Target-Specific Expression of Presynaptic Mossy Fiber Plasticity

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Mossy fiber synaptic transmission at hippocampal CA3 pyramidal cells and interneurons was compared in rat brain slices to determine whether mossy terminals are functionally equivalent. Tetanic stimulation of mossy fibers induced long-term potentiation in pyramidal neurons but was either without effect or it induced depression at synapses onto interneurons. Unlike transmission onto pyramidal neurons, transmission onto interneurons was not potentiated after adenosine 3′,5′-monophosphate (cAMP) activation. Furthermore, metabotropic glutamate receptor depression of transmission onto interneurons did not involve cAMP-dependent pathways. Thus, synaptic terminals arising from a common afferent pathway do not function as a single compartment but are specialized, depending on their postsynaptic target.

The axons of dentate gyrus granule cells, the mossy fibers, release glutamate onto postsynaptic α-amino-3-hydroxy-5-methylisoxazolepropionic acid receptors to generate fast synaptic transmission in CA3 pyramidal neurons (*1*). Long-term potentiation (LTP) of transmission between mossy fibers and CA3 pyramidal neurons (mossy fiber LTP) is *N*-methyl-D-aspartate (NMDA) receptor–independent and is triggered by a rise in calcium and subsequent formation of adenosine 3′,5′-monophosphate (cAMP) in the mossy presynaptic terminals (*2–4*). Mossy fibers also make synaptic contact with inhibitory interneurons (*5*, *6*). A monosynaptic form of LTP onto hippocampal interneurons has not been described (*7*, *8*), as has been done for pyramidal cells.

Mossy fibers terminate onto their postsynaptic targets with anatomically distinct types of terminals (*9*). Synaptic contacts between mossy fibers and interneurons are restricted to the filopodial terminal types, whereas large mossy terminals synapse only onto pyramidal cells (*10*). Do these structural differences between mossy fiber synaptic inputs onto pyramidal neurons and inhibitory interneurons imply a functional difference in the properties of synaptic transmission? Functional heterogeneity of hippocampal presynaptic terminals from a common afferent pathway has been suggested, on the basis of a differential localization of presynaptic autoreceptors (*11*); however, a physiological demonstration is still missing. Can high-frequency stimulation of mossy fibers induce presynaptic LTP at synapses onto both pyramidal cells and interneurons? The issue of synaptic plasticity onto interneurons is of considerable interest because such a mechanism will affect the computational properties of the hippocampal system (*12*).

Hippocampal slices from rat brains were prepared as described previously (*13*). The mossy fiber pathway was activated by low-intensity stimulation of the dentate gyrus or stratum lucidum (*14*). In a typical experiment, tetanic stimulation induced LTP in a CA3 pyramidal cell (Fig. 1A). Moreover, excitatory postsynaptic currents (EPSCs) were blocked by the group II–selective metabotropic glutamate receptor (mGluR) agonists (2*S*,1′*R*,2′*R*,3′*R*)-2-(2,3-dicarboxylcyclopropyl)-glycine (DCG-IV, 1 μM) or 1*S*,3*R*-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD, 10 μM) (9.2 ± 3.3% of control, $n = 6$), confirming that they originated from mossy fibers (*15*). LTP in pyramidal neurons was accompanied by a decrease in the failure rate (44.6 ± 5% in the control period versus 17.3 ± 5.4% after LTP, $n = 5$ cells) (Fig. 1B), which is consistent with a presynaptic mechanism for LTP expression (*16*). The same protocol applied to interneurons elicited two distinct responses. High-frequency stimulation produced either

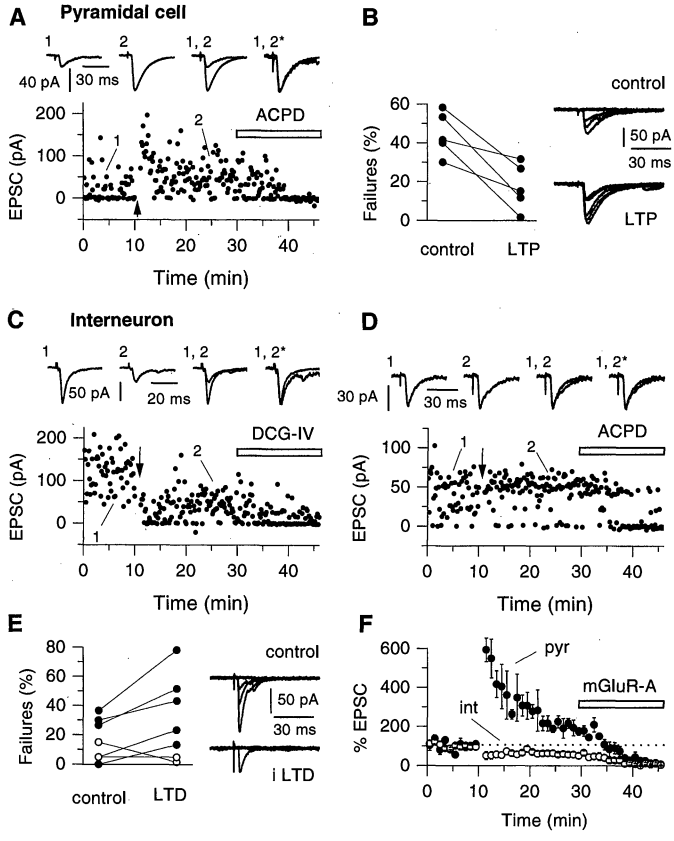

Fig. 1. Differential synaptic plasticity at mossy fiber–pyramidal cell and mossy fiber–interneuron synapses. (**A**) A single experiment showing the effect of tetanic stimulation (arrow) of mossy fibers on EPSCs on a CA3 pyramidal neuron. LTP results from a decrease in the failure rate. EPSCs were blocked by 10 μM ACPD, confirming their mossy fiber origin. Insets show averaged EPSC traces before LTP (1), after LTP (2), superimposed (1, 2), and superimposed after scaling of the peak amplitudes to demonstrate that the EPSC kinetics are unchanged (1, 2*). (**B**) Summary plot showing the decrease in the failure rate after LTP in all cells (44.6 ± 5% before LTP versus 17.3 ± 5.4% after LTP, $n = 5$, $P < 0.05$). Superimposed EPSC traces from a representative experiment show baseline transmission (control) and the reduction in failure rate after LTP induction (LTP). (**C** and **D**) Single experiments on interneurons. In (C), tetanic stimulation leads to LTD; in (D), no plasticity was observed. However, in both cases mGluR agonists block the EPSC, confirming their mossy fiber origin. (**E**) The effects of high-frequency stimulation on the failure rate in interneurons; the average change was from 16.9 ± 5.4% to 30.9 ± 10.6% after tetanic stimulation ($n = 7$). Solid circles indicate interneurons undergoing LTD; open circles indicate two cells that did not show plasticity. On the right, representative traces before (control) and after (i LTD) plasticity induction highlight the increased number of failures observed after tetanic stimulation. (**F**) Summary plot demonstrating the differential response to tetanic stimulation in pyramidal cells (pyr, solid circles) and interneurons (int, open circles) ($n = 5$ pyramidal cells and 9 interneurons). mGluR-A, metabotropic receptor agonists.

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a long-lasting depression (n = 6, Fig. 1C) or no change in EPSC amplitude (n = 3, Fig. 1D). In all cases, EPSCs were subsequently blocked by ACPD or DCG-IV (14.3 ± 4.5% of control amplitude, n = 9), confirming their mossy fiber origin (*15*). The EPSC failure rate increased in interneurons undergoing a depression (solid circles in Fig. 1E) and remained unchanged or was slightly decreased in cells lacking any form of plasticity (open circles in Fig. 1E). The average values obtained for the failure analysis were 16.9 ± 5.4% in the control period versus 30.9 ± 10.6% after the tetani. The summary graphs for both pyramidal cells and interneurons are very different (Fig. 1F). Pyramidal cell EPSCs displayed strong post-tetanic potentiation (PTP) followed by LTP (380 ± 30% and 200 ± 10% of control amplitude during the first and second 10-min periods after tetanic stimulation, respectively). EPSCs onto interneurons lacked PTP and in contrast underwent depression (60 ± 3% and 60 ± 1% of control amplitude in the first and second 10-min periods after the tetanus, respectively).

Because mossy fiber LTP onto pyramidal neurons is critically dependent on cAMP levels within the mossy fiber terminal (*4*), we next determined whether mossy fiber synapses onto interneurons were similarly sensitive to cAMP elevation. Application of 50 to 100 μM forskolin, a diterpene that directly activates adenylyl cyclase, irreversibly increased both the peak amplitude and the charge transfer of mossy fiber–evoked EPSCs onto pyramidal cells (from 48.7 to 242 pA and from 0.61 to 7.7 pC, respectively) (Fig. 2A). The same experiment performed on interneurons (Fig. 2B) revealed a depression of the EPSC peak by forskolin, whereas the EPSC charge transfer (*17*) was increased (from 31.0 to 21.6 pA and 0.46 to 1.7 pC, respectively) (Fig. 2C). The increased charge transfer and kinetic change probably reflect the recruitment of a polysynaptic recurrent circuit due to the potentiation of synapses between the mossy fibers and pyramidal cells (*18*). The summary plots for both pyramidal cells and interneurons (Fig. 2, C and D) show that forskolin increased pyramidal cell EPSCs to 340 ± 10% of control amplitude (n = 6) but was without effect on interneurons (102 ± 3% of control amplitude, n = 5). In contrast, the normalized charge transfer was significantly increased in both pyramidal cells and interneurons (520 ± 10%, n = 6, and 230 ± 10%, n = 5, respectively).

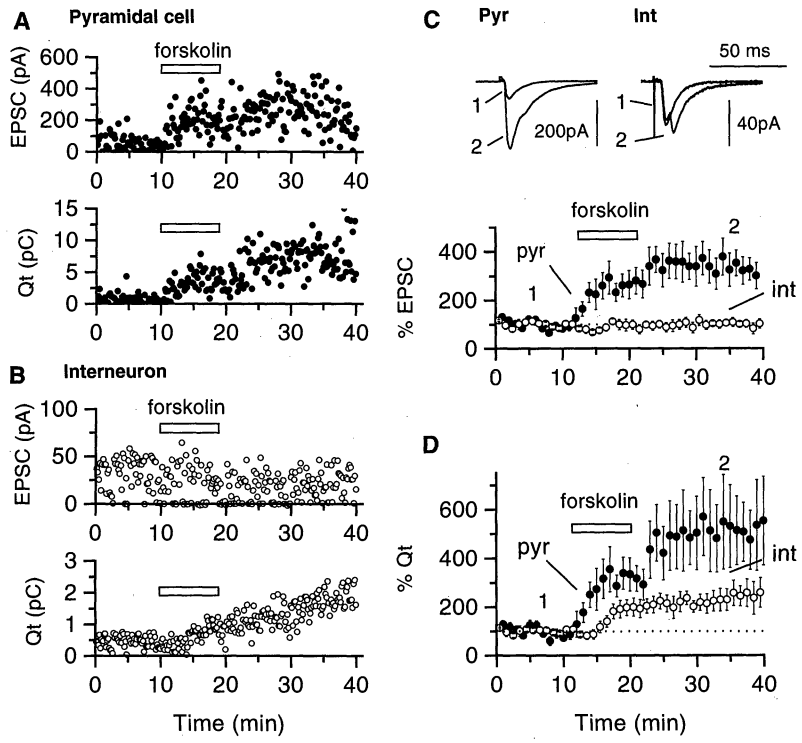

Fig. 2. A lack of cAMP-dependent LTP at mossy fiber synapses onto interneurons. (**A**) A single experiment on a CA3 pyramidal cell showing the effect of bath application of forskolin (50 mM, bar) on both the EPSC peak (upper panel) and on the calculated charge transfer (lower panel; Qt, charge transfer). Both variables are robustly and irreversibly potentiated by forskolin. (**B**) Similar experiment as that in (A) but performed on a CA3 interneuron. Forskolin is without effect on the EPSC peak (upper plot) compared to the clear potentiation of the charge transfer (lower plot). (**C** and **D**) Summary graphs obtained from pyramidal cells (pyr, solid circles; n = 6) and interneurons (int, open circles; n = 5). (C) Forskolin increased the average EPSC to 336 ± 9% in pyramidal cells but was ineffective in interneurons (105 ± 3%, n = 5). In the upper panel is shown the change in EPSC kinetics after application of forskolin in both the pyramidal neuron and the interneuron; traces are the average of 20 EPSCs. (D) The normalized charge transfer is increased by forskolin in both pyramidal cells and interneurons (to 518 ± 9% and 231 ± 6%, respectively).

Any functional differences in mossy fiber terminals may be reflected in the degree of paired pulse facilitation observed in pyramidal and inhibitory neurons, similar to that observed at excitatory synapses onto neocortical neurons (*19*). Paired pulse facilitation is a presynaptic phenomenon that depends on residual calcium levels in the presynaptic terminal (*20*). Pyramidal cells displayed marked facilitation of the second EPSC as compared with the first (the ratio of the second EPSC to the first = 1.9 ± 0.2, n = 5) (Fig. 3A). In contrast, a significantly smaller facilitation was observed on interneurons (the ratio of the second EPSC to the first = 1.1 ± 0.2, n = 7, $P < 0.05$). Although these data suggest differences in the sensitivity to cAMP formation and in the degree of paired pulse facilitation of mossy fiber terminals onto different target cells, in both cell types transmission was blocked by activation of mGluRs (Fig. 1). Presynaptically located group II mGluRs suppress transmission between mossy fibers and CA3 pyramidal neurons by a mechanism involving cAMP-dependent pathways (*15*). We next determined whether modulation of mossy fiber transmission onto interneurons and pyramidal neurons by the group II–selective agonist DCG-IV was similarly occluded by activation of cAMP-dependent processes. The block of the mossy fiber–evoked EPSC onto interneurons by a submaximal concentration of DCG-IV (100 nM) was similar in the absence (57.0 ± 3.9% of baseline, n = 6) or presence of forskolin (51.1 ± 4.7% of baseline, n = 6) (Fig. 3, C and D). In contrast, the block of mossy fiber EPSCs onto pyramidal neurons by DCG-IV was occluded after application of forskolin (76.4 ± 2.4% of baseline compared to 38.0 ± 3.4% of baseline in the control period, n = 6) (Fig. 3, E and F). Thus, mGluR modulation of mossy fiber synaptic transmission onto interneurons, unlike transmission onto pyramidal neurons, does not involve cAMP-dependent processes.

These data suggest that the functional properties of mossy fiber terminals are shaped by their postsynaptic target cell. Although LTP was reliably evoked in pyramidal neurons, we were unable to elicit any form of long-lasting potentiation in interneurons. The fact that LTP in pyramidal neurons was induced using EGTA-loaded recording elec-

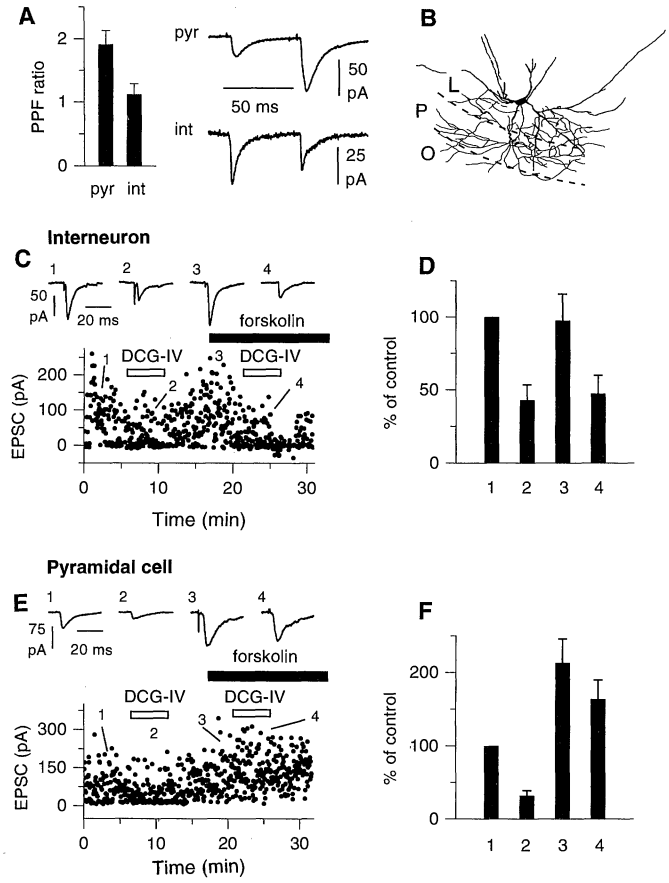

Fig. 3. (**A**) Summary histograms demonstrating that paired pulse facilitation of mossy fiber–evoked EPSCs is significantly reduced in interneurons (int; $n = 7$) as compared with pyramidal cells (pyr; $n = 5$). Averages from 10 traces are shown on the right. The interpulse interval was 50 ms; the upper and lower traces are from a representative pyramidal cell and interneuron, respectively. (**B**) Camera lucida drawing of a typical interneuron used in this study; the axon ramifies primarily in the stratum pyramidale (P). O, stratum oriens; L, stratum lucidum. (**C**) The DCG-IV block of mossy fiber–evoked EPSCs onto interneurons is not occluded by forskolin application. In a single experiment on an interneuron, DCG-IV (open bar) blocked mossy fiber–evoked EPSCs under control conditions and in the presence of forskolin (solid bar) by a similar amount. Insets show the average from 30 traces under control conditions (1), after DCG-IV application (2), after forskolin application (3), and in the presence of both DCG-IV and forskolin (4). (**D**) Summary histograms for six experiments under each of the four different conditions. Numbers under bars indicate the time period from which data were obtained. (**E**) In contrast to (C), the block of mossy fiber EPSCs onto pyramidal cells by DCG-IV was markedly reduced after application of forskolin as compared with the block of EPSCs by DCG-IV under control conditions. Insets show averaged traces like those shown in (C). (**F**) Summary histograms like those shown in (D) for pyramidal neuron experiments ($n = 7$) under each of the four conditions

trodes is consistent with a presynaptic induction mechanism (*16*). An attractive hypothesis to explain the lack of potentiation in interneurons is that the smaller filopodial terminals, which only contact interneurons, lack a component of the adenylyl cyclase–cAMP cascade. Furthermore, our data show that mossy fiber synaptic responses onto interneurons were modulated by presynaptic mGluRs, but the mechanism involved is cAMP-independent, unlike mossy fiber EPSCs onto pyramidal neurons. These differential properties will ensure that synaptic efficacy is either unaltered or depressed at presynaptic terminals onto interneurons during protocols that induce LTP at principal cell synapses, profoundly affecting the properties of the hippocampus. In addition, these data demonstrate that presynaptic terminals from a common afferent pathway do not behave as a single computational unit but are functionally specialized, depending on their postsynaptic targets.

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13. Methods were similar to those of Maccaferri and McBain (7). Transverse hippocampal slices from Sprague-Dawley rats 14 to 20 days old were prepared on a Vibratome and incubated at room temperature for at least 1 hour before use. Animals were killed with volatile anesthesia, according to NIH animal welfare guidelines. This age range of animals was chosen because mossy fibers expansions are close to their mature form (9). The bathing solution contained 130 mM NaCl, 24 mM $NaHCO_3$, 3.5 mM KCl, 1.25 mM NaH_2PO_4, 3 mM $CaCl_2$, 1.5 mM $MgSO_4$, 10 mM glucose, 0.005 mM bicuculline, and 95% O_2/5% CO_2 (pH 7.4). Voltage clamp recordings were performed at room temperature (~24°C) at a holding potential of −60 mV. The intracellular pipette solution contained 100 mM Cs-gluconate, 5 mM $MgCl_2$, 10 mM Hepes, 30 mM EGTA, and 0.4% biocytin (pH = 7.3), and electrode resistances were 2 to 3 megohms. Series resistance was monitored by delivery of a −2-mV voltage step after each evoked EPSC. Interneurons were selected on the basis of their location in the stratum lucidum or at the border of the stratum lucidum and the stratum radiatum (Fig. 3B). Mossy fibers were activated by low-intensity stimulation (<25 mA for 100 to 250 ms) through a patch pipette positioned either in the granule cell layer or the stratum lucidum to minimize the possibility of contamination of the evoked response by activation of non–mossy fiber axons. Stimulating electrodes were filled with oxygenated extracellular solution. The NMDA receptor antagonist D-2-amino-5-phosphonovaleric acid (D-AP5, 50 μM) was added to the recording medium, whole-cell pipettes were loaded with 30 mM EGTA, and both adenosine triphosphate and guanosine triphosphate were omitted to prevent NMDA receptor–dependent synaptic plasticity arising from non-mossy fibers. EPSCs were filtered at 1 kHz, acquired at 10 kHz, and analyzed with the Pclamp6 suite of programs (Axon Instruments, Foster City, CA). Recorded slices were processed with an ABC kit for morphological identification (Fig. 3B). All cells corresponded to interneurons described in (5).
14. EPSC amplitudes were determined from the response during a 1- to 2-ms window that included the peak of the waveform, and the amplitude of the baseline in a similar time window was subtracted. Failures were identified visually, averaged, and used (when present) to correct for the stimulus artifact.
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17. Measurement of charge transfer not only allows the contribution of polysynaptic components to be assessed but provides a positive control indicating that forskolin is working at other synaptic locations despite there being no change in the monosynaptic input to interneurons.
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