

# Role for Rapid Dendritic Protein Synthesis in Hippocampal mGluR-Dependent Long-Term Depression

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A hippocampal pyramidal neuron receives more than  $10^4$  excitatory glutamatergic synapses. Many of these synapses contain the molecular machinery for messenger RNA translation, suggesting that the protein complement (and thus function) of each synapse can be regulated on the basis of activity. Here, local postsynaptic protein synthesis, triggered by synaptic activation of metabotropic glutamate receptors, was found to modify synaptic transmission within minutes.

Two mechanistically distinct forms of long-term depression (LTD) coexist at synapses in the CA1 region of hippocampus (1). Induction of one form depends on activation of *N*-methyl-D-aspartate receptors (NMDARs) (2, 3), and induction of the other depends on activation of metabotropic glutamate receptors (mGluRs) (1, 4). Much has been learned about the induction and expression mechanisms of NMDAR-LTD (5). In contrast, little is known about the mechanisms of mGluR-LTD, although postsynaptic group 1 mGluRs and protein kinase C (PKC) have been implicated. Biochemical studies indicate that one consequence of group 1 mGluR activation in synaptoneurosomes is the synthesis of new proteins, including the fragile X mental retardation protein (6, 7). In this study we tested the hypothesis that dendritic mRNA translation is required for mGluR-LTD.

One reason for the slow progress on the mechanism of mGluR-LTD is that, until recently, it has been difficult to elicit reliably with synaptic stimulation (1, 8). Thus, in our initial studies, we took advantage of the selective group 1 mGluR agonist DHPG [(*RS*)-3,5-dihydroxyphenylglycine] to induce LTD. Application of DHPG (5 min, 50  $\mu$ M) to hippocampal slices reliably induces LTD of evoked excitatory synaptic responses by transiently activating mGluR5, the major postsynaptic group 1 mGluR in CA1 (9, 10).

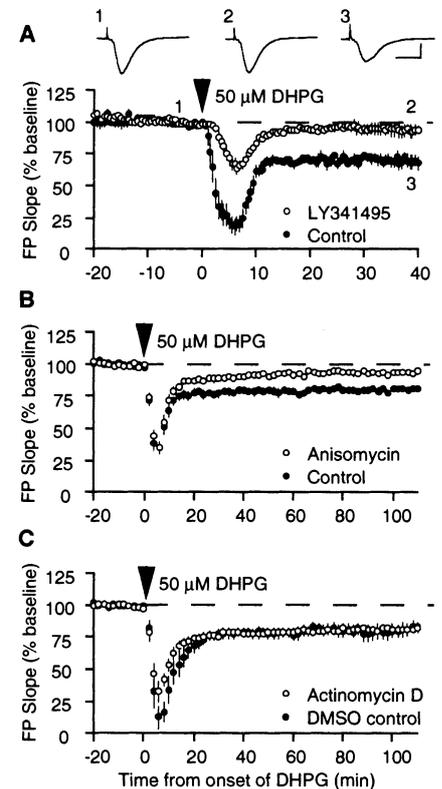
DHPG caused an acute depression of synaptic transmission (11), followed, upon washout, by the relaxation of synaptic responses to a stable level that was significantly reduced relative to baseline (Fig. 1A). Both the acute response and the resulting LTD were inhibited when DHPG was applied in the presence of the

selective mGluR antagonist LY341495 (10  $\mu$ M). To investigate the possible requirement for protein synthesis in the establishment of LTD, we incubated hippocampal slices in the translation inhibitor anisomycin (20  $\mu$ M) for 1 hour before application of DHPG. This treatment had no effect on baseline synaptic transmission or on the acute effect of DHPG, but it strongly inhibited the establishment of LTD relative to interleaved controls (Fig. 1B). Inhibition was apparent immediately after washing out the DHPG, suggesting a deficit in the induction rather than the consolidation of LTD (12). To explore the possible contribution of mRNA synthesis to the LTD, we repeated the experiment in the presence of the transcription inhibitor actinomycin-D (25  $\mu$ M). In contrast to the translation inhibitor, actinomycin-D had no effect on LTD (Fig. 1C).

Unlike many translation inhibitors, anisomycin is not known to interfere with synaptic function by actions other than the inhibition of protein synthesis (12). The fact that the acute effect of DHPG was unaffected by anisomycin, for example, confirms that the drug was not acting directly on mGluRs. Nonetheless, to confirm the requirement for mRNA translation, and to assess if this occurred specifically in the postsynaptic CA1 pyramidal neurons, we performed additional experiments in which cells were loaded with mRNA cap analogue (*m*<sup>7</sup>GpppG) before DHPG application. The cap analogue inhibits protein synthesis by competing with endogenous capped mRNA for the cap binding protein eIF-4E (13, 14). Evoked excitatory postsynaptic currents (EPSCs) were recorded with whole-cell pipettes containing the cap analogue or control solution, and the effects of DHPG were investigated (15). Although the acute depression of synaptic transmission was still observed, stable LTD failed to occur in response to DHPG in cells loaded with the cap analogue (Fig. 2A). This finding contrasts with the LTD of evoked EPSCs observed in the interleaved control neurons and in simulta-

neously recorded field potentials (FPs). Thus, mRNA translation in postsynaptic CA1 pyramidal neurons is required for mGluR-LTD.

Although there is evidence that mRNA can be translated in the dendrites of hippocampal neurons (16, 17), the major site of protein synthesis is the cell body. To test the hypothesis that protein synthesis within dendrites is sufficient to support mGluR-LTD, we performed experiments in which the cell body layer of CA1 was excised, leaving only the neuropil of the stratum radiatum (18–20). This reduced preparation supported synaptic



**Fig. 1.** mGluR-dependent LTD is dependent on protein synthesis, but not mRNA synthesis. (A) Preincubation of slices in the mGluR antagonist LY341495 (20 min, 10  $\mu$ M) blocked LTD induced by a brief application of DHPG (arrowhead; 5 min, 50  $\mu$ M;  $94 \pm 4\%$  of pre-DHPG baseline;  $n = 8$ ,  $P > 0.2$ ) (○). A second application of DHPG after the washout of LY341495 induced a robust depression ( $69 \pm 4\%$ ;  $n = 5$ ,  $P < 0.001$ ) (●). Representative FPs (2 min, average) were taken at the times indicated on the graph. Calibration: 0.5 mV, 5 ms. (B) Preapplication of the protein synthesis inhibitor anisomycin (1 hour, 20  $\mu$ M) blocked the long-lasting but not acute depression caused by DHPG [ $93 \pm 4\%$  (measured 100 min after DHPG application);  $n = 9$ ] (○) compared with interleaved control slices ( $80 \pm 2\%$ ;  $n = 9$ ,  $P < 0.001$ ) (●). (C) Preapplication of the transcription inhibitor actinomycin D (30 to 60 min, 25  $\mu$ M) did not affect DHPG-LTD ( $81 \pm 4\%$ ;  $n = 11$ ) (○) compared with vehicle-treated control slices (0.2% dimethylsulfoxide;  $83 \pm 4\%$ ;  $n = 5$ ,  $P > 0.7$ ) (●).

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## REPORTS

transmission, and the isolated dendrites still supported DHPG-induced LTD (Fig. 2B). Thus, the protein synthetic machinery and mRNA required for induction of mGluR-LTD is present in postsynaptic dendrites.

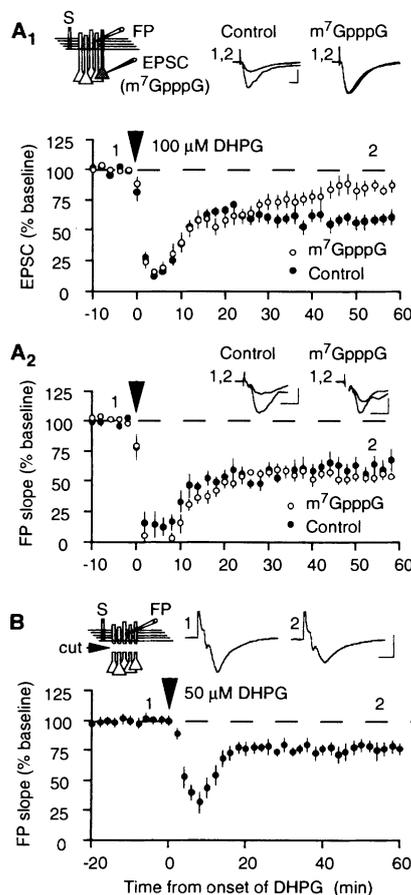
The DHPG experiments demonstrated a requirement for postsynaptic, dendritic mRNA translation in the induction of mGluR-LTD. However, these experiments did not address the possibility of synapse-specific control of protein synthesis because DHPG acts simultaneously at all CA1 synapses; nor did they demonstrate that synaptically released glutamate can activate these protein synthesis-dependent mechanisms. Thus, we adapted a recently published protocol (8) to investigate the role of mRNA translation in synaptically evoked mGluR-LTD. Delivery of paired-pulse stimulation (50-ms interstimulus interval) at 1 Hz for 15 min produced a stable form of LTD with three important characteristics (Fig. 3). First, LTD induction did not depend on activation of NMDARs, as it was induced in the presence of the NMDAR antagonist D,L-2-amino-5-phosphonovaleric acid (APV; 50  $\mu$ M) (Fig. 3A). Second, LTD induction was homosynaptic, as it was confined to the synapses receiving the paired-pulse, low-frequency stimulation (PP-LFS); unstimulated control inputs converging on the same population of

postsynaptic neurons did not express the LTD (Fig. 3A). Third, LTD induction (in the presence of APV) required activation of mGluRs, as it was completely blocked by LY341495 (100  $\mu$ M) (Fig. 3B).

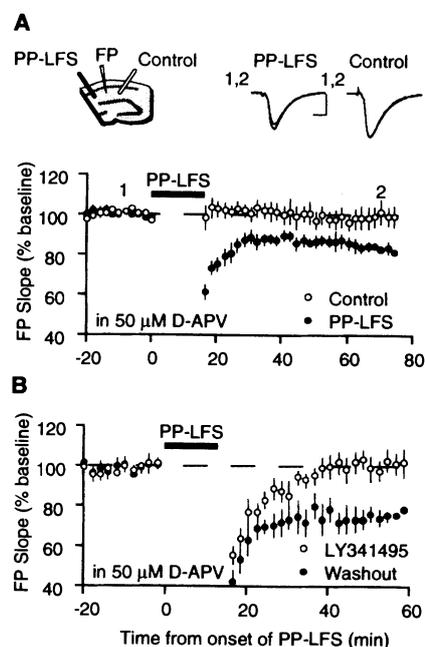
To confirm that homosynaptic mGluR-LTD also required mRNA translation, we investigated the consequences of applying anisomycin (Fig. 4A). As in the case of DHPG-induced LTD, we found that a 1-hour preincubation in anisomycin (20  $\mu$ M) was sufficient to completely inhibit induction of LTD with PP-LFS. Again, inhibition relative to control was apparent immediately after completion of the conditioning stimulation. Similar results were obtained with the translation inhibitor cycloheximide (60  $\mu$ M) (Fig. 4B). However, as expected from the previous findings with DHPG, preincubation in the transcription inhibitor actinomycin-D had no effect on homosynaptic mGluR-LTD (25  $\mu$ M,  $n = 6$ ). Thus, an essential step for LTD induction with PP-LFS appears to be translation of preexisting mRNA in the postsynaptic neuron.

These findings are consistent with the idea that activation of postsynaptic mGluRs during the PP-LFS stimulates mRNA translation at the synapse, resulting in LTD. However, an alternative hypothesis is that a postsynaptic protein molecule with a high turnover rate is required for LTD induction. Preincubation in anisomy-

**Fig. 2.** mGluR-dependent LTD relies on postsynaptic, dendritic protein synthesis. **(A<sub>1</sub>)** LTD induced by DHPG (5 min, 100  $\mu$ M) was prevented by postsynaptic injection of the cap analogue  $m^7$ GpppG. Whole-cell voltage-clamp recordings were performed from CA1 pyramidal neurons. Inclusion of  $m^7$ GpppG in the recording pipette reduced the magnitude of LTD measured 1 hour after DHPG ( $84 \pm 4\%$ ;  $n = 11$ ) as compared with interleaved control cells ( $58 \pm 4\%$ ;  $n = 10$ ,  $P < 0.001$ ). **(Inset)** Schematic of experimental design showing placement of stimulating electrode (S), intracellular recording and injection electrode (EPSC), and field potential (FP) electrode. Representative EPSCs (2 min, average) are from a control cell and a cell containing  $m^7$ GpppG at the times indicated by the numbers on the graph. Calibration: 50 pA, 5 ms. **(A<sub>2</sub>)** In a subset of experiments, FPs were also recorded simultaneously. In all cases, FPs showed that LTD was reliably induced by DHPG ( $m^7$ GpppG:  $53 \pm 3\%$ ;  $n = 6$ ; control:  $63 \pm 8\%$ ;  $n = 6$ ). Representative FPs (2 min, average) were taken at the times indicated. Calibration: 0.25 mV, 0.5 mV, 5 ms. **(B)** DHPG induced LTD in isolated dendrites. **(Inset)** Schematic of microsurgical cut made at the border of the CA1 cell body layer to disconnect the somata from the dendritic layer. FPs were elicited by stimulation (S) of the Schaffer collateral axons. DHPG application (5 min, 50  $\mu$ M) to isolated dendrites induced robust LTD ( $77 \pm 6\%$ ;  $n = 6$ ,  $P < 0.005$ ). Representative FPs (2 min, average) were taken at the times indicated by the numbers on the graph. Calibration: 0.25 mV, 5 ms.

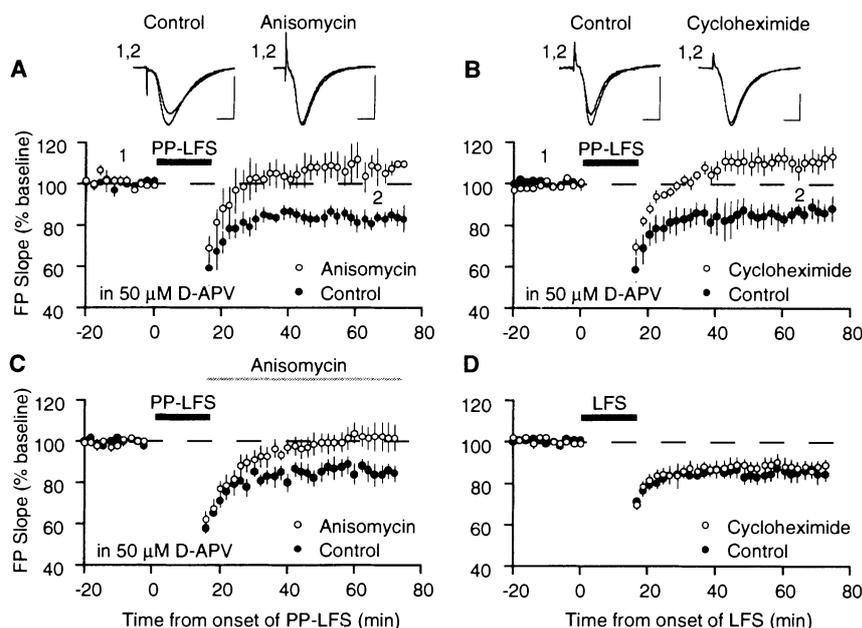


cin for 1 hour might lead to depletion of this necessary protein. We thus repeated the experiment, beginning the anisomycin treatment at the conclusion of PP-LFS. This experiment revealed that blockade of mRNA translation immediately after conditioning stimulation was sufficient to inhibit the expression of mGluR-LTD (Fig. 4C). The fact that inhibition of LTD emerged rapidly upon anisomycin treatment suggests that the necessary protein synthesis occurs within minutes of the cessation of PP-LFS. Moreover, because the drug was not present during conditioning stimulation, the inhibition of LTD by anisomycin cannot be due to a direct effect on mGluRs.



**Fig. 3.** Paired-pulse low-frequency stimulation (PP-LFS) induces homosynaptic, mGluR-dependent LTD. **(A)** **(Inset)** Schematic of two independent pathway recording configurations. Two stimulating electrodes placed on either side of an extracellular recording electrode (FP) were stimulated in alternation. Independence of pathways was tested by delivering a stimulus pulse (at 50% of maximal response) to one stimulating electrode and then to the other in rapid succession (50-ms interstimulus interval) to test for paired-pulse facilitation across pathways. Experiments were performed in the presence of D-APV (50  $\mu$ M). PP-LFS delivered to one pathway, indicated by the black bar, induced LTD only in that pathway ( $83 \pm 2\%$ ;  $n = 5$ ,  $P < 0.02$ ) (●). The control pathway, in which stimulation was turned off during PP-LFS, was not depressed ( $100 \pm 5\%$ ;  $n = 5$ ) (○). Representative FPs (2 min, average) were taken at the times indicated by the numbers on the graph. Calibration: 0.5 mV, 5 ms. **(B)** Preapplication of the mGluR antagonist LY341495 (20 min, 100  $\mu$ M) blocked the induction of LTD with PP-LFS ( $98 \pm 4\%$ ;  $n = 6$ ,  $P > 0.6$ ) (○). A second episode of PP-LFS after the washout of LY341495 induced LTD ( $76 \pm 2\%$ ;  $n = 3$ ,  $P < 0.01$ ) (●).

## REPORTS



**Fig. 4.** Induction of mGluR-dependent LTD by PP-LFS requires protein synthesis. **(A)** Preincubation in the protein synthesis inhibitor anisomycin (1 hour, 20  $\mu$ M) blocked the induction of LTD induced with PP-LFS ( $111 \pm 6\%$ ;  $n = 4$ ) (○) as compared with interleaved control slices ( $83 \pm 4\%$ ;  $n = 5$ ,  $P < 0.01$ ) (●). **(B)** Preincubation in the protein synthesis inhibitor cycloheximide (30 min, 60  $\mu$ M) blocked the induction of LTD induced with PP-LFS ( $111 \pm 4\%$ ;  $n = 5$ ) (○) as compared with interleaved control slices ( $87 \pm 4\%$ ;  $n = 4$ ,  $P < 0.01$ ) (●). Representative FPs in **(A)** and **(B)** are from control and inhibitor-treated slices at the times indicated by the numbers on the graphs. Calibration: 0.5 mV, 5 ms. **(C)** Application of anisomycin (20  $\mu$ M; gray line) immediately after PP-LFS blocked the induction of LTD ( $103 \pm 6\%$ ;  $n = 5$ ) (○) as compared with interleaved control slices ( $84 \pm 3\%$ ;  $n = 5$ ,  $P < 0.05$ ) (●). **(D)** NMDAR-dependent LTD, induced with 900 pulses of stimulation at 1 Hz in the absence of APV, was unaffected by 60  $\mu$ M cycloheximide ( $87 \pm 3\%$ ;  $n = 8$ ) (○) as compared with controls ( $85 \pm 3\%$ ;  $n = 8$ ,  $P > 0.5$ ) (●).

The current findings, when considered with previous data on mGluRs (1, 6–8, 21), suggest a model in which activation of postsynaptic mGluR5, either with a selective group 1 mGluR agonist (DHPG) or with synaptically released glutamate (PP-LFS), triggers LTD by a mechanism that requires local, postsynaptic translation of preexisting dendritically localized mRNA. Because mGluR-LTD induced with PP-LFS is homosynaptic, synapse-specific protein synthesis is strongly suggested. This mechanism appears to be completely distinct from that of NMDAR-dependent homosynaptic LTD, which does not require mGluR5 activation (10, 22, 23) or mRNA translation (Fig. 4D). Moreover, because the consequence is LTD, this mechanism is also distinct from those proposed to link protein synthesis to synaptic potentiation in mammals (20) and invertebrates (24, 25).

Two points are considered axiomatic in the neurobiology of memory: first, that the elementary unit of information storage is the synapse, and second, that information is stored as a physical change in the brain. The discovery of polyribosomes at the base of individual synapses has long invited speculation that local protein synthesis is involved in activity-dependent, synapse-specific structural modifications that store information (26). Our results demon-

strate that local protein synthesis is involved in the coupling of mGluR activity to an enduring, synapse-specific modification of synaptic transmission. The mGluR-LTD model should be useful for dissecting the mechanisms of synaptic mRNA translation regulation and their contribution to structural plasticity and information storage in the mammalian brain.

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11. Hippocampal slices from postnatal day 21 to 30 Long-Evans rats (Charles River, Cambridge, MA) were prepared as described (27). Slices were allowed to recover for 1 hour at 30°C, and three additional hours at room temperature, in ACSF containing (in mM) NaCl (124), KCl (5), NaH<sub>2</sub>PO<sub>4</sub> (1.25), NaHCO<sub>3</sub> (26), MgCl<sub>2</sub> (1),

CaCl<sub>2</sub> (2), and dextrose (10), saturated in 95% O<sub>2</sub>, 5% CO<sub>2</sub>. For recording, slices were placed in a submersion recording chamber, maintained at 30°C, and perfused with ACSF at a rate of 2 ml/min. FPs were evoked, recorded, and analyzed as described (27). Average values ( $\pm$ SEM) are expressed as the percentage of initial baseline response measured 55 to 60 min after DHPG or conditioning stimulation, unless otherwise indicated.

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15. For voltage-clamp experiments, picrotoxin (25  $\mu$ M) was added to the ACSF to isolate EPSCs. Whole-cell pipettes (3 to 7 megohm) were fabricated from thick-wall (outer diameter, 1.5 mm; inner diameter, 0.86 mm; Sutter Instruments) borosilicate glass and filled with (in mM) K-gluconate (134), KCl (6), NaCl (4), HEPES (10), EGTA (0.2), MgATP (4), tris-GTP (0.3), and phosphocreatine (14). The pH of the internal solution was adjusted to 7.25 with KOH and the osmolarity was adjusted to 300 mOsm with H<sub>2</sub>O or sucrose. Series resistance was monitored throughout the experiment by measuring the peak of the fast-current transient (filtered at 30 kHz; electrode capacitance was compensated for) in response to a 5-mV step. Average series resistance was  $36 \pm 3$  megohm for cap cells and  $28 \pm 3$  megohm for controls. Average input resistance was  $153 \pm 10$  megohm for cap cells and  $134 \pm 11$  megohm for controls. Only experiments in which there was less than a 15% change in series resistance were included in the analysis. Cap analog experiments were performed in two series, one with 100  $\mu$ M m<sup>7</sup>GpppG ( $n = 5$ ) and the second with 250  $\mu$ M m<sup>7</sup>GpppG ( $n = 6$ ). Both concentrations produced comparable inhibition of LTD relative to control cells (300  $\mu$ M GTP;  $n = 5$  and 6, respectively), so the data were combined. To ensure a reliable and robust LTD in all cases, we used 100  $\mu$ M DHPG to induce LTD.
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18. For experiments on isolated CA1 dendrites, an incision was made at the border of the stratum pyramidale and stratum radiatum with a microdissection knife visualized under a dissection microscope (20). Slices were left to recover from the dendritic isolation procedure for at least 2 hours at 30°C. For recording, slices were placed in a submersion recording chamber, maintained at 30°C, and perfused with ACSF at a rate of 2 ml/min. Higher stimulation intensities (20 to 60  $\mu$ A) were required to elicit FPs in isolated dendrites. Slices were used only if a slope of at least 0.1 mV/ms was obtained with a stimulation intensity of 100  $\mu$ A. In these experiments, the absence of a population spike in response to high-intensity stimulation (150  $\mu$ A) confirmed that the CA1 dendrites were isolated from their somata.
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