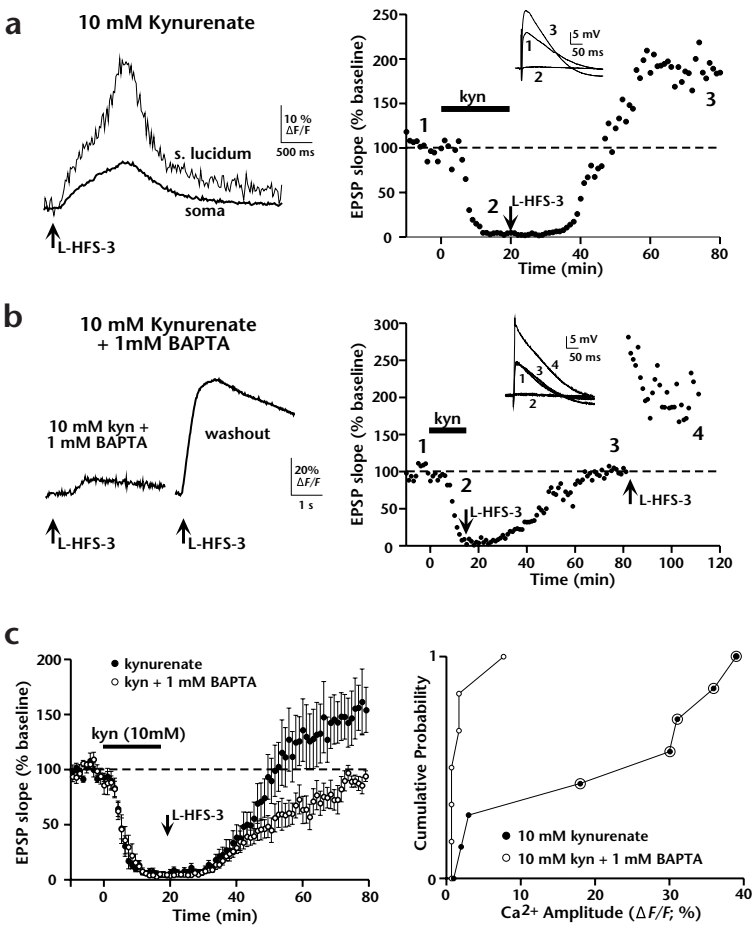

Fig. 6. Suppressing synaptic transmission alone does not prevent mossy fiber LTP. **(a)** Individual experiment showing that the glutamate antagonist kynurenate (10 mM) in the perfusion medium blocked fast synaptic transmission but did not prevent an L-HFS-3-evoked rise in postsynaptic $[Ca^{2+}]_i$ (left, average of all experiments, 20.4 ± 5.5% ΔF/F, $n = 8$), nor did it prevent mossy fiber LTP (right). **(b)** Combining kynurenate and a low dose of postsynaptic BAPTA (1 mM) prevented a rise in $[Ca^{2+}]_i$ (left, average of all experiments, 2.3 ± 1.0% ΔF/F, $n = 7$) and prevented LTP induction (right). After kynurenate washout, L-HFS-3 evoked a robust Ca^{2+} transient (left), resulting in mossy fiber LTP (right). **(c)** Right, time course and magnitude of potentiation induced in the presence of 10 mM kynurenate or 1 mM BAPTA and 10 mM kynurenate ($p < 0.05$). Left, cumulative probability graph showing L-HFS-evoked Ca^{2+} responses when either kynurenate or kynurenate and BAPTA were present. Circled dots represent cases in which LTP was induced in the presence of kynurenate; in all of these cases, a Ca^{2+} transient was observed.

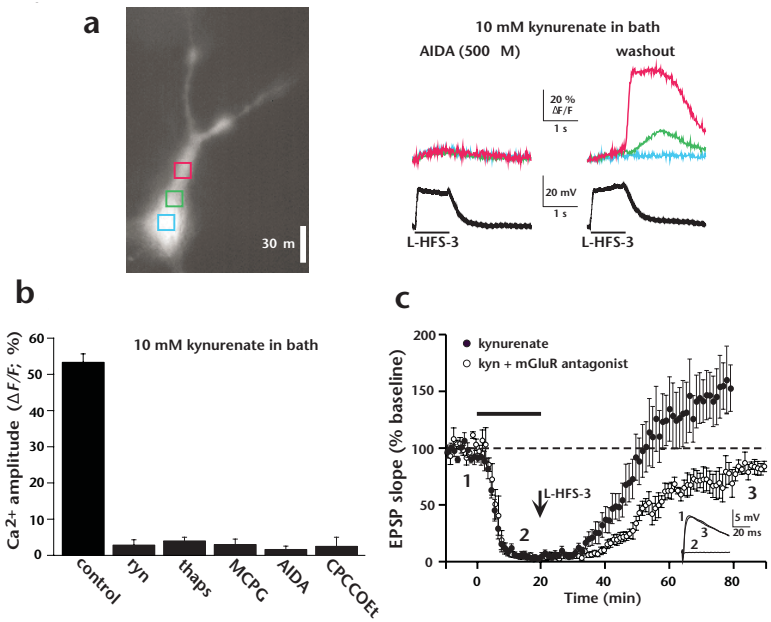

Fig. 7. Ca^{2+} release from internal stores when fast synaptic transmission is blocked. **(a)** In the presence of kynurenate (8–10 mM), L-HFS-3 elicited a rise in $[Ca^{2+}]_i$. An individual experiment in which the mGluR1 antagonist AIDA (500 μM) blocked the rise in $[Ca^{2+}]_i$ during L-HFS. After washout (30 min), a large rise in $[Ca^{2+}]_i$ was evoked in the proximal dendrite by L-HFS. In this case, a sustained depolarization was observed both with and without AIDA. (Waveforms are from single stimulation trains.) **(b)** Ryanodine (ryn; $n = 3$), thapsigargin (thaps; $n = 2$), MCPG ($n = 3$), AIDA ($n = 6$) or CPCCOEt ($n = 3$) blocked the rise in postsynaptic $[Ca^{2+}]$, consistent with the block of Ca^{2+} release from internal stores. **(c)** AIDA ($n = 3$) or CPCCOEt ($n = 3$) blocked mossy fiber LTP when fast synaptic transmission was blocked (83.1 ± 4.3%, $n = 6$; data were combined for the two drugs). These results were significantly different from LTP induced without mGluR antagonists present ($p < 0.05$). Horizontal bar indicates drug application.

tions. Furthermore, mossy fiber synapses show tremendous frequency facilitation, leading to plateau depolarizations and large, prolonged rises in $[Ca^{2+}]_i$ during long stimulus trains (see Fig. 2a and 4a). Part of this plateau depolarization may also be contributed by kainate receptors[34,35], which represent a substantial proportion of glutamate receptors on mossy fiber terminals. Whatever the cause of the prolonged depolarization, it is not surprising that postsynaptic $[Ca^{2+}]$ is difficult to buffer during multiple L-HFS protocols with concentrations of BAPTA or EGTA that are effective in preventing a rise in $[Ca^{2+}]_i$ at other synapses[3,12,36]. High concentrations of BAPTA are also required to block certain forms of synaptic plasticity in Purkinje cells[22], and 100 mM EGTA is required to block mossy fiber LTP in single granule cell cultures[37]. Although an initial rise in postsynaptic $[Ca^{2+}]$ may be a required step for mossy fiber LTP induction, maintenance or expression of this LTP seems to involve presynaptic changes[16,17,38], suggesting that a retrograde signal is involved in linking these two events.

The CA3 region, and s. lucidum in particular, are heavily innervated by norepinephrine-containing fibers, and β-adrenoceptors acting through the cAMP signal transduction cascade are important in modulating mossy fiber synaptic plasticity, including LTP and long-term depression (LTD)[30,39,40]. Consistent with these findings, our results suggest that postsynaptic activation of PKA might be required for the full expression of mossy fiber LTP. This second messenger cascade could be triggered by a rise in postsynaptic $[Ca^{2+}]$ during HFS of mossy fibers, including co-activation of norepinephrine-containing fibers[30], as mice with mutations in the Ca^{2+}-stimulated type I adenylyl cyclase (AC1) gene have an impaired ability to express mossy fiber LTP[41]. There is, however, good evidence for presynaptic regulation of mossy fiber synaptic transmission by cAMP-dependent mechanisms[18], as well as data showing a retrograde signaling system in which postsynaptic excitation of PKA can influence presynaptic transmitter release[42]. Therefore, both pre- and postsynaptic cAMP pathways are likely to regulate these synapses.

The differences between brief trains and long trains of synaptic activation for inducing synaptic plasticity have been particularly intriguing in both the present and previous experiments[15]. B-HFS-induced LTP can be prevented when L-type Ca^{2+} channels are blocked[19], whereas L-HFS-3 LTP is not prevented. Here we found that even with synaptic transmission mostly blocked by the low-affinity, competitive antagonist kynurenate, a rise in postsynaptic $[Ca^{2+}]$ still occurred and could initiate LTP. These results suggest that multiple mechanisms for increasing $[Ca^{2+}]$ in the postsynaptic neuron are engaged by different stimulus protocols[24,26]. Voltage-gated Ca^{2+} channels seem to dominate for shorter trains, whereas the

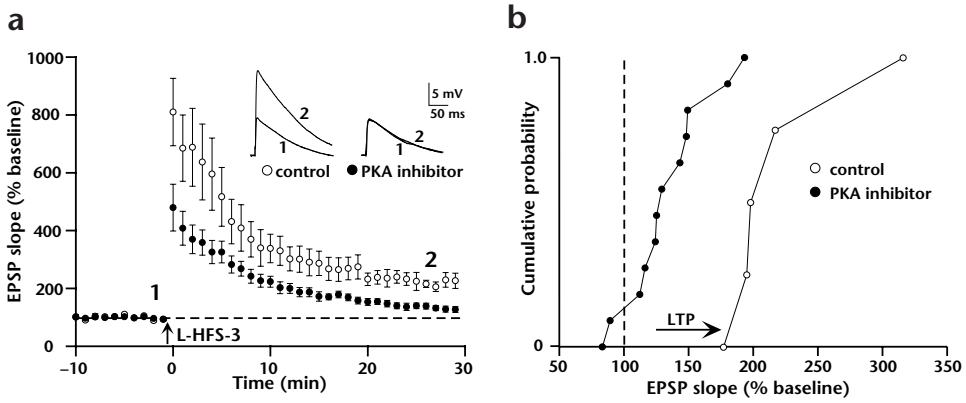

Fig. 8. Postsynaptic inhibition of PKA blocked mossy fiber LTP. **(a)** PKA inhibitor peptide (5-24; 30–100 μM) in the recording pipet significantly reduced potentiation compared to control (133 ± 9%, $n = 12$ and 221 ± 22%, $n = 5$, respectively; $p < 0.05$). **(b)** Cumulative probability.

longer trains may favor other routes of Ca^{2+} entry and/or Ca^{2+} release. In support of this possibility, prolonged stimulation demonstrates kainate receptor and mGluR activation at mossy fiber synapses[25,34,35]. Furthermore, one report has suggested that, under some conditions, mGluR blockade may inhibit mossy fiber LTP[43].

One point of agreement among studies of mossy fiber plasticity is that LTP induced with long trains of stimulation cannot be prevented by hyperpolarizing the postsynaptic membrane potential[3,14] and that L-HFS LTP does not require additional (or even much) depolarization of the neuron for its induction. These conclusions are supported by the kynurenate experiments reported here and elsewhere[15,24], and strengthen the argument that B-HFS-induced LTP may require an associative interaction with another input (experimentally, a paired depolarization), whereas L-HFS-induced LTP does not depend on either a Hebbian or cooperative mechanism[15,44]. Because of the paucity of innervation of CA3 pyramidal cells by granule cells, it has been suggested[46] that no more than one mossy fiber synapse on a given CA3 neuron is active at any one time during some behaviors of the animal. Because granule cells have been observed *in vivo* to fire in both brief bursts and long trains[47], this suggests that LTP is not likely to occur with brief periods of mossy fiber excitation unless the bursting is associated with concomitant input from C/A or perforant path input. In contrast, long periods of granule cell firing could elicit LTP even if only a single mossy fiber were active and there were no other associative synaptic inputs to the cell. The results presented here provide plausible mechanisms for how different firing patterns of granule cells can alter postsynaptic $[Ca^{2+}]$ and thereby increase mossy fiber synapse strength.

Methods

Preparation of slices and solutions. Hippocampal slices (400 μm) were prepared from Sprague-Dawley rats (21–28 days) as described[19]. All experimental procedures were approved by the Animal Research Committee of Baylor College of Medicine. A Zeiss Axioskop, fitted with a 40× Zeiss water-immersion objective and differential interference contrast (DIC) optics, was used to view slices. The bathing solution contained 125 mM NaCl, 2.5 mM KCl, 25 mM $NaHCO_3$, 4 mM $MgCl_2$, 5 mM $CaCl_2$ and 10 mM dextrose with 95% O_2, 5% CO_2. NMDA receptor antagonists D,L-APV (50 μM) and MK-801 (20 μM), plus $GABA_A$ receptor antagonists (–)-bicuculline methiodide (10–20 μM) and picrotoxin (10 μM) were present in the bathing solution during recording, unless stated otherwise. Where specified, one or more of the following drugs was included in the media: DCG-IV (Tocris), prepared from a stock solution dissolved in 10 mM DMSO; kynurenate (Sigma), dissolved directly into the media (pH 7.2 with NaOH); ryanodine or thapsigargin (RBI), prepared from stock solutions dissolved in 20 mM or 5 mM DMSO, respectively; MCPG (RBI), AIDA (Tocris), or CPCCOEt (Tocris), dissolved directly into the media.

Recording and stimulating. Whole-cell patch-recording pipets (3–6 MΩ) were pulled from borosilicate glass and filled with 110–120 mM K-gluconate, 20 mM KCl, 10 mM HEPES, 2 mM $MgCl_2$, 4 mM ATP (disodium salt), 0.3 mM Tris-GTP and 7 mM phosphocreatine (pH 7.3). When high concentrations of BAPTA (> 30 mM) were added to the recording pipet, it was necessary to use tetrapotassium BAPTA ($4K^+$-BAPTA; Sigma) as the charge carrier (instead of K-gluconate) to maintain proper osmolarity and ionic balance in the whole-cell pipets. In these experiments, three different solutions were used: 50 mM $4K^+$-BAPTA (n = 3), 30 mM $4K^+$-BAPTA and 20 mM BAPTA (n = 4) or 30 mM $4K^+$-BAPTA (n = 3). Whole-cell patch-clamp recordings were made from the soma of visually identified CA3 pyramidal neurons with either an Axoclamp 2A amplifier in bridge mode or a SEC 05L amplifier (Adams and List Associates) in bridge or discontinous voltage-clamp modes. The resting membrane potential was between –60 and –75 mV. LTP experiments were done in current clamp because EPSP slopes were less sensitive than EPSCs to changes in series resistance. In addition, coating electrodes with sylgard and using the capacitance compensation circuitry of the SEC amplifier significantly reduced the sensitivity of both the slope and peak amplitude measurements to changes in series resistance.

Mossy fiber axons were stimulated electrically with a bipolar microelectrode (glass pipet with tip diameter of ~4 μm, filled with media solution, glued to a fine tungsten rod) placed in s. lucidum (30–50 μm from the edge of the pyramidal cell layer) at a lateral distance of 75–200 μm from the recording pipet. In some experiments, a stimulating electrode was placed in s. radiatum (150–200 μm from s. pyramidale) to activate C/A afferents. Test pulses were delivered every 10–20 seconds; a hyperpolarizing current pulse (20 pA, 300 ms) was injected into the cell between test pulses to monitor input resistance and series resistance. Because of convergence of multiple afferent systems onto CA3 neurons (for example, mossy fiber, C/A and perforant path) and the relative sparseness of mossy fiber input, interpretation of synaptically evoked CA3 responses as exclusively monosynaptic is problematic[48,49]. For example, placement of a stimulating electrode at any site in the hippocampus capable of directly activating mossy fibers may also excite neighboring afferents. In addition, excitation of CA3 neurons sufficient to generate an action potential can lead to polysynaptic activation via the powerful excitatory recurrent pathways. Therefore, amplitude measurements were made on the initial portion (1 ms from onset) of EPSPs that were elicited by stimulation in s. lucidum and that conformed to previously developed rise time criteria; rapid rise times of mossy fiber responses (EPSP < 2.5 ms; EPSC < 1.5 ms, 20–80%) are due to the proximity of the synapses to the soma; in contrast, activation of C/A afferents, terminating more distally, have slower kinetics (see ref. 19). The amplitude range of evoked EPSPs was ~5–15 mV (< 50% of threshold for generating an action potential). LTP was operationally defined as >20% increase above baseline for the slope of the EPSP rising phase from 21–25 min after high-frequency stimulation, and unless stated otherwise, one-way ANOVAs with Fishers PLSD *post-hoc* analysis were used to determine significance. Data are reported as mean ± s.e.

In experiments evaluating release of Ca^{2+} from internal stores, a loading protocol was sometimes used to ensure that Ca^{2+} stores were replenished after L-HFS. As described[27], we gave 3–4 depolarizing pulses (2–3 nA; 1-second duration), sufficient to evoke a train of approximately 20 action potentials.

Fluorescence imaging. To measure changes in $[Ca^{2+}]_i$, the fluorescence indicator fura-2 (80–200 μM) was included in the pipet solution and allowed to diffuse into the neuron for at least 15 min before optical recordings began. Using a cooled charge-coupled device (CCD) camera (Photometrics, Tucson, Arizona) in sequential frame transfer mode[50], we recorded high-speed fluorescence images. Relative changes in $[Ca^{2+}]_i$ were quantified as changes in $\Delta F/F$, where F is fluorescence intensity before stimulation (after subtracting autofluorescence) and ΔF is the change from this value during neuronal activity (corrected for bleaching during optical recording). The bleaching correction was determined by measuring fluorescence in the neuron without stimulation. Tissue autofluorescence was determined by measuring fluorescence at an equivalent measurement at a parallel location in the slice that was away from the dye-filled cell. We used 380-nm light (13-nm bandpass filter; Omega Optical) to excite fura-2. The sequential frame rate was 50 Hz, and pixels were binned in a 10 by 10 array.

Acknowledgements

We thank Tom Brown, Jeff Magee, Nick Poolos and Bob Zucker for comments on the manuscript and Rick Gray for help through various stages of this project. We also thank Nathan Urban for help with L-HFS LTP, David Sweatt for advice on the PKA inhibitor experiments and Rob Gereau for advice on the mGluR experiments. This work was supported by NIH grants MH11390 (MFY) and MH44754 and MH48432 (D.J.), HFSP and the Hankamer Foundation.

RECEIVED 22 APRIL; ACCEPTED 25 MAY 1998

1. Bliss, T. V. & Collingridge, G. L. A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361, 31–39 (1993).
2. Johnston, D., Williams, S., Jaffe, D. & Gray, R. NMDA-receptor-independent long-term potentiation. *Annu. Rev. Physiol.* 54, 489–505 (1992).
3. Zalutsky, R. A. & Nicoll, R. A. Comparison of two forms of long-term potentiation in single hippocampal neurons. *Science* 248, 1619–1624 (1990).
4. Yang, S.-N., Tang, Y.-G. & Zucker, R. S. Selective induction of LTP and LTD by postsynaptic $[Ca^{2+}]_i$ elevation. *J. Neurophysiol.* 81, 781–787 (1999).
5. Grover, L. M. & Teyler, T. J. N-methyl-D-aspartate receptor-independent long-term potentiation in area CA1 of rat hippocampus: input-specific induction and preclusion in a non-tetanized pathway. *Neuroscience* 49, 7–11 (1992).
6. Wilsch, V. W., Behnisch, T., Jaeger, T., Reymann, K. G. & Balschun, D. When are class I metabotropic glutamate receptors necessary for long-term potentiation? *J. Neurosci.* 18, 6071–6080 (1998).
7. Bolshakov, V. Y., Golan, H., Kandel, E. R. & Siegelbaum, S. A. Recruitment of new sites of synaptic transmission during the cAMP-dependent late phase of LTP at CA3-CA1 synapses in the hippocampus. *Neuron* 19, 635–651 (1997).
8. Stevens, C. F. & Wang, Y. Changes in reliability of synaptic function as a mechanism for plasticity. *Nature* 371, 704–707 (1994).
9. Isaac, J. T., Nicoll, R. A. & Malenka, R. C. Evidence for silent synapses: implications for the expression of LTP. *Neuron* 15, 427–434 (1995).
10. Chicurel, M. E. & Harris, K. M. Three-dimensional analysis of the structure and composition of CA3 branched dendritic spines and their synaptic relationships with mossy fiber boutons in the rat hippocampus. *J. Comp. Neurol.* 325, 169–182 (1992).
11. Claiborne, B. J., Amaral, D. G. & Cowan, W. M. A light and electron microscopic analysis of the mossy fibers of the rat dentate gyrus. *J. Comp. Neurol.* 246, 435–458 (1986).
12. Katsuki, H., Kaneko, S., Tajima, A. & Satoh, M. Separate mechanisms of long-term potentiation in two input systems to CA3 pyramidal neurons of rat hippocampal slices as revealed by the whole-cell patch-clamp technique. *Neurosci. Res.* 12, 393–402 (1991).
13. Williams, S. & Johnston, D. Long-term potentiation of hippocampal mossy fiber synapses is blocked by postsynaptic injection of calcium chelators. *Neuron* 3, 583–588 (1989).
14. Jaffe, D. & Johnston, D. The induction of long-term potentiation at hippocampal mossy fibers follows a Hebbian rule. *J. Neurophysiol.* 64, 948–960 (1990).
15. Urban, N. N. & Barrionuevo, G. Induction of hebbian and non-hebbian mossy fiber long-term potentiation by distinct patterns of high-frequency stimulation. *J. Neurosci.* 16, 4293–4299 (1996).
16. Castillo, P. E., Janz, R., Südhof, T. C., Tzounopoulos, T., Malenka, R. C. & Nicoll, R. A. Rab3A is essential for mossy fibre long-term potentiation in the hippocampus. *Nature* 388, 590–593 (1997).
17. Xiang, Z., Greenwood, A. C., Kairiss, E. W. & Brown, T. H. Quantal mechanism of long-term potentiation in hippocampal mossy-fiber synapses. *J. Neurophysiol.* 71, 2552–2556 (1994).
18. López-García, J. C., Arancio, O., Kandel, E. R. & Baranes, D. A presynaptic locus for long-term potentiation of elementary synaptic transmission at mossy fiber synapses in culture system. *Proc. Natl. Acad. Sci. USA* 93, 4712–4717 (1996).
19. Kapur, A., Yeckel, M. F., Gray, R. & Johnston, D. L-type calcium channels are required for one form of hippocampal mossy fiber LTP. *J. Neurophysiol.* 79, 2181–2190 (1998).
20. Kamiya, H., Shinozaki, H. & Yamamoto, C. Activation of metabotropic receptor type 2/3 suppresses transmission at rat hippocampal mossy fibre synapses. *J. Physiol. (Lond.)* 493, 447–455 (1996).
21. Shigemoto, R. *et al.* Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. *J. Neurosci.* 17, 7503–7522 (1997).
22. Llano, I., Leresche, N. & Marty, A. Calcium entry increases the sensitivity of cerebellar Purkinje cells to applied GABA and decreases inhibitory synaptic currents. *Neuron* 6, 565–574 (1991).
23. Bliss, T. V. & Lynch, M. A. in *Long-Term Potentiation: From Biophysics to Behavior* (eds. Landfield, P. W. & Deadwyler, S. A.) 3–72 (Liss, New York, 1988).
24. Castillo, P. E., Weisskopf, M. G. & Nicoll, R. A. The role of Ca^{2+} channels in hippocampal mossy fiber synaptic transmission and long-term potentiation. *Neuron* 12, 261–269 (1994).
25. Pozzo Miller, L. D., Petrozzino, J. J., Golarai, G & Connor, J. A. Ca^{2+} release from intracellular stores induced by afferent stimulation of CA3 pyramidal neurons in hippocampal slices. *J. Neurophysiol.* 76, 554–562 (1996).
26. Berridge, M. J. Neuronal calcium signaling. *Neuron* 21, 13–26 (1998).
27. Jaffe, D. B. & Brown, T. H. Metabotropic glutamate receptor activation induces calcium waves within hippocampal dendrites. *J. Neurophysiol.* 78, 10–18 (1997).
28. Huang, Y.-Y., Li, X.-C. & Kandel, E. R. cAMP contributes to mossy fiber LTP by initiating both a covalently mediated early phase and macromolecular synthesis-dependent late phase. *Cell* 79, 69–79 (1994).
29. Weisskopf, M. G., Castillo, P. E., Zalutsky, R. A. & Nicoll, R. A. Mediation of hippocampal mossy fiber long-term potentiation by cyclic AMP. *Science* 265, 1878–1882 (1994).
30. Hopkins, W. F. & Johnston, D. Noradrenergic enhancement of long-term potentiation at mossy fiber synapses in the hippocampus. *J. Neurophysiol.* 59, 667–687 (1988).
31. Bao, J.-X., Kandel, E. R. & Hawkins, R. D. Involvement of presynaptic and postsynaptic mechanisms in a cellular analog of classical conditioning at Aplysia sensory-motor neuron synapses in isolated cell culture. *J. Neurosci.* 18, 458–466 (1998).
32. Murphy, G. G. & Glanzman, D. L. Mediation of classical conditioning in *Aplysia californica* by long-term potentiation of sensorimotor synapses. *Science* 278, 467–471 (1997).
33. Westenbroek, R. E., Ahlijanian, M. K. & Catterall, W. A. Clustering of L-type Ca^{2+} channels at the base of major dendrites in hippocampal pyramidal neurons. *Nature* 347, 281–284 (1990).
34. Castillo, P. A., Malenka, R. C. & Nicoll, R. A. Kainate receptors mediate a slow postsynaptic current in hippocampal CA3 neurons. *Nature* 388, 182–186 (1997).
35. Vignes, M. & Collingridge, G. L. The synaptic activation of kainate receptors. *Nature* 388, 179–182 (1997).
36. Langdon, R. B., Johnson, J. W. & Barrionuevo, G. Posttetanic potentiation and presynaptically induced long-term potentiation at the mossy fiber synapse in rat hippocampus. *J. Neurobiol.* 26, 370–385 (1995).
37. Tong, G., Malenka, R. C. & Nicoll, R. A. Long-term potentiation in cultures of single hippocampal granule cells: a presynaptic form of plasticity. *Neuron* 16, 1147–1157 (1996).
38. Staubli, U., Larson, J. & Lynch, G. Mossy fiber potentiation and long-term potentiation involve different expression mechanisms. *Synapse* 5, 333–335 (1990).
39. Huang, Y. Y. & Kandel, E. R. Modulation of both the early and the late phase of mossy fiber LTP by the activation of β-adrenergic receptors. *Neuron* 16, 611–617 (1996).
40. Kobayashi, K., Manabe, T. & Takahashi, T. Presynaptic long-term depression at the hippocampal mossy fiber-CA3 synapse. *Science* 273, 648–650 (1996).
41. Villacres, E. C., Wong, S. T., Chavkin, C. & Storm, D. R. Type I adenylyl cyclase mutant mice have impaired mossy fiber long-term potentiation. *J. Neurosci.* 18, 3186–3194 (1998).
42. Davis, G. W., DiAntonio, A., Peterson, S. A. & Goodman, C. S. Postsynaptic PKA controls quantal size and reveals a retrograde signal that regulates presynaptic transmitter release in Drosophila. *Neuron* 20, 305–315 (1998).
43. Bashir, Z. I. *et al.* Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. *Nature* 363, 347–350 (1993).
44. Zalutsky, R. A. & Nicoll, R. A. Mossy fiber long-term potentiation shows specificity but no apparent cooperativity. *Neurosci. Lett.* 138, 193–197 (1992).
45. Acsády, L., Kamondi, A., Sík, A., Freund, T. & Buzsáki, G. GABAergic cells are the major postsynaptic targets of mossy fibers in the rat hippocampus. *J. Neurosci.* 18, 3386–3403 (1998).
46. Amaral, D. G., Ishizuka, N. & Claiborne, B. in *Understanding the Brain Through the Hippocampus: The Hippocampal Region as a Model for Studying Structures and Function* (eds. Storm-Mathisen, J., Zimmer, J. & Ottersen, O. P.) 1–11 (Elsevier, Amsterdam, 1990).
47. Jung, M. W. & McNaughton, B. L. Spatial selectivity of unit activity in the hippocampal granular layer. *Hippocampus* 3, 165–182 (1993).
48. Claiborne, B. J., Xiang, Z. & Brown, T. H. Hippocampal circuitry complicates analysis of long-term potentiation in mossy fiber synapses. *Hippocampus* 3, 115–122 (1993).
49. Yeckel, M. F. & Berger, T. W. Spatial distribution of potentiated synapses in hippocampus: dependence on cellular mechanisms and network properties. *J. Neurosci.* 18, 438–450 (1998).
50. Lasser-Ross, N., Miyakawa, H., Lev-Ram, V., Young, S. R. & Ross, W. N. High time resolution fluorescence imaging with a CCD camera. *J. Neurosci. Methods* 36, 253–261 (1991).