

Chemical Induction of mGluR5- and Protein Synthesis-Dependent Long-Term Depression in Hippocampal Area CA1

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Huber, Kimberly M., John C. Roder, and Mark F. Bear. Chemical induction of mGluR5- and protein synthesis-dependent long-term depression in hippocampal area CA1. *J Neurophysiol* 86: 321–325, 2001. Recent work has demonstrated that specific patterns of synaptic stimulation can induce long-term depression (LTD) in area CA1 that depends on activation of metabotropic glutamate receptors (mGluRs) and rapid protein synthesis. Here we show that the same form of synaptic modification can be induced by brief application of the selective mGluR agonist (RS)-3,5-dihydroxyphenylglycine (DHPG). DHPG-LTD 1) is a saturable form of synaptic plasticity, 2) requires mGluR5, 3) is mechanistically distinct from *N*-methyl-D-aspartate receptor (NMDAR)-dependent LTD, and 4) shares a common expression mechanism with protein synthesis-dependent LTD evoked using synaptic stimulation. DHPG-LTD should be useful for biochemical analysis of mGluR5- and protein synthesis-dependent synaptic modification.

INTRODUCTION

Homosynaptic long-term depression (LTD) is a widely expressed form of synaptic plasticity in the brain. The best understood type of LTD is induced in hippocampal area CA1 by low-frequency synaptic stimulation (LFS) via an *N*-methyl-D-aspartate (NMDA) receptor-dependent rise in postsynaptic intracellular Ca²⁺ and the activation of a protein phosphatase cascade (Bear and Abraham 1996). Under the appropriate circumstances, pharmacological activation of NMDA receptors (NMDARs) can also induce this type of LTD. This “chem-LTD” approach has been useful for the biochemical characterization of the mechanism, revealing, for example, that NMDAR-dependent LTD is associated with dephosphorylation of the GluR1 subunit of the postsynaptic α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor (Lee et al. 1998).

Recent work has shown that mechanistically distinct types of LTD can also be induced in CA1 by other types of synaptic stimulation. For example, paired-pulse stimulation repeated at 1 Hz for 15 min (PP-LFS) induces LTD that is independent of NMDARs and requires activation of metabotropic glutamate receptors (mGluRs) (Huber et al. 2000; Kemp and Bashir 1999). This mGluR-dependent form of LTD is of particular interest because it also requires rapid translation of preexisting mRNA (Huber et al. 2000). A “chem-LTD” approach could be particularly useful for dissecting this novel mechanism. Indeed, reports from several groups indicate that transient activation of

group 1 mGluRs with the selective agonist (RS)-3,5-dihydroxyphenylglycine (DHPG) can induce LTD (Camodeca et al. 1999; Fitzjohn et al. 1999; Huber et al. 2000; Palmer et al. 1997). However, it is clear that not all protocols are equivalent; for example, some are effective only under conditions of low Mg²⁺ and are partially dependent on NMDARs (Palmer et al. 1997; Schnabel et al. 1999).

Here we characterize a chemical induction protocol that reliably produces protein synthesis-dependent LTD (Huber et al. 2000). We show that mGluR5 is required for LTD induction and provide novel evidence that this chemically induced LTD shares a common saturable expression mechanism with LTD induced using PP-LFS. We anticipate that the method we describe here will be useful for understanding how mGluR activation regulates mRNA translation and the expression of synaptic LTD.

METHODS

All animals were used in accordance with procedures approved by the Brown University Institutional Animal Care and Use Committee. Hippocampal slices were prepared from *postnatal day 21–30* (P21–30) Long Evans rats (Charles River, Cambridge, MA) and mGluR5 knockout mice (Lu et al. 1997) as described previously (Huber et al. 2000). For most experiments, CA3 was removed immediately after sectioning. Slices recovered for 1–2 h at room temperature (rats) or at 30°C (mice) in artificial cerebrospinal fluid (ACSF) containing (in mM) 124 NaCl, 5 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 1 MgCl₂, 2 CaCl₂, and 10 dextrose, saturated with 95% O₂-5% CO₂. For recording, slices were placed in a submersion recording chamber and perfused with 30°C ACSF at a rate of 2 ml/min.

Synaptically evoked field potentials (FPs) were recorded from area CA1 as described previously (Huber et al. 2000). Sharp microelectrode and whole cell voltage-clamp recordings were made using Axoclamp 2B and Axopatch 1D amplifiers (Axon Instruments), respectively. Sharp electrodes (80–120 M Ω) were filled with 3 M K-acetate and 10 mM KCl; patch pipettes (3–7 M Ω) were filled with (in mM) 134 K-gluconate, 6 KCl, 4 NaCl, 10 HEPES, 0.2 EGTA, 4 MgATP, 0.3 TrisGTP, and 14 phosphocreatine. The pH of the internal solution was adjusted to 7.25 with KOH, and the osmolarity was adjusted to 300 mOsm with H₂O or sucrose. Only experiments in which there was less than a 15% change in series resistance were included in the analysis. Waveforms were filtered at 2 kHz and acquired and digitized at 10 kHz on a PC using Experimenter's Workbench (DataWave Systems, Boulder, CO).

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Baseline responses were collected every 10–30 s using a stimulation intensity (10–30 μ A; 0.2 ms) yielding 50–60% of the maximal response. Experiments in which there was a >5% drift in the response magnitude during the 20-min baseline period before DHPG or LFS were excluded from further analysis. All experiments with mGluR5 KO mice used wildtype littermates as controls and were performed blind to the genotype, later determined by Therion (Troy, NY). LFS consisted of 900 pulses at 1 Hz. PP-LFS consisted of 900 pairs of stimuli (50-ms interstimulus interval) delivered at 1 Hz. In saturation experiments, stimulus duration was increased from 0.2 to 0.4 ms during PP-LFS.

The group data were analyzed as follows: 1) the initial slopes of the FPs and excitatory postsynaptic potentials (EPSPs), or the amplitude of the excitatory postsynaptic currents (EPSCs), for each experiment were expressed as percentages of the preconditioning or DHPG base-

line average, 2) the time scale in each experiment was converted to time from the onset of conditioning or DHPG, and 3) the time-matched, normalized data were averaged across experiments and expressed in the text and figures as the means \pm SE. Significant differences between groups were determined using an independent *t*-test or ANOVA performed on a 5-min average taken 1 h after LFS or DHPG application.

R,S-DHPG and D-2-amino-5-phosphonopentanoic acid (D-AP5) was purchased from Tocris (St. Louis, MO); all other chemicals were from Sigma Chemical (St. Louis, MO). DHPG was prepared as a 100 times stock in H₂O, aliquoted and stored at -20° C. Fresh stocks were made once a week. A 10 times stock of AP5 was prepared in ACSF and stored at 4° C. These stocks were diluted in ACSF to achieve their final concentrations. Picrotoxin was dissolved directly into ACSF immediately before use.

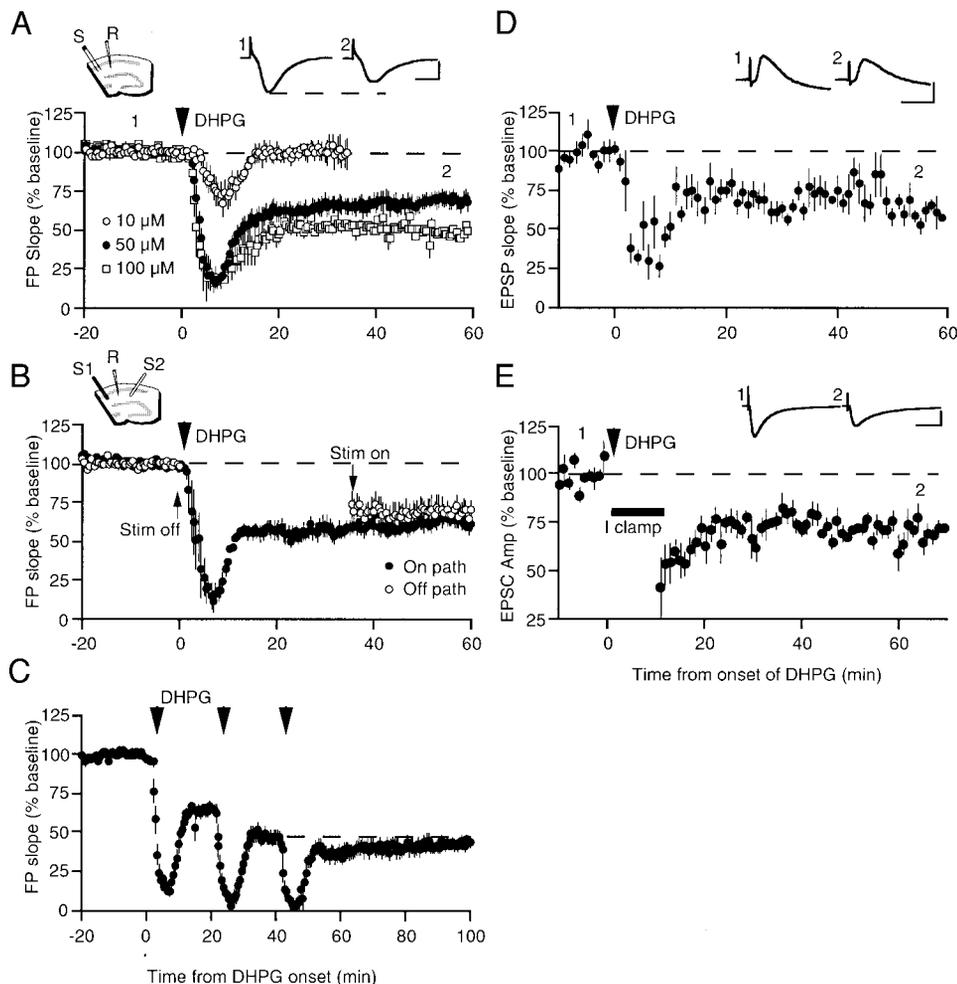


FIG. 1. Properties of (RS)-3,5-dihydroxyphenylglycine (DHPG)-induced long-term depression (LTD). **A:** dose dependence of effects of DHPG application (5 min; indicated by the downward arrow) on field potential (FP) slope values (10 μ M DHPG; $n = 5$; 50 μ M; $n = 11$; 100 μ M DHPG; $n = 4$). *Inset:* schematic of placement of stimulating (S) and extracellular recording (R) electrodes in an isolated CA1 hippocampal slice. Representative field potentials (2-min average) from a slice treated with 50 μ M DHPG and taken at the times indicated by the numbers on the graph. Calibration: 0.5 mV, 5 ms. **B:** DHPG-LTD is stimulation independent. *Inset:* placement of stimulating electrodes (S1 and S2) that stimulated 2 independent inputs in alternation. Stimulation to 1 pathway (OFF path; \circ) was turned off immediately prior to DHPG application and resumed 30 min after DHPG wash out, while the other (ON path; \bullet) input was stimulated at baseline frequency (0.067 Hz) for the duration of the experiment. A similar magnitude of depression was observed in both the ON and OFF paths ($n = 4$). **C:** DHPG-LTD is saturable. Two applications of DHPG are sufficient to saturate LTD. A 3rd DHPG application did not induce any further depression ($n = 8$). **D:** DHPG (50 μ M; 5 min) application induces a persistent depression of average excitatory postsynaptic potential (EPSP) slope values ($n = 6$). Representative EPSP waveforms (2-min average) taken from an experiment at times indicated by numbers on the graph. Calibration: 5 mV, 10 ms. **E:** DHPG (50 μ M; 5 min) application decreases excitatory postsynaptic current (EPSC) amplitudes. Cells were voltage clamped at -70 mV. Recording mode was switched from voltage clamp to current (I) clamp during and 5 min after DHPG application as indicated by the bar. Representative EPSCs (2-min average) taken from an experiment at the times indicated by the numbers on the graph. Calibration: 125 pA, 25 ms.

RESULTS

Application of DHPG for 5 min produced an acute, dose-dependent depression of evoked FPs (Fig. 1A). At concentrations $\geq 50 \mu\text{M}$, the FP did not fully recover after drug wash out. Instead, the synaptic responses stabilized at a depressed level ($50 \mu\text{M}$: $69 \pm 5\%$, means \pm SE, of pre-DHPG baseline; $n = 11$; $100 \mu\text{M}$: $48 \pm 1\%$; $n = 4$). In all subsequent studies $50 \mu\text{M}$, DHPG (5 min) was used to induce what we will refer to as DHPG-LTD. Application of another group 1 mGluR agonist, quisqualic acid (5 min; $5 \mu\text{M}$), also resulted in LTD ($81 \pm 2\%$; $n = 4$), confirming that the effect is not peculiar to DHPG. Two-pathway experiments ($n = 4$), in which only one input was stimulated during DHPG, indicated that DHPG-LTD does not require concurrent synaptic stimulation (stimulated: $62 \pm 4\%$; unstimulated: $68 \pm 5\%$, $P > 0.2$; Fig. 1B). DHPG-LTD also showed evidence of saturation; two applications of $50 \mu\text{M}$ DHPG were sufficient to produce maximal depression (Fig. 1C).

Intracellular recordings confirmed that the DHPG-LTD of FPs reflects diminished synaptic transmission. Both sharp electrode recording of EPSPs and whole cell voltage-clamp recording of EPSCs (recorded at -70 mV) revealed stable LTD (EPSP: $61 \pm 5\%$; $n = 6$; Fig. 1D; EPSC: $69 \pm 5\%$; $n = 5$; Fig. 1E). In contrast, there were no significant long-term changes in membrane potential, input resistance, or membrane excitability measured 1 h after DHPG (data not shown). Thus DHPG-LTD is a long-lasting modification of synaptic transmission.

The competitive NMDAR antagonist AP5 ($50 \mu\text{M}$) had no effect on the magnitude of DHPG-LTD as compared with interleaved control slices (AP5: $83 \pm 3\%$, $n = 5$; control: $85 \pm 3\%$, $n = 4$; $P > 0.3$; Fig. 2A). LTD induced with $5 \mu\text{M}$ quisqualic acid was also unaffected by AP5 ($79 \pm 2\%$; $n = 3$). Therefore LTD induced by pharmacological activation of group 1 mGluRs under these experimental conditions does not require concurrent NMDAR activation.

To assess the involvement of mGluR5, the major group 1 mGluR in area CA1 pyramidal neurons (Romano et al. 1995), DHPG-LTD was attempted in mice lacking this receptor. DHPG-LTD was absent in the mGluR5 homozygous mutants ($98 \pm 3\%$ measured 1 h after DHPG application; $n = 8$; Fig. 2A). An intermediate amount of LTD was observed in heterozygous mutants ($84 \pm 4\%$; $n = 6$), as compared with wild-type littermate controls ($77 \pm 2\%$; $n = 9$; Fig. 2B). A one-way ANOVA revealed a significant effect of genotype [$F(2,19) = 10.33$, $P < 0.001$]. A subsequent Tukey test revealed that both the wild-type and heterozygotes were significantly different from homozygotes ($P < 0.025$). Although there is a trend for DHPG-LTD in the heterozygotes to be less than wildtypes, this is not significant ($P = 0.5$). Thus DHPG-LTD strictly relies on mGluR5, and the presence of one allele for mGluR5 is sufficient for LTD induction. In contrast to DHPG-LTD, normal NMDAR-dependent LTD, induced with LFS, was observed in the homozygous mutants ($87 \pm 2\%$; $n = 6$; $P > 0.6$; Fig. 2C) as compared with the wild type mice ($89 \pm 5\%$; $n = 6$). These results indicate that there are two distinct routes of LTD induction in area CA1: one that relies on NMDARs and another on mGluR5.

The results from the mGluR5 knockouts indicate that the induction mechanisms of NMDAR-dependent LTD and DHPG-LTD are different. The next experiment was designed

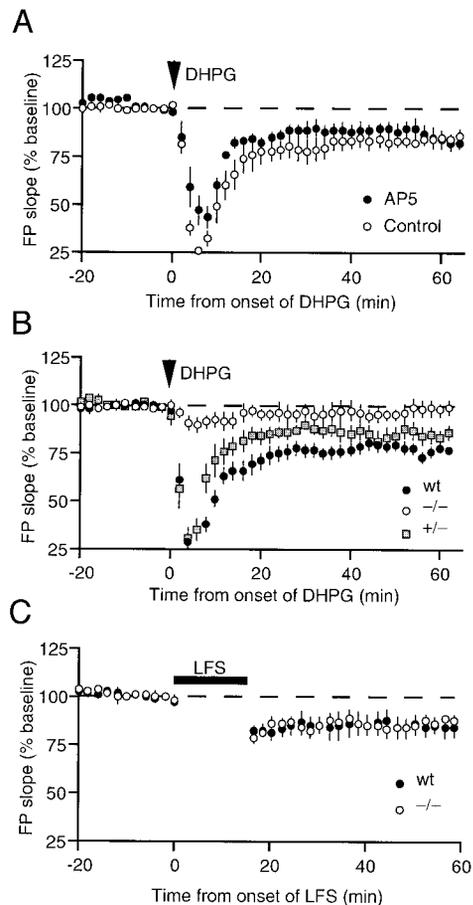


FIG. 2. DHPG-LTD, but not *N*-methyl-D-aspartate receptor (NMDAR)-dependent LTD, require mGluR5. **A**: DHPG-LTD is NMDAR independent. Preincubation of slices in D-2-amino-5-phosphopentanoic acid (AP5; $50 \mu\text{M}$; ●; $n = 5$) does not affect the magnitude of DHPG-LTD as compared with interleaved control slices (○; $n = 4$). **B**: DHPG-LTD requires mGluR5. DHPG application to slices from homozygote mGluR5 knockout mice ($-/-$; ○; $n = 8$) does not induce LTD. Intermediate LTD is observed in heterozygotes ($+/-$; ■; $n = 6$) as compared with slices from wild-type mice (wt; ●; $n = 9$). **C**: Low-frequency synaptic stimulation (LFS)-induced LTD does not require mGluR5. LFS induces a similar magnitude of LTD in both homozygote knockout mice ($-/-$; ○; $n = 6$) as compared with wild-type mice (wt, ●; $n = 6$).

to test whether these two forms of LTD utilize similar expression mechanisms. Repeated episodes of LFS were delivered to saturate NMDAR-dependent LTD (Fig. 3A). DHPG then was then applied, and the magnitude of LTD was measured by renormalizing FP slope values to a pre-DHPG baseline. If NMDAR-dependent LTD and DHPG-LTD utilize a common expression mechanism, then previous saturation of NMDAR-dependent LTD should reduce or occlude DHPG-LTD. However, DHPG still significantly depressed synaptic responses ($81 \pm 5\%$ of pre-DHPG baseline; $n = 5$; $P < 0.05$; Fig. 3B), suggesting that NMDAR-dependent LTD and DHPG-LTD use distinct expression mechanisms.

The same approach was used to assess whether DHPG-LTD employs the same saturable expression mechanism as synaptically evoked mGluR-dependent LTD. PP-LFS in the presence of the NMDAR antagonist D-AP5 ($50 \mu\text{M}$) was used to saturate mGluR-dependent LTD, and DHPG ($50 \mu\text{M}$) was then applied to the slice (Fig. 3C). In contrast to the previous occlusion experiment, DHPG application after saturation of LTD with PP-LFS did not induce any further LTD ($100 \pm 5\%$ of pre-

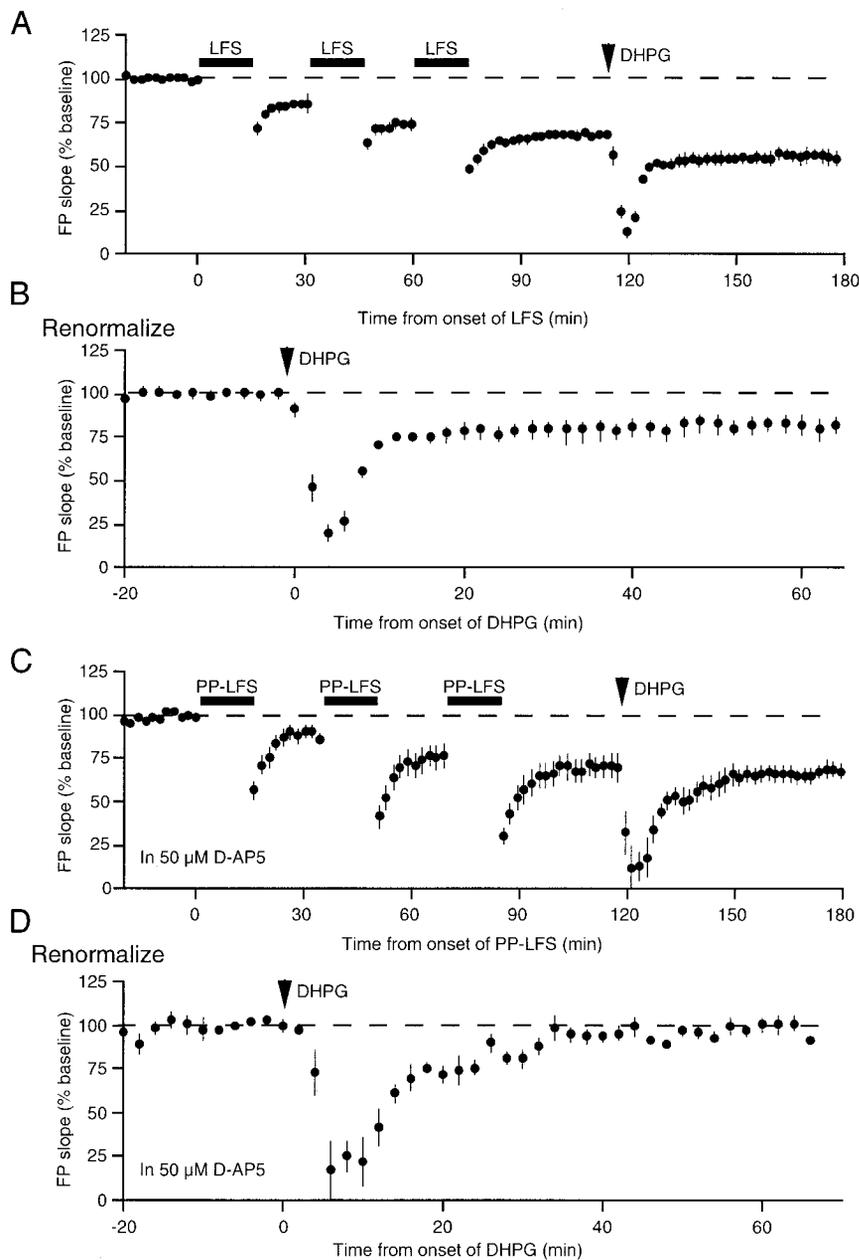


FIG. 3. DHPG-LTD is occluded by mGluR-dependent LTD induced with PP-LFS, but not NMDAR-dependent LTD. *A*: repeated episodes of LFS were delivered to saturate NMDAR-dependent LTD. DHPG (downward arrow) was then applied to the slice. *B*: renormalized FP slope values to the pre-DHPG baseline ($n = 8$). *C*: repeated episodes of PP-LFS were delivered to saturate mGluR-dependent LTD. DHPG (downward arrow) was then applied to the slice. The entire experiment was performed in $50 \mu\text{M}$ D-AP5 to prevent induction of NMDAR-dependent LTD. *D*: renormalized FP slope values to the pre-DHPG baseline ($n = 5$).

DHPG baseline; $n = 5$; $P > 0.5$; Fig. 3D). These results provide strong evidence that mGluR-LTD induced with DHPG and PP-LFS share common expression mechanisms.

DISCUSSION

A number of different protocols have been introduced to induce homosynaptic LTD in CA1 (Berretta and Cherubini 1998; Camodeca et al. 1999; Dudek and Bear 1992; Fitzjohn et al. 1999; Huber et al. 2000; Kemp and Bashir 1999; Oliet et al. 1997; Overstreet et al. 1997; Palmer et al. 1997). Although mGluR involvement has been suggested for many of these, the constellation of findings is confusing and not entirely consistent with a single mGluR-dependent form of LTD. For example, it has been reported that application of $100 \mu\text{M}$ DHPG for 10 min to adult hippocampal slices elicits little LTD unless slice excitability is increased by removing Mg^{2+} from the

extracellular medium (Palmer et al. 1997; Schnabel et al. 1999). The resulting LTD is partially blocked by NMDAR antagonists. Moreover, PP-LFS in adult hippocampal slices can apparently elicit LTD via activation of either group 1 mGluRs or activation of AMPA/kainate receptors (Kemp and Bashir 1999). In contrast, we recently demonstrated that in *P21–30* rats, both PP-LFS and DHPG ($50 \mu\text{M}$, 5 min) induce LTD that is 1) independent of NMDAR activation, 2) blocked entirely by mGluR antagonists, and 3) dependent on a transient phase of mRNA translation (Huber et al. 2000). The latter finding is of particular importance, as this mGluR-LTD model should be useful for elucidating the regulation and function of dendritic protein synthesis, which may be defective in fragile-X mental retardation (Jin and Warren 2000).

Because of the diverse effects of DHPG and PP-LFS, it could not be assumed that previous findings under different experimental conditions would apply to our model. Therefore

it was necessary to characterize the protein synthesis-dependent form of mGluR-LTD. We have shown here that DHPG-LTD is a saturable form of synaptic plasticity, that it requires mGluR5, that it is mechanistically distinct from NMDAR-dependent LTD, and, importantly, that it shares a common saturable expression mechanism with the LTD evoked using PP-LFS. Because DHPG-LTD does not require concurrent synaptic stimulation, it is a form of "chem-LTD" (Lee et al. 1998) that should be useful for biochemical and biophysical studies.

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