Presynaptic Long-Term Depression at the Hippocampal Mossy Fiber–CA3 Synapse

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Long-term potentiation (LTP) and long-term depression (LTD) of synaptic strength may underlie learning and memory in the brain. The induction of LTP occurs in postsynaptic cells in the hippocampal CA1 region but is presynaptic in CA3. LTD is also well characterized in CA1 but not in CA3. Low-frequency stimulation of mouse hippocampal slices caused homosynaptic LTD at the mossy fiber–CA3 synapse, which may be induced presynaptically by activation of metabotropic glutamate receptors. Thus, the efficacy of mossy fiber–CA3 synapses can be regulated bidirectionally, which may contribute to neuronal information processing.

In the hippocampal CA1 region, LTP and LTD at Schaffer collateral-commissural synapses are induced postsynaptically through activation of *N*-methyl-D-aspartate (NMDA) receptors [(*1–3*), but see (*4*)]. In contrast, a distinct type of LTP occurs at mossy fiber–CA3 synapses (*5*) that is independent of NMDA receptor activation (*6, 7*) and is believed to be induced [(*7–9*), but see (*10*)] and expressed [(*7, 11–13*), but see (*14*)] entirely presynaptically. However, there is little information about LTD in the CA3 region (*15*). In the present study, we established that LTD can be induced at mossy fiber–CA3 synapses and examined its induction mechanism.

In the CA1 region (*2, 3*) and the visual cortex (*16*), homosynaptic LTD can be induced by prolonged low-frequency stimulation (LFS). We found that the same conditioning protocol was equally effective in inducing LTD at the mossy fiber–CA3 synapse. The field excitatory postsynaptic potential (EPSP) was recorded in the CA3 region of hippocampal slices while dentate granule cells were stimulated at 0.1 Hz (*17*). When the stimulus frequency was raised to 1 Hz for 15 min, the EPSP was markedly facilitated in amplitude (to 488 ± 35%; n = 8) with a gradual decline during the LFS (311 ± 24% at the end of LFS; n = 8) (Fig. 1A). After the stimulus frequency was returned to 0.1 Hz, EPSP amplitude rapidly decayed to a value below the baseline. After a slight recovery, EPSPs remained at a stable amplitude about 20% smaller than the baseline for more than 60 min (Fig. 1A). The magnitude of LTD 60 min after LFS was 21.0 ± 2.2% (n = 8) (*18*). This LTD was not due to a sustained decrease in presynaptic excitability because it was not accompanied by a reduction in amplitude of the fiber volley (Fig. 1A) (*19*).

The mossy fiber EPSP can be depressed heterosynaptically by tetanization of neighboring fibers (*20*). To examine whether mossy fiber LTD is specific to the stimulated fibers, we stimulated two independent mossy fiber pathways alternately (*21*) and applied LFS to only one pathway. LTD was observed in the conditioned pathway but not in the control pathway (Fig. 1B) (n = 4), which indicates that mossy fiber LTD is input specific. This input specificity excludes the possibility that the LTD is due to a nonspecific damage in postsynaptic cells caused by LFS.

One might argue that this LTD reflects an irreversible damage of the synapses to which repetitive stimulation was delivered. However, the following observations are against this argument. First, the same number of conditioning stimulations applied at a higher frequency induced almost no depression at 2 Hz and even evoked large potentiation at 5 or 10 Hz (Fig. 1C). Second, LTD seemed to be saturated (depression to 57.0 ± 2.3% of the baseline; n = 4) after repeated application of LFS (Fig. 2A). Third, a high-frequency stimulation applied after establishment of LTD induced LTP that stabilized above the initial baseline (Fig. 2B) (n = 5), which suggests that depressed synapses could still exhibit LTP.

During LFS, EPSPs were facilitated (see Fig. 1A), presumably because of enhanced release of glutamate transmitter (*22*). We investigated the involvement of glutamate receptors in the induction of mossy fiber LTD. A competitive NMDA receptor antagonist, D,L-2-amino-5-phosphonovaleric acid (APV), was applied during LFS. In the presence of APV (*23*), LFS caused LTD (16.7 ± 4.0%, 60 min after LFS; n = 5) that was comparable in magnitude to that of the control (Fig. 3A). Thus, the induction of mossy fiber LTD is independent of NMDA receptors.

Metabotropic glutamate receptors (mGluRs) have been reported to be essential for the induction of LTD in the CA1 region [(*4*), but see (*24*)], the visual cortex (*25*), and the cerebellum (*26*). We tested the effect of a competitive antagonist for mGluRs, (+)-α-methyl-4-carboxyphenylglycine (MCPG) (*27*), on mossy fiber LTD. MCPG had no effect on basal synaptic

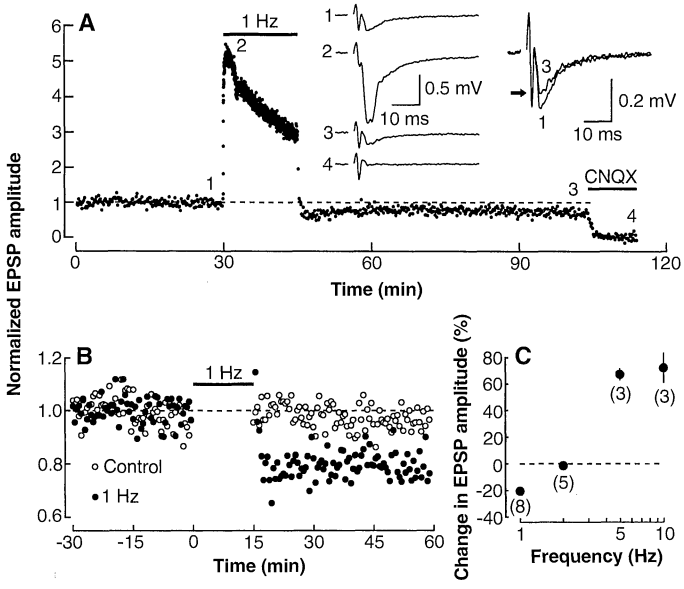

Fig. 1. Homosynaptic LTD at mossy fiber–CA3 synapses induced by LFS. (**A**) An example of the time course of mossy fiber LTD. Horizontal bar at left indicates conditioning LFS at 1 Hz for 15 min. At the end of the experiment, CNQX was applied (horizontal bar at right). Sample traces are averages of 10 consecutive records obtained at the times shown by numbers 1 through 4 in the graph. Two traces are superimposed on the right with expanded amplitude. Fiber volley components indicated by the arrow, which represent presynaptic activity, are followed by EPSPs. Stimulus artifacts are truncated. The horizontal dashed line indicates the average value of the normalized amplitude during the control period [also in (B)]. (**B**) An experiment showing input specificity of LTD. LFS was applied to one (solid circles) of two independent pathways but not to the other (control; open circles). Each point shows an average of three consecutive responses. Horizontal bar indicates conditioning LFS at 1 Hz for 15 min. (**C**) Frequency dependence of mossy fiber synaptic plasticity. A change in EPSP amplitude 60 min after the conditioning stimulation (900 pulses) applied at various frequencies (1, 2, 5, and 10 Hz) is plotted against the frequency used. The number of slices examined at each frequency is shown in parentheses.

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transmission (Fig. 3, C and D) (*28*). In the presence of MCPG, EPSPs were facilitated by LFS (Fig. 3D) and gradually decreased, as in the control (from 458 ± 42% to 283 ± 23%; $n = 5$). After the LFS, EPSPs were initially depressed to a level similar to that in the control, but they gradually returned toward the baseline (Fig. 3C). The magnitude of depression in the presence of MCPG was 5.5 ± 2.9% (60 min after LFS; $n = 6$) (*29*), which was significantly less than that in the control ($P < 0.01$, t test). Thus, mGluRs are crucial for the induction of mossy fiber LTD. The small depression remaining in the presence of MCPG might be due to incomplete blockade of mGluRs by MCPG or to possible contributions of other neurotransmitter receptors to LTD induction.

An NMDA receptor–independent and mGluR-dependent form of LTD has been reported in the CA1 region of young animals (3 to 7 days old) [(*4*), but see (*3*, *24*)], which is suppressed by an L-type Ca^{2+} channel blocker. In the presence of nicardipine, an L-type Ca^{2+} channel blocker, LTD was induced normally (15.8 ± 3.8% depression 60 min after LFS; $n = 5$) (Fig. 3B), which suggests that L-type Ca^{2+} channels are not involved in the induction of mossy fiber LTD, in contrast to LTD in the CA1 region.

We next tested whether depolarization in the postsynaptic cell is required for the induction of mossy fiber LTD by using 20 mM kynurenic acid, which antagonizes both non-NMDA and NMDA receptors. This concentration of kynurenic acid completely blocked EPSPs within a few minutes of bath application (Fig. 4A), and no depolarizing response was observed during LFS (Fig. 4A). After kynurenic acid was washed out, EPSPs stabilized below the baseline (19.6 ± 2.6% depression 120 min after LFS; $n = 4$). This depression cannot be due to incomplete washout of kynurenic acid, because the EPSP returned to the baseline within 90 min in the absence of LFS (Fig. 4A) ($n = 3$). Therefore, neither postsynaptic depolarization nor the ionotropic glutamate receptor was required for the induction of mossy fiber LTD.

Our results indicate that activation of mGluRs, but not of ionotropic glutamate receptors, is required for LTD induction. To test whether mGluR activation alone can induce LTD, a nonselective mGluR agonist, (1S, 3R)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD), was applied at a concentration (10 μM) that activates both postsynaptic mGluRs (*30*) and presynaptic mGluRs (*31*, *32*). To eliminate a possible secondary effect due to ACPD-induced bursting activity of CA3 neurons (*33*), a low concentration of kynurenic acid was applied together with ACPD. After prolonged application of ACPD (15 min), EPSPs completely recovered to the baseline level (Fig. 4B) ($n = 5$), which indicates that mGluR activation alone was not sufficient for the LTD induction.

In the present study, we demonstrated that LTD can be induced by prolonged low-frequency stimulation at the mossy fiber–CA3 synapse in an input-specific manner. Activation of mGluRs was crucial for the LTD induction. However, mGluR activation alone did not induce LTD, which indicates the requirement of an additional factor or factors. As in the case for mossy fiber LTP (*9*, *34*), the LTD was not influenced by blockade of postsynaptic depolarization, L-type Ca^{2+} channels, or ionotropic glutamate receptors, which suggests that presynaptic activity is important for the LTD induction (*35*). Therefore, we conclude that mossy fiber LTD is induced presynaptically by pairing of presynaptic activity and the activation of presynaptic mGluRs. In the mossy fiber–CA3 synapse, mGluR 2/3/7 subtypes are exclusively expressed at the presynaptic site, whereas

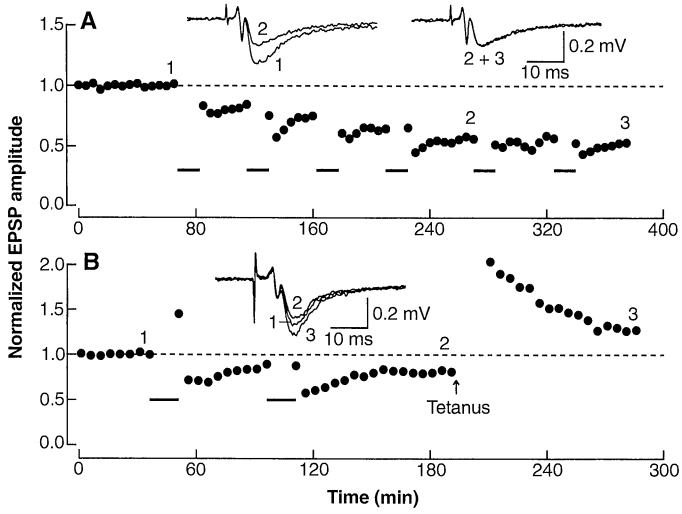

Fig. 2. Saturation and reversal of mossy fiber LTD. (**A**) An experiment showing saturation of LTD. LFS (shown by horizontal bars) was delivered several times at intervals from 30 to 50 min. Each data point is an average of responses for 5 min [also in (B)]. (**B**) Representative data showing reversal of LTD. LFS (bars) was applied twice and then tetanic stimulation was given at the time indicated by the upward arrow (at 100 Hz for 1 s, twice). EPSPs showed the slowly decaying potentiation that is typical of mossy fiber LTP [see (*7*, *13*)]. Data points for about 15 min after the tatanus are not shown in the figure because of large post-tetanic potentiation (over six times the initial baseline).

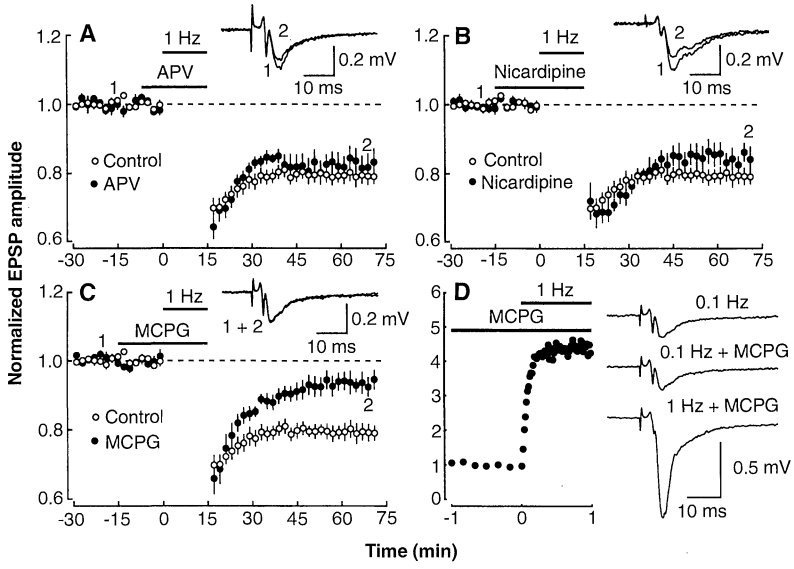

Fig. 3. Pharmacological properties of mossy fiber LTD. The time course of LTD induced in the absence (open circles in A, B, and C; $n = 8$) or presence (solid circles) of (**A**) APV (25 μM, $n = 5$), (**B**) nicardipine (10 μM, $n = 5$), and (**C**) MCPG (1 mM, $n = 6$). Horizontal bars indicate drug application in bath. Sample records were obtained in the experiment in which the drug was applied. Each data point indicates an average of the data from all the slices examined in each experimental condition. (**D**) Facilitation of EPSPs during LFS in the presence of MCPG. Sample traces were recorded before [top trace, identical to trace 1 in (C)] and after (middle trace) addition of MCPG, and during LFS in the presence of MCPG (bottom trace).

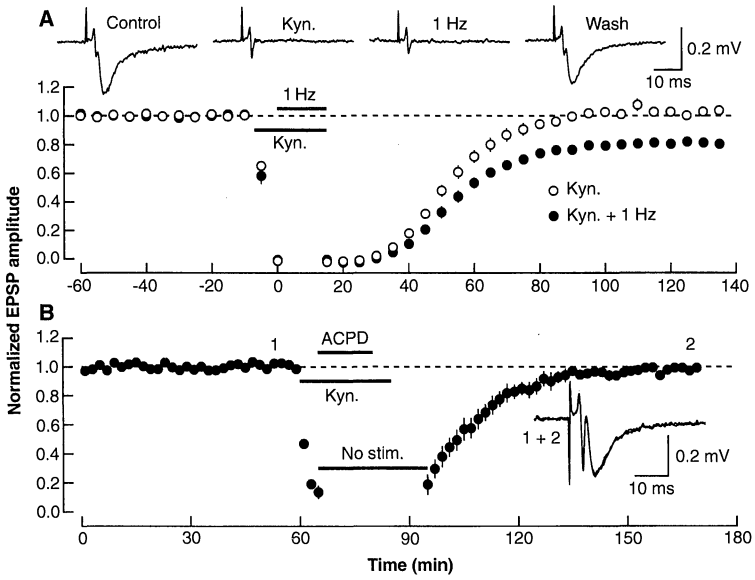

Fig. 4. Effects of LFS and ACPD in the presence of kynurenic acid (kyn.). (**A**) Mossy fiber LTD induced in the absence of excitatory synaptic transmission. After complete blockade of EPSPs with kynurenic acid, LFS was applied (solid circles, $n = 4$) or stimulation was continued at a baseline frequency (open circles, $n = 3$). Washout of kynurenic acid was started at the end of the LFS or at the corresponding time in the control experiment. Sample traces were obtained before (control), during the application of kynurenic acid, during LFS in kynurenic acid (1 Hz), and 120 min after the LFS (wash). (**B**) ACPD application alone did not induce LTD. Horizontal bars indicate application of ACPD and kynurenic acid (1 to 2 mM). In order to avoid a possible synergistic action of mGluR activation and synaptic activity, no stimulation was delivered during ACPD application and for 15 min after washout of ACPD.

mGluR 1/5 subtypes are expressed in the postsynaptic cell (*31*, *36*). To assess the contribution of presynaptic mGluRs to the LTD induction more directly, we studied mossy fiber synaptic transmission in mice lacking mGluR2 (*36*). Mossy fiber LTD was greatly reduced in these mice, confirming our conclusion here. This type of LTD is distinct from that in the CA1 region, the visual cortex, and the cerebellum, which are induced postsynaptically (*2*, *4*, *16*) [see (*26*) for a review]. In the CA1 region, a rise in Ca^{2+} concentrations in postsynaptic cells is essential for the induction of both LTP and LTD (*1*, *2*, *4*). An increase in presynaptic Ca^{2+} concentrations caused by LFS (*22*), in combination with mGluR activation, may also contribute to the induction of mossy fiber LTD.

Mossy fiber LTP is induced presynaptically (*7*–*9*). Our present study, together with the previous work on LTP, suggests that presynaptic mechanisms regulate long-term changes of mossy fiber–CA3 synaptic transmission in both up and down directions. This long-term bidirectional modification of synaptic efficacy at the mossy fiber–CA3 synapse may be important for hippocampal information processing.

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17. Transverse hippocampal slices (400 μm thick) were prepared from adult mice (>6 weeks old) (Charles River: ICR mouse) after mice were decapitated under halothane anesthesia. Slices were maintained in a holding chamber for at least 1 hour and were then transferred to a recording chamber and submerged beneath a continuously superfusing solution. Mossy fiber field EPSPs were recorded in the stratum lucidum with a glass microelectrode filled with 2 or 3 M NaCl (pipette resistance, 1 to 5 megohm) as described previously [Y. Takei *et al.*, *J. Cell Biol.* **131**, 1789 (1995)]. At the end of each experiment, 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 20 μM) was added to the bath to assess a fiber volley component (see Fig. 1A), which was subtracted from the EPSP on analysis. All data were expressed as means ± SEM. CNQX, APV, MCPG, ACPD, and kynurenic acid were purchased from Tocris Cookson (Bristol, U.K.). Nicardipine (Sigma) was dissolved in dimethyl sulfoxide (DMSO) and diluted to the superfusing solution (final concentration of DMSO was 0.02% v/v). An Axopatch 1D amplifier was used to record field EPSPs. Records were filtered at 1 kHz, digitized at 10 kHz, and collected on a computer (466/LV, Dell). All experiments were carried out at room temperature (24° to 28°C).
18. It is quite unlikely that mossy fiber LTD is contaminated with the depression of nonmossy fiber EPSPs because LFS induced only very small LTD (<5%) at associational-commissural synapses ($n = 3$).
19. LFS sometimes caused an increase in amplitude of the fiber volley, resulting in an apparent reduction in magnitude of LTD. When the increase was more than 5%, data were excluded from analysis.
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28. On a few occasions, a clear enhancement of EPSPs was observed during MCPG application. Because this enhancement occurred for unknown reasons and was not observed consistently, these data were excluded from analysis. Fiber volleys were not altered by MCPG when it was applied either alone or during LFS.
29. In four out of six slices, MCPG blocked LTD almost completely (less than 5% depression). In some experiments, LFS was applied twice, initially in the presence of MCPG and then after MCPG was washed out. The first LFS caused almost no depression (2.9 ± 1.0%) but the second induced robust depression (16.0 ± 3.6%) ($n = 3$).
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35. Our preliminary results showed that LTD of the excitatory postsynaptic current could be induced in voltage-clamped (around −65 mV) CA3 pyramidal cells loaded with a Ca^{2+} chelator BAPTA (10 mM) through the whole-cell patch pipette (22.5 ± 11.3% depression 30 min after LFS; $n = 3$).
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