Differential roles for group 1 mGluR subtypes in induction and expression of chemically-induced hippocampal long-term depression

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Running head: mGluR1 and long-term depression
Text pages: 40 Figures: 5 Tables: 0
Word count: Abstract: 235

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Abstract

Although mGluR1 and mGluR5 are often found to have similar functions, there is considerable evidence that the two receptors also serve distinct functions in neurons. In hippocampal area CA1, mGluR5 has been most strongly implicated in long-term synaptic depression (LTD), whereas, mGluR1 has been thought to have little or no role. Here we demonstrate that simultaneous pharmacological blockade of mGluR1 and mGluR5 is required to block LTD induced by the group 1 mGluR agonist, (RS)-3,5-Dihydroxyphenylglycine (DHPG). Blockade of mGluR1 or mGluR5 alone has no effect on LTD induction suggesting that activation of either receptor can fully induce LTD. Consistent with this conclusion, mGluR1 and mGluR5 both contribute to activation of ERK which has previously been shown to be required for LTD induction. In contrast, selective blockade of mGluR1, but not mGluR5, reduces the expression of LTD and the associated decreases in AMPA surface expression. LTD is also reduced in mGluR1 knockout mice confirming the involvement of mGluR1. This demonstrates of a novel role for mGluR1 in long-term synaptic plasticity in CA1 pyramidal neurons. In contrast to DHPG-induced LTD, synaptically induced LTD with paired pulse low frequency stimulation (PP-LFS) persists in the pharmacological blockade of group 1 mGluRs and in mGluR1 or mGluR5 knockout mice. This suggests different receptors and/or upstream mechanisms for chemically and synaptically induced LTD.

Key Words: mGluR1, mGluR5, long-term depression, AMPA receptor, CA1
Introduction

Group 1 metabotropic glutamate receptors (mGluRs) are composed of two subtypes, mGluR1 and mGluR5. Both receptors are coupled to the Gq subtype of heterotrimeric G proteins and activate phospholipase C. mGluR1 and mGluR5 both contribute to increased neuronal excitability, intracellular Ca\textsuperscript{2+} increases (Ireland and Abraham 2002; Thuault et al. 2002; Rae and Irving 2004), synaptic plasticity (Sung et al. 2001; Gubellini et al. 2003), and pain (Karim et al. 2001). Although they have similar signaling cascades, there is evidence that mGluR1 and mGluR5, expressed in the same neuron, can serve distinct functions (Mannaioni et al. 2001; Merlin 2002) (Thuault et al. 2002; Kettunen et al. 2003) for review see (Valenti et al. 2002) In hippocampal CA1 pyramidal neurons mGluR5 is the most highly expressed subtype (Romano et al. 1995; Lujan et al. 1996; Shigemoto et al. 1997). However, a role for mGluR1 has recently been elucidated using the selective mGluR1 antagonist LY367385 (Clark et al. 1997). In particular, mGluR1 contributes to many functions in CA1 pyramidal neurons including cell depolarization, intracellular Ca\textsuperscript{2+} increases, decreases in the after-hyperpolarization potential (AHP), short term depression of excitatory postsynaptic currents (EPSCs) and Extracellular signal-Regulated Kinase (ERK) activation (Mannaioni et al. 2001; Ireland and Abraham 2002; Berkeley and Levey 2003; Rae and Irving 2004).

Group 1 mGluR activation of CA1 pyramidal neurons with the selective agonist, DHPG, or with low frequency (1-5 Hz) synaptic stimulation causes long-term depression of excitatory synaptic transmission (mGluR-LTD) (Bolshakov and Siegelbaum 1994; Oliet et al. 1997; Palmer et al. 1997; Fitzjohn et al. 1999; Huber et al. 2000; Huber et al. 2001). mGluR-LTD in mature rodents is mediated by a persistent reduction in the
number of postsynaptic amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors (AMPAR) (Snyder et al. 2001; Xiao et al. 2001; Nosyreva and Huber 2005). In addition, there is evidence for presynaptic contributions to mGluR-LTD (Bolshakov and Siegelbaum 1994; Oliet et al. 1997; Fitzjohn et al. 2001; Watabe et al. 2002; Zakharenko et al. 2002; Feinmark et al. 2003; Rammes et al. 2003). One of the most interesting properties of mGluR-LTD is that it relies on rapid (within minutes) dendritic protein synthesis (Huber et al. 2000). Likewise, the long-term decrease in AMPAR surface expression requires protein synthesis (Snyder et al. 2001; Nosyreva and Huber 2005). Recent work has revealed the signaling pathways that activate translation in response to mGluRs. The ERK and PI3K/mTOR pathways regulate translation initiation in many cell types, including neurons, are activated by DHPG and required for mGluR-LTD (Gallagher et al. 2004; Hou and Klann 2004) for reviews see (Kelleher et al. 2004; Klann and Dever 2004). The fact that LTD induced with either DHPG or synaptic stimulation (paired pulse low frequency stimulation; PP-LFS) are both blocked by the broad range mGluR antagonist, LY341495, occlude each other, and both rely on protein synthesis and ERK activation suggest that these two methods of LTD induction represent the same or a similar LTD mechanism (Huber et al. 2000; Huber et al. 2001; Gallagher et al. 2004).

mGluR-LTD is absent in mGluR5 knockout (KO) mice suggesting a requirement for this subtype for either induction or expression of LTD (Huber et al. 2001). Consistent with this work, the mGluR5 antagonist MPEP has been shown to reduce or abolish mGluR-LTD in rats and mice (Gasparini et al. 1999; Faas et al. 2002; Hou and Klann 2004; Huang et al. 2004; Huang and Hsu 2005). In contrast, selective mGluR1
blockade has been reported to have either a partial or no effect on DHPG-induced LTD in area CA1 (Fitzjohn et al. 1999; Faas et al. 2002; Hou and Klann 2004). Recent work has demonstrated that mGluR1 activity is required for the acute, short-term, depression of excitatory synaptic transmission induced with DHPG (Mannaioni et al. 2001) (Faas et al. 2002). Therefore, mGluR1 and mGluR5 both regulate excitatory synaptic transmission onto CA1 neurons. Remarkably, the expression of DHPG-induced LTD can be reversed by broad mGluR antagonists, even when applied hours after the induction stimulus (Palmer et al. 1997; Fitzjohn et al. 1999; Watabe et al. 2002). Therefore, sustained mGluR activation is required for the expression of mGluR-LTD. MGlur-LTD is enhanced in the mouse model of fragile X syndrome and it has been suggested that group 1 mGluR antagonists may serve as potential therapies for Fragile X Syndrome patients (Huber et al. 2002; Bear et al. 2004). Therefore, determination of the specific mGluR subtypes required for LTD induction and expression may facilitate development of pharmaceutical treatments for Fragile X Syndrome or other forms of mental retardation.

In light of accumulating data for mGluR1 function in CA1 pyramidal neurons and synapses, we evaluated the role of mGluR1 in LTD of their synapses. Unexpectedly, we find a role for mGluR1 in the induction and expression of DHPG-induced LTD. Our data demonstrate that activation of mGluR1 or mGluR5 alone can induce the full complement of LTD. Consequently, simultaneous blockade of mGluR1 and mGluR5 is required to abolish the induction of DHPG LTD and the associated ERK activation. mGluR1, however, is selectively required for the expression of DHPG-induced LTD. In contrast, synaptically-induced LTD (with PP-LFS), is unaffected by blockade or genetic
knockout of group 1 mGluRs. These data provide evidence for new and additional roles for mGluR1 at hippocampal CA1 synapses and suggests that different neurotransmitter receptors induce chemically and synaptically induced LTD.

Materials and Methods

Drugs

D,L-AP5 (Tocris; Ellisville, MO) was prepared fresh in ACSF. R,S-DHPG or S-DHPG, LY367385, LY341495, MPEP (Tocris), tetraethylammonium chloride and tetrodotoxin (Sigma) were prepared as stocks in water or equimolar NaOH (LY367385 and LY341495), aliquoted and frozen for no more that 10 days. We observed no differences in the effectiveness of fresh or frozen drugs.

Electrophysiology.

Hippocampal slices were prepared from Long Evans hooded or Sprague Dawley rats, or mGluR1 or mGluR5 knockout (KO) or wildtype mice. Rats were obtained from Charles River Laboratories (Boston, MA). mGluR1 KO mice were originally from Francois Conquet (Conquet et al. 1994). mGluR5 KO mice were from John Roder (Lu et al. 1997) and were mated with wildtype C57BL/6 mice (Jackson Laboratories; Bar Harbor, ME) to obtain heterozygotes. Experiments in KO mice were compared to those in WT littermates. Hippocampal slices (400μm) were prepared from 19 to 55-day old animals. Rats or mice were anesthetized with the barbiturate pentobarbital (50mg/kg) and decapitated soon after the disappearance of corneal reflexes. The brain was removed, dissected and sliced in ice-cold dissection buffer containing (in mM): 2.6 KCl, 1.25 NaH₂PO₄, 26 NaHCO₃, 0.5 CaCl₂, 5 MgCl₂, 212 sucrose, and 10 dextrose, using a vibratome (Leica VT 1000S, Germany). The slices were transferred into a reservoir
chamber filled with artificial cerebrospinal fluid (ACSF) containing (in mM): 124 NaCl, 5 KCl, 1.25 NaH2PO4, 26 NaHCO3, 2 CaCl2, 1 MgCl2, and 10 dextrose. Slices were allowed to recover for 2-5 hours at 30°C. ACSF and dissection buffer were equilibrated with 95% O2-5% CO2.

For recording, slices were transferred to a submerged recording chamber, maintained at 30°C and perfused continuously with ASCF at a rate of 2.5-3 ml/min. Field potentials (FPs) were recorded with extracellular recording electrodes (1MΩ) filled with ACSF and placed in stratum radiatum of area CA1. FPs were evoked by monophasic stimulation (200 µs duration) of Schaffer collateral/commissural afferents with a concentric bipolar tungsten stimulating electrode (FHC, Bowdoinham, ME). Stable baseline responses were collected every 30 sec using a stimulation intensity (10-30 μA) yielding 50-60% of the maximal response. The initial slope of the FP was used to measure stability of synaptic responses and quantify the magnitude of LTD. Chemically-induced mGluR-LTD was elicited by application of 100 μM DHPG for 5 or 20 min as indicated. Synaptically induced LTD was induced using paired pulse low frequency stimulation (PP-LFS; paired (50ms interstimulus interval) pulses at 1 Hz for 15 (rats) or 20(mice) min. The group data were analyzed as follows: (1) the initial slope of the FP were expressed as percentages of the pre- DHPG baseline average, (2) the time scale in each experiment was converted to time from the onset of DHPG, and (3) the time-matched, normalized data were averaged across experiments and expressed in the text and figures as the means (± SEM). The effects of all pharmacological treatments on LTD were evaluated by comparing interleaved control and treated slices. Significant differences were determined by a Student’s independent t-test. Paired t-tests were
used to determine significance of reversal effects on LTD expression (Fig. 2). Probability values of $p<0.05$ were considered to represent significant differences.

For $I_{\text{AHP}}$ measurements, whole cell voltage clamp recordings were performed from visualized CA1 pyramidal neurons under IR-DIC optics. Whole cell pipettes (3-7 MΩ) were fabricated from thick wall (1.5 mm O.D., 0.86 mm I.D., Sutter Instruments) borosilicate glass and filled with (in mM) K-methanesulfonate, 135; KCl, 8; NaCl, 4; HEPES, 10; MgATP, 4; and TrisGTP, 0.4, pH 7.25, 300 mOsm. To elicit and measure the $I_{\text{AHP}}$, cells were voltage clamped at -50 mV and depolarizing steps (+60 mV; 200 mS) were applied every 30 sec to elicit an unclamped Ca$^{2+}$ action current. The resulting outward tail current (10-15 msec after the offset of the depolarizing step) was measured as the $I_{\text{AHP}}$. This would be considered the medium AHP as previously described (Pedrazani and Storm 1993; Mannaioni et al. 2001). Series and input resistance were monitored throughout the experiment. Only cells which maintained a stable series resistance (<15% change) were included in the analysis. FP and $I_{\text{AHP}}$ records were filtered at 2 kHz, acquired and digitized at 10 kHz on a PC using custom software (Labview; National Instruments, Austin, TX). Paired t-tests were used to make within cell comparisons of the effects of MPEP on DHPG-induced suppression of $I_{\text{AHP}}$.

Biochemical measurements of surface expressed AMPA receptors and ERK phosphorylation.

Hippocampal slices were prepared as for electrophysiology experiments. After a 2-3 hr recovery period in ACSF, slices (containing area CA1 and dentate gyrus, CA3 was cut off) were maintained in a static incubation chamber in ACSF (containing 100
μM D,L-AP5) at 30°C and aerated with 95% O₂/5% CO₂. Slices were preincubated in antagonist and then treated with DHPG (5 min) or ACSF (control). For P-ERK measurements, slices were frozen immediately after DHPG treatment and stored at -80°C as previously described (Gallagher et al. 2004). Slices were homogenized in lysis buffer containing 50 mM HEPES, pH 7.3, 150 mM NaCl, 1.5mM MgCl₂, 1mM EGTA, 10% glycerol, 0.2mM NaVO₄, 100mM NaF, 50mM β-glycerophosphate, 1mM dithiothreitol, 1mM benzamidine, 0.01mg/ml leupeptin, 0.1 mg/ml aprotonin, 0.5 μg/ml pepstatin A, and 1% Triton. Protein concentrations were measured with a BCA Protein Assay (Pierce). Samples containing 20-35 μg of protein were resolved on 10% SDS-PAGE in duplicate and transferred to nitrocellulose. Membranes were blocked and incubated with phosphospecific (P)-ERK (Thr202/Tyr204, Promega; 1:5000 dilution) or total ERK (1:1000; Cell Signaling Technologies) according to manufacturer’s protocol.

Biotinylation experiments were performed as previously described (Chung et al. 2000; Heynen et al. 2003; Nosyreva and Huber 2005). From each rat, two to three slices were pooled together for one condition. Fifteen minutes after DHPG treatment, slices were placed on ice to stop endocytosis, then washed with ice-cold ACSF and incubated in ACSF containing 1 mg/ml Sulfo-NHS-LC-Biotin (Pierce, Rockford, IL) for 10 min on ice. To quench the biotin reaction slices were washed three times with Tris-buffered saline (TBS) and homogenized in a modified radioimmunoprecipitation (RIPA) buffer containing: 50 mM Tris-HCl, pH 7.4, 1% Triton X100, 0.1 % SDS, 0.5% Na-deoxycholate, 150 mM NaCl, 2 mM EDTA, 50 mM NaH₂PO₄, 50 mM NaF, 10 mM Na₄P₂O₇, 1mM Na₃VO₄, and protease inhibitor cocktail III (Calbiochem, La Jolla, CA). The homogenates were centrifuged at 14,000x g for 10 min at 4°C. 15 μg of protein
was removed for total (T) protein measurements. 150 µg of protein were then mixed with 150 µl of UltraLink immobilized NeutrAvidin beads (Pierce) by rotating for 2 h at 4°C. The beads were washed with 10 volumes of RIPA buffer and proteins were eluted with SDS-PAGE sample buffer supplemented with 50 mM dithiothreitol for 20 min at 90°C. Both total and biotinylated proteins were resolved by SDS-PAGE transferred to nitrocellulose membranes and probed with anti-GluR1 C-terminal antibody (1:5000, Upstate Biotechnology). Immunoreactive bands were visualized by enhanced chemiluminescence (ECL), captured on autoradiography film (Kodak). Digital images, produced by densitometric scans of autoradiographs on a ScanJet 4300C (Hewlett Packard), and quantified using Scion Image software. Multiple film exposures were performed and quantified to insure that the values were in the linear range of the ECL reaction. A subset of GluR1 biotinylation experiments were quantified both using chemiluminescence (ECL) and chemifluorescence using ECL Plus (Amersham) and quantified using a Storm 860 scanner (Molecular Dynamics) which yielded similar results.

The P-ERK/total ERK or surface/total GluR1 ratio was calculated for each condition. For the GluR1 ratios, duplicate conditions within one animal were averaged to obtain an animal average for that condition. Therefore, the n values for the biotinylation experiments (Fig 4) represent the number of rats as opposed to slices. Significant differences between surface/total ratios of treated slices and within animal control slices were determined using a Wilcoxon signed rank test. Although the raw ratio values were used for statistical comparisons, the group data are presented in
Figure 4 as a percent of condition control to compare across different treatment conditions.

**Results**

**Combined blockade of mGluR1 and mGluR5 is necessary to block DHPG-induced LTD**

MGlur-LTD can be induced chemically with the specific group 1 mGluR agonist, R,S-dihydroxyphenylglycine (DHPG) (Ito et al. 1992). Previously, we found that LTD induced with 5 min of DHPG was absent in mGluR5 KO mice (Huber et al. 2001). We first attempted to confirm that pharmacological blockade of mGluR5 inhibited DHPG-induced LTD (Faas et al. 2002; Hou and Klann 2004; Huang et al. 2004). MPEP is the most potent and selective commercially available mGluR5 antagonist with an IC<sub>50</sub> = 32 nM for mGluR5 and > 100 μM for mGluR1 (Gasparini et al. 1999). We first tested the ability of MPEP to block DHPG-induced LTD in hippocampal slices prepared from Long Evans rats. Extracellular field potential recordings elicited by Schaffer collateral stimulation were obtained in area CA1. We performed these and all subsequent LTD experiments in the NMDA receptor antagonist D,L-AP5 (100 μM) to prevent induction of NMDAR dependent LTD (Dudek and Bear 1992). AP5 had no effect on DHPG-induced LTD (100 μM; 20 min; data not shown) (Huber et al. 2001). Surprisingly, in contrast to previous reports, MPEP (10 μM), applied during the baseline and DHPG application, had no effect on the acute or long-lasting depression induced with DHPG (100 μM; 5 min) (MPEP = 77 ± 2% of pre-DHPG baseline, n = 6, measured 60-65 min after DHPG application; interleaved control slices = 77 ± 3%, n = 6, p=0.99; Fig. 1A). To test the role of mGluR1 in the induction of LTD, we applied the mGluR1 antagonist LY367385
(100 μM) before and during DHPG (100 μM; 5 min). As previously described, LY367385 reduced the acute depression observed during DHPG application, but did not affect LTD (acute depression; LY367385 = 64 ± 4%, n = 5; interleaved controls = 37% ± 5%, n = 7, p < 0.01; LTD; LY367385 = 82 ± 4%, n = 5; interleaved control; 82 ± 4%, n = 7, p = 0.92; Fig. 1B) (Fitzjohn et al. 1999; Mannaioni et al. 2001; Hou and Klann 2004). Therefore, mGluR1 or mGluR5 blockade alone does not affect the induction of mGluR-LTD. However, preincubation in both MPEP (10 μM) and LY367385 (100 μM) completely blocks mGluR-LTD induced with DHPG (LY367385 + MPEP = 97 ± 2%, n = 7; interleaved controls = 83 ± 4%, n = 6, p < 0.01; Fig. 1C). These data suggest that activation of mGluR1 or mGluR5 is sufficient to induce LTD and therefore inhibition of both receptors is required to block LTD induction. Interestingly, LY367385 and MPEP did not completely block the acute depression observed during DHPG application (60 ± 6% of pre-DHPG baseline, n = 7; Fig. 1C). It is unlikely that the residual acute depression is due to sub-saturating concentrations of LY367385 or MPEP, as increasing LY367385 to 150 μM did not further inhibit the acute depression during DHPG as compared to 100 μM LY367385 (62% ± 6%, n=6; p = 0.8) and MPEP had no affect on the acute depression when applied alone (Fig. 1A) or in the presence of LY367385 (compare Fig. 1B and Fig. 1C).

mGluR1 and mGluR5 contribute to DHPG induced phosphorylation of ERK

DHPG induces phosphorylation of the mitogen-activated kinase, ERK, in area CA1 (Roberson et al. 1999; Berkeley and Levey 2003; Gallagher et al. 2004) and ERK activation is required for mGluR-LTD (Gallagher et al. 2004). Therefore, ERK activation may be a biochemical measure of the LTD induction signaling cascade. Because both
mGluR1 and mGluR5 contribute to the induction of LTD, we predicted that both receptors contribute to ERK activation and that activation of mGluR1 or mGluR5 alone would induce phosphorylation of ERK. Application of LY367385 and MPEP together blocked DHPG-induced (100 μM; 5 min) phosphorylation of ERK (Gallagher et al. 2004). In contrast to their effects on DHPG-induced LTD, MPEP or LY367385 alone inhibited DHPG-induced ERK phosphorylation compared to DHPG alone (ASCF + DHPG; 470 ± 64% of basal levels, n = 6; MPEP + DHPG = 195 ± 24%, n = 5; LY367385 + DHPG = 214 ± 45%, n = 7; Fig. 1D). A one-way ANOVA and subsequent multiple comparison test (Fisher’s PLSD) indicated that the ACSF (DHPG only) group was different from either MPEP or LY367385 treated groups (F(2,15) = 11.01, P = 0.002; MPEP; p = 0.005; LY367385; p = 0.005). Although LY367385 and MPEP reduce the levels of phosphorylated ERK, DHPG still increased ERK phosphorylation over basal levels in the presence of each drug (MPEP, p = 0.01; LY367385, p = 0.04; t-test). Taken together, the data in Fig.1 suggest that this moderate level ERK activation is sufficient to induce the full level of LTD.

**mGluR1 activity is required for LTD expression**

Previous work has demonstrated that blockade of mGluR1 with LY367385 during DHPG application has either no effect on LTD induction or a partial effect (Fitzjohn et al. 1999; Faas et al. 2002; Hou and Klann 2004) (Fig. 1B). These studies differ with regard to the duration of DHPG (5 or 20 min) and/or LY367385 application. We found that the presence of LY367385 throughout the experiment reduced DHPG-LTD by approximately 50% using either brief (5 min) or prolonged (20 min) DHPG applications (5 min; LY367385; 91± 1%; n = 6; Control 79 ± 2%; n = 7; p = 0.002; Fig. 4A; 20 min;
LY367385 = 88 ± 4% measured at 75-80 min., n=6; controls = 66 ± 2%, n=6, p < 0.01; (Fig. 2A). Because DHPG-LTD is unaffected when LY367385 is washed out of the slice immediately after DHPG (Fig. 1B), this suggests a role for mGluR1 in LTD expression. Previous reports have shown that DHPG-induced LTD can be transiently “reversed” by applying broad range mGluR antagonists after LTD has been established (Palmer et al. 1997; Fitzjohn et al. 1999; Watabe et al. 2002). To further examine the role of mGluR1 in expression of LTD, we tested the ability of LY367385 to reverse LTD. LY367385 was applied for 20 min. beginning 60 min. after DHPG application. LY367385 reversed approximately 50% of LTD expression (LTD, 55-60 min post DHPG = 69 ± 2%; LY367385 reversal, 75-80 min post DHPG = 86 ± 4%, n = 12; p < 0.01; Fig. 2B). After LY367385 washout, LTD was re-established and not different from LTD before LY367385 (100-105 min. post DHPG = 73 ± 4%, n = 12, p = 0.1). LY367385 did not facilitate baseline synaptic transmission before DHPG application (95 ± 2%; n = 8; p = 0.07; Fig. B2), suggesting that LY367385 is specifically reversing an LTD process. To confirm the role for mGluR1 in expression of DHPG-induced LTD, we evaluated LTD in mGluR1 knockout mice. In agreement with our pharmacological data, the DHPG-induced acute depression and LTD are reduced by approximately 50% in mGluR1 knockout mice (acute depression measured 15-20 min: mGluR1 KO = 59 ± 2%; mGluR1 WT littermates = 34 ± 4%, p < 0.01; LTD: mGluR1 KO = 81 ± 3%; mGluR1 WT littermates = 64 ± 4%, p = 0.01 measured 75-80 min after DHPG application; Fig. 2C). We also examined the ability of the mGluR1 antagonist LY367385 to reverse LTD in WT and mGluR1 KO mice. Consistent with our data in rats, LY367385 transiently reverses LTD in WT mice when it is applied after LTD has been established (80-100 min after
DHPG application) (mGluR1 WT, reversal; 79 ± 5% measured at 95-100 min after DHPG application; p < 0.05 compared to LTD at 75-80 min.). However, LY367385 had no effect in mGluR1 KO mice, confirming that LY367385 is selective for mGluR1 (mGluR1 KO reversal = 83 ± 3% measured at 95-100 min after DHPG application; p = 0.44 compared to LTD at 75-80 min.; Fig. 2C). Interestingly, LY367385 reverses LTD in the mGluR1 WT up to the level of LTD in the mGluR1 KO such that there is no difference in the magnitude of LTD between the mGluR1 KO and WT during LY367385 application (p = 0.61; Fig. 2C). Overall, these results implicate sustained mGluR1 activity in the expression of LTD.

In contrast to mGluR1, mGluR5 blockade with 10µM MPEP throughout the experiment had no effect on LTD (MPEP = 64 ± 3%, n = 7; interleaved controls = 62 ± 4%, n = 4; p = 0.76; Fig. 2D). In addition, mGluR5 blockade with MPEP 60-80 min after DHPG failed to reverse LTD expression (LTD, 55-60 min post DHPG application = 53 ± 4%; MPEP reversal, 75-80 min post DHPG = 55 ± 4%, n = 10; p = 0.5). Prolonging the MPEP application from 20 to 60 min also did not reverse LTD expression (LTD, 55-60 min post DHPG = 57 ± 4%; MPEP reversal, 115-120 min post DHPG = 64 ± 8%, n = 5; p = 0.29; Fig. 2E). LTD is completely blocked by the presence of MPEP and LY367385 throughout the experiment (Fig. 1C). Therefore, we predicted that their combined application would completely reverse LTD. This was not the case. MPEP and LY367385 perfusion after DHPG, only partially reversed LTD (LTD, 55-60 min post DHPG = 67 ± 3%; MPEP + LY367385 reversal, 75-80 min post DHPG = 89 ± 3%, n = 8; p < 0.01; Fig. 2F) and was not different than reversal with LY367385 alone (p = 0.78).
MPEP inhibits the DHPG-induced suppression of $I_{\text{AHP}}$ in hippocampal CA1 neurons but not DHPG-induced LTD

Here, we have demonstrated that MPEP inhibits ERK phosphorylation (Fig. 1D) and blocks LTD induction when combined with mGluR1 blockade (Fig 1C). However, in light of our negative results with MPEP alone on LTD, we wanted to confirm an effect of MPEP on an electrophysiological measure in our slice preparation. We tested the ability of MPEP to blocked DHPG-induced suppression of the after-hyperpolarization (AHP). The AHP can be divided into 3 components, a fast, medium and slow AHP, which are mediated by activation of different potassium conductances (Storm 1990). DHPG suppresses both the medium and slow AHP which are either partially or completely blocked by MPEP (Mannaioni et al. 2001; Ireland and Abraham 2002). Whole cell patch clamp recordings were obtained from visualized CA1 neurons from rat hippocampal slices. Cells were voltage clamped at -50 mV and the Ca$^{2+}$ activated potassium current which mediates the AHP ($I_{\text{AHP}}$) was elicited by applying a depolarizing step to +60 mV. An outward tail current was measured as the $I_{\text{AHP}}$ as described (Pedarzani and Storm 1993; Mannaioni et al. 2001) (Fig. 3A). DHPG application (100 μM) reduced the $I_{\text{AHP}}$ (baseline = 382 ± 32 pA; DHPG = 221 ± 21 pA, n = 10; p < 0.01). MPEP (10 μM) applied in the presence of DHPG reversed the effects of DHPG on the $I_{\text{AHP}}$ (DHPG+MPEP = 266 ± 19 pA, n = 10; p < 0.01). Similar results were observed with 30 μM DHPG (baseline = 366 ± 32 pA; DHPG = 161 ± 27 pA, p < 0.01 compared to baseline; DHPG+MPEP = 229 ± 29 pA, n = 9, p < 0.01 compared to DHPG alone. Although MPEP did not completely reverse the effects of DHPG in every cell tested, on average it reduced the DHPG-induced suppression of the $I_{\text{AHP}}$. Our
result is consistent with that of Ireland et al., (2002) who found that both mGluR1 and mGluR5 mediate IAHP suppression in CA1 neurons and suggests that our inability to block LTD is not due to inactive MPEP.

Strain variations in LTD and mGluR expression have been noted in mice and rats (Manahan-Vaughan 2000, 2000; Chen et al. 2005). In order to determine if our inability to block LTD with MPEP is due to the particular rat strain used in our experiments we tested the effects of MPEP on LTD in a different strain of rats and in mice. Previous work has demonstrated that MPEP blocks LTD in hippocampal slices prepared from Sprague Dawley (SD) rats induced with the mixed isomer (R,S) DHPG (100 μM; 20 min) or the active form S-DHPG (50 μM; 5 min) (Faas et al. 2002; Huang et al. 2004). Consistent with our data in Long Evans rats, we find that in slices prepared from Sprague Dawley rats MPEP (10 μM) has no effect on LTD induced with R,S-DHPG (100 μM; 20 min) (MPEP = 60 ± 4%, n = 8; interleaved controls = 62 ± 3%, n = 8, p = 0.78; Fig. 3B) or S-DHPG (50 μM; 5 min) (MPEP = 85 ± 9%, n = 2; control = 88 ± 2%, n = 2, p = 0.73). We also see no effect of MPEP on DHPG-induced LTD (100 μM; 20 min) in slices prepared from C57BL6 mice (MPEP = 74 ± 5%, n = 8; control = 69 ± 5%, n= 4, p = 0.55; Fig. 3C). Finally, slices (from Long Evans rats) were perfused with 25 μM MPEP throughout the experiment. This treatment also had no effect on DHPG-induced LTD (100μM; 5min; MPEP = 87 ± 3%, n = 6; control = 84 ± 2%, n= 8, p = 0.44). Therefore, we find that under several experimental conditions in both mice and rats, MPEP is ineffective in blocking mGluR-LTD.
mGluR1 is required for DHPG-induced decreases in AMPA receptor surface expression

DHPG treatment of hippocampal neurons results in a rapid endocytosis and persistent decrease in the surface expression of postsynaptic AMPA receptors which is thought to mediate LTD (Snyder et al. 2001; Xiao et al. 2001; Nosyreva and Huber 2005). The mGluR subtype which mediates DHPG-induced decreases in AMPAR surface expression is unknown. To investigate the role of mGluR1 and mGluR5, we tested the effects of LY367385 and MPEP on DHPG-induced decreases in GluR1 surface expression in slices using receptor biotinylation (Nosyreva and Huber 2005). Slices were preincubated in antagonists (15-30 min) before DHPG or ACSF application and throughout the experiment. Slices were treated with DHPG (100 μM; 5 min) and surface proteins were biotinylated 15 min after DHPG application. Figure 4A illustrates the time course for receptor biotinylation overlaid onto the electrophysiological equivalent of Fig. 4D. Like LTD, the combined application of LY367385 and MPEP blocked DHPG-induced decreases in GluR1 surface expression (LY367385 + MPEP + DHPG = 109 ± 12% of control slices, n = 7, p = 0.7; Fig. 4C). Because LTD is reduced by the continued presence of LY367385, but not MPEP, we would predict that decreases in GluR1 surface expression should also rely on mGluR1. Incubation in LY367385, but not MPEP, blocked DHPG induced decreases in GluR1 surface expression (MPEP + DHPG = 84 ± 4% of condition control, n = 9, p = 0.02; ACSF + DHPG = 77 ± 4 %, n = 8, p = 0.02; Fig. 4C) (LY367385 + DHPG = 106 ± 12% of condition control, n = 12, p = 0.53; ACSF + DHPG = 80 ± 5%, n = 10, p < 0.01; Fig. 4D). These results are consistent with our findings that mGluR1 antagonists alone reduce
expression of DHPG-LTD (Fig. 2A), while blockade of mGluR5 alone has no effect (Fig. 2D).

**Synaptically-induced LTD does not require Group I mGluR activation**

Previously it was shown that mGluR dependent LTD can be elicited by synaptic stimulation using paired pulses (50ms inter-stimulus interval) of low frequency (1 Hz) stimulation (PP-LFS) (Kemp and Bashir 1999; Huber et al. 2000). This conclusion was based on the finding that PP-LFS-induced LTD is blocked by the broad range mGluR antagonist LY341495 (100µM; Fig. 5E; (Huber et al. 2000)). However, there is very little data addressing the specific mGluR(s) required for PP-LFS-induced LTD (but see (Faas et al. 2002)). We next examined the role of group 1 mGluRs in synaptically induced LTD using PP-LFS. All experiments were performed in 100µM D,L-AP5. PP-LFS-induced LTD is normal in mGluR1 KO mice (mGluR1 KO = 85 ± 3%, n = 15; mGluR1 WT littermates = 84 ± 3%, n = 11, p = 0.78; Fig. 5A) and in mGluR5 KO mice (mGluR5 KO = 73 ± 6%, n = 5; mGluR5 WT littermates = 75 ± 6%, n = 6, p = 0.77; Fig. 5B1). This result is in stark contrast to the effect of mGluR1 KO (Fig. 2C) and mGluR5 KO (mGluR5 KO = 96 ± 1%, n=5; mGluR5 WT littermates = 61 ± 4%, p < 0.01, Fig. 5B2; (Huber et al. 2001) on DHPG-induced LTD. We next sought to determine if inhibition of both mGluR1 and mGluR5 was necessary to block PP-LFS-induced LTD. Blockade of mGluR1 with 100µM LY367385 in mGluR5 KO mice had no effect on PP-LFS-induced LTD (mGluR5 KO + LY367385 = 81 ± 5%, n=10, p = 0.2 compared to mGluR5 KO without antagonists, Fig. 5C). In addition, pharmacological blockade of group I mGluRs in Long Evans rats with 10µM MPEP and 100µM LY367385 had no effect on PP-LFS-
induced LTD (LY367385 + MPEP = 76 ± 4%, n=11; interleaved controls = 79 ± 3%, 
n=11, p =0.47; Fig. 5D). In light of the surprising finding that group I mGluR activation is 
not required for induction of PP-LFS LTD, we confirmed that LY341495 (100µM) 
inhibited PP-LFS-induced LTD (100µM LY341495 = 93 ± 4%, n = 5; interleaved controls 
= 61 ± 4%, n = 5, p < 0.01; Fig. 5E). Taken together, these data suggest that specific 
blockade of group II and III mGluRs or combined blockade of all 3 mGluR groups is 
required to block PP-LFS induced LTD. While LY341495 inhibits Group I, II, and III 
mGluRs at 100µM, it is effective against primarily group II and III mGluRs at 20µM 
(Kingston et al. 1998; Capogna 2004). Selective blockade of group II and III mGluRs 
with 20µM LY341495 has no effect on PP-LFS LTD in Long Evans rats (20µM 
LY341495 = 76 ± 3%, n = 6; Fig. 5F). A cocktail of 10 µM MPEP, 100µM LY367385, 
and 20 µM LY341495 was used to block all 3 mGluR groups and also was ineffective 
against PP-LFS-induced LTD (20µM LY341495 + LY36785 + MPEP = 71 ± 5%, n = 6, p 
= 0.45 compared to 20µM LY341495 alone; Fig. 5F) suggesting that blocking all 
mGluRs may not be sufficient to block PP-LFS-induced LTD and that 100 µM LY341495 
may have effects at other as yet unidentified mGluRs or other neurotransmitter 
receptors. Future experiments are required to determine the identity of these receptors.

Discussion

Our data indicate a novel and unexpected role for mGluR1 in DHPG induced 
synaptic plasticity at CA1 excitatory synapses. We find that activation of either mGluR1 
or mGluR5 can induce the full complement of LTD. Consistent with these data, both 
mGluR1 and mGluR5 induce activation of ERK, which is required for mGluR-LTD
(Gallagher et al. 2004). Furthermore, mGluR1 is specifically required for the expression of DHPG-induced LTD and the associated decrease in AMPAR surface expression.

Previous work in the hippocampus and other brain regions has demonstrated that mGluR1 and mGluR5 can mediate similar functions in neurons (Karim et al. 2001; Ireland and Abraham 2002; Lee et al. 2002; Merlin 2002; Gubellini et al. 2003; Rae and Irving 2004). However, in these studies mGluR1 or mGluR5 blockade alone had a partial or complete effect on the physiological or behavioral measure. Here, we find that blockade of either mGluR1 or mGluR5 has no effect on LTD induction, whereas the combined blockade completely prevents LTD (Fig. 1). This result suggests that the mGluR1 and mGluR5 can fully substitute or compensate for each other and both can activate the signaling cascades required for LTD induction. The fact that both mGluR1 and mGluR5 induce ERK activation supports this conclusion and suggests that this submaximal level of ERK activation is sufficient for LTD induction.

In our attempts to block DHPG-induced LTD with MPEP, we tested mice and two different rat strains, used 5 or 20 min of DHPG application, used either R,S-DHPG or S-DHPG, different durations of MPEP application and 2 different concentrations (10 and 25 μM). All of these conditions yielded no effect of MPEP on LTD. Like previous studies, we used 10 μM MPEP for most of these experiments which is almost 300 times the IC₅₀ value for MPEP against mGluR5. Furthermore, MPEP is a noncompetitive antagonist for mGluR5 and at 10 μM its effects on agonist-stimulated phosphoinositide turnover are saturating. MPEP (10 μM) inhibits >95% of agonist stimulated phosphoinositide turnover at cloned mGluR5 and >75 % in rat hippocampus (Gasparini et al. 1999). We do not think the differences are due to developmental changes in the
mGluR subtype required for LTD, because the age range of our rats and mice is similar as that used in previous studies (3-6 weeks) (Faas et al. 2002; Hou and Klann 2004; Huang et al. 2004; Huang and Hsu 2005). Our inability to block DHPG-induced LTD with MPEP can not be explained by inactive MPEP in our preparation because we observed effects of MPEP on ERK activation (Fig. 1D), suppression of the \( I_{\text{AHP}} \) (Fig. 2), and on LTD induction when combined with LY367385 (Fig. 1C). There are other examples or reports which differ in their findings of the contribution of mGluR1 and mGluR5 to other mGluR-dependent functions in CA1 and striatal neurons (Gubellini et al. 2001; Mannaioni et al. 2001; Sung et al. 2001; Ireland and Abraham 2002; Rae and Irving 2004). Although there may be technical reasons for these differences, we suggest that mGluR1 function is either upregulated or maintained in our slice preparation such that it is able to substitute for mGluR5 activation. In contrast to results with MPEP, LTD induced with a either a brief (Huber et al. 2001) or prolonged DHPG application is completely absent in mGluR5 KO mice (Fig. 5B2). These results suggest that mGluR1 can compensate for mGluR5 when it is blocked pharmacologically, but not in the absence of mGluR5 protein. Our findings also suggest that there may be alterations in mGluR1 expression, localization or function in CA1 neurons of mGluR5 KO mice.

Although our data demonstrate that mGluR1 and mGluR5 can both induce LTD, we find that only mGluR1 is important for expression of LTD and surface GluR1 decreases (Figs. 2, 4). This finding is consistent with studies which have found mGluR1 and mGluR5 mediate distinct functions in neurons (for review see (Valenti et al. 2002). The reversal of DHPG-induced LTD with nonselective mGluR antagonists has been
previously shown (Palmer et al. 1997; Fitzjohn et al. 1999; Watabe et al. 2002). Data with pharmacological blockade or genetic knockout of mGluR1 indicate that activation of mGluR1 is important for the expression of LTD (Fig. 2). These data suggest that persistent mGluR1 activity contributes to LTD expression and that there is another component of LTD expression that does not require group I mGluR activity. Other studies have discovered that mGluR1 activity is required for the expression of long-term potentiation in medial vestibular neurons and epileptiform bursts in CA3 suggesting that mGluR1 may be a common mechanism to sustain mGluR-dependent plasticity in the brain (Grassi et al. 2002; Merlin 2002). Our findings support studies which have demonstrated a role for mGluR1 in hippocampal dependent learning and suggest that LTD in CA1 may contribute to these behaviors (Aiba et al. 1994; Petersen et al. 2002; Maciejak et al. 2003).

Previous work using single cell recordings of CA1 pyramidal neurons has demonstrated that mGluR1 has many functions in these neurons including acute depression of EPSCs, cell depolarization (or inward current), increases in intracellular [Ca2+] and suppression of the I_{AHP} (Mannaioni et al. 2001; Ireland and Abraham 2002; Rae and Irving 2004). Furthermore, an immunohistochemical study reported that DHPG induced phosphorylation of ERK in CA1 pyramidal cell bodies and was inhibited by LY367385 or MPEP, consistent with our western blotting results (Berkeley and Levey 2003). These studies and our present work have relied on the specificity of LY367385 to make conclusions regarding mGluR1 function (Clark et al. 1997; Valenti et al. 2002). The fact that LTD reversal is not observed with LY367385 in the mGluR1 KO mouse strongly supports that its effects are specific for mGluR1 (Fig. 2C). Many studies have
demonstrated a functional role of mGluR1 in CA1 pyramidal neurons, but demonstrating the presence of mGluR1 protein has been more elusive (Martin et al. 1992; Lujan et al. 1996; Ferraguti et al. 2004). The many functions attributed to mGluR1 in CA1 neurons may be mediated by a low diffuse expression of mGluR1. Alternatively, the currently available antibodies may not detect the relevant mGluR1 isoforms.

We and others have previously demonstrated that DHPG results in an endocytosis and persistent decrease in the surface expression of AMPARs which is thought to mediate LTD in mature neurons (Snyder et al. 2001; Xiao et al. 2001; Nosyreva and Huber 2005). Here we observed that mGluR1 blockade completely blocked DHPG-induced decreases in GluR1 surface expression (Fig. 4). From our LTD data, we expected that LY367385 would only partially block the decrease in GluR1 surface expression. It is likely that we are unable to detect partial effects of LY367385 on GluR1 surface expression because of large variability in the receptor biotinylation assay as compared to LTD measurements. Alternatively, the mGluR1-dependent GluR1 endocytosis may only mediate one half of the LTD. Based on the effects of mGluR1 blockade on LTD (Fig. 4A), we would interpret this result as an effect of LY367385 on the expression (as opposed to induction) of GluR1 surface decreases.

Results to date indicate that DHPG and PP-LFS-induced LTD (in AP5) represent the same LTD mechanism. Both are blocked by LY341495, are absent in the Gαq knockout mouse, rely on protein synthesis and ERK activation, are enhanced in the Fragile X Syndrome mouse model, and are similarly developmentally regulated (Huber et al. 2000; Huber et al. 2001; Kleppisch et al. 2001; Huber et al. 2002; Zho et al. 2002; Nosyreva and Huber 2005). These data and the fact that PP-LFS induced LTD
occludes DHPG-induced LTD indicate that the two forms of plasticity converge on a common protein synthesis dependent mechanism (Huber et al. 2001). Because PP-LFS induced LTD is absent in G_{aq} knockout mice, it is possible that other G_{q} coupled neurotransmitter receptors are sufficient to induce LTD when group 1 mGluRs are blocked (Kirkwood et al. 1999; Kleppisch et al. 2001; Scheiderer et al. 2004). It is unclear why 100 μM LY341495 blocks PP-LFS induced LTD. At high concentrations LY341495 may have non-specific effects at these other receptor types may act at other mGluR subtypes yet to be identified (Fitzjohn et al. 1998).

**Acknowledgments:**

We would like to thank Sean Gallagher for technical assistance and members of the Huber lab for helpful discussions.

**Grants**

This research was supported by the National Institutes of Health Grant NS045711, McKnight Foundation and FRAXA Research Foundations (KMH) and the National Science Foundation (LV). K.M.H. is a Southwestern Medical Foundation endowed scholar in biomedical research.

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**Figure Legends**

**Figure 1. Activation of mGluR1 or mGluR5 is sufficient to induce LTD and activate ERK.** In all experiments NMDA receptors were blocked with 100 μM D,L-AP5.

A-C. Plotted are the average (± SEM) initial slope values of field potentials (FP) normalized to the pre-DHPG baseline. LTD was induced with a 5 min. application of 100 μM DHPG. A, Acute application of the mGluR5-specific antagonist MPEP (10 μM; solid line) during the baseline and DHPG application had no effect on LTD induction. B, Likewise, acute application of the mGluR1-specific antagonist LY367385 (100 μM; solid line) during the baseline and DHPG application had no effect on LTD induction. C, Combined, acute blockade of mGluR1 and mGluR5 (10 μM MPEP + 100 μM LY367385; solid line) abolished LTD. Inset: FPs (averages of 4-10 waveforms) from a representative experiment were taken at the times indicated by the numbers on the graph. For all panels scale bars = 0.5mV/ 5msec. D, Representative western blot of phosphorylated (P)-ERK and Total ERK under basal (B; untreated) or DHPG (D; 100
μM; 5 min) treated conditions in the absence of antagonist (ACSF) or in the presence of mGluR1 (LY367385) or mGluR5 (MPEP) antagonists. Group data illustrate that the DHPG-induced increase in the ratio of P-ERK/Total ERK is reduced by LY367385 or MPEP alone and the combined application of LY367385 and MPEP (*, p < 0.01: LY367385, MPEP, or LY367385 + MPEP as compared to ACSF). However, in the presence of LY367385 or MPEP, DHPG induces a significant increase in P-ERK (#, p < 0.05). Data demonstrating that LY367385 + MPEP block the DHPG-induced increase in P-ERK is replotted from Gallagher et al., 2004 for comparison to each antagonist alone.

**Figure 2. mGluR1 activity is required for LTD expression.** In all experiments NMDA receptors were blocked with 100 μM D,L-AP5. **A-B1, C-F,** Plotted are the average (± SEM) initial slope values of field potentials (FP) normalized to the pre-DHPG baseline. LTD was induced with a 20 min. application of 100 μM DHPG. **A,** The presence of the mGluR1-specific antagonist LY367385 (100 μM) for the duration of the experiment reduces DHPG-induced LTD **B1,** LY367385 (100 μM) application 60-80 min after DHPG transiently reverses LTD. **B2,** LY367385 (20min., 100 µM) was applied during the baseline (open circles) and 60-80 min. after DHPG (filled circles) in the same slice. Plotted are the average (± SEM) initial slope values of field potentials (FP) normalized to the pre-LY baseline. LY367385 (100 μM) has no significant effect on basal synaptic transmission (open circles). In contrast, LY367385 significantly increases synaptic transmission after LTD has been induced with DHPG (closed circles). **C,** LTD is reduced in mGluR1 KO mice. LY367385 significantly reverses LTD in WT mice, but not in mGluR1 KO mice. During LY367385 application LTD is not different in mGluR1 KO
vs. WT mice.  

D, The presence of the mGluR5-specific antagonist MPEP (10 μM) for the duration of the experiment has no effect on DHPG-induced LTD.  

E, Application of MPEP (60 min) does not reverse LTD expression.  

F, Reversal of LTD by co-application of LY367385 and MPEP is not different from LY367385 alone (as in B).  

Inset: FPs (averages of 4-10 waveforms) from a representative experiment are taken at the times indicated by the numbers on the graph.  For all panels scale bars = 0.5mV/ 5msec.

**Figure 3. MPEP is effective at blocking the DHPG-induced suppression of I_{AHP} but not DHPG-induced LTD.**

A<sub>1</sub>-A<sub>2</sub>, Time course of I_{AHP} in a representative experiment.  I_{AHP} was elicited by applying a 110mV (-50- to +60mV) voltage step in the presence of 0.5 μM TTX and 1 mM TEA.  

Example waveforms (A<sub>1</sub>) indicate that I_{AHP} was measured as the outward current peak immediately (10-15 msec) following the voltage step.  100 μM DHPG significantly reduced the I_{AHP} and subsequent wash-on of MPEP reversed this effect in the continued presence of DHPG.  

A<sub>3</sub>, Plot of average I_{AHP} values from each cell in 100 μM DHPG and upon reversal in MPEP (*, p< 0.01: DHPG vs. DHPG + MPEP).  

B-C, LTD induced with 20 min DHPG is not affected by MPEP in multiple rat strains (B, Sprague Dawley (SD) rats, and Long Evans hooded rats as in Figures 1A and 2D) or in mice (D, C57BL/6 mice).

**Figure 4. mGluR1 is required for DHPG-induced decreases in AMPA receptor surface expression.**  

A, The presence of LY367385 (100 μM) before, during and after DHPG (100 μM; 5 min) reduces LTD.  

The time course of biotin application (open bar)
and slice homogenization (↓) is indicated. B, Sample western blots of total (T) and surface (S) GluR1 subunits of the AMPA receptor for each condition. C-D, Plotted are the average ratios of surface to total GluR1 normalized to condition control slices taken 15 min after DHPG application (100 μM; 5 min). Control slices, like DHPG-treated slices, were preincubated in ACSF or antagonists. Number of experiments per group is denoted on each bar. (*) denotes significantly different from within animal condition control (untreated) slices (p < 0.05). DHPG treatment of hippocampal slices results in a decrease of GluR1 surface expression using receptor biotinylation. Preincubation in LY367385 (100 μM), but not MPEP (10 μM) blocks DHPG-induced decreases in GluR1 surface expression.

Figure 5. Group I mGluR activation is not required for synaptically-induced LTD.

In all experiments NMDA receptors were blocked with 100 μM D,L-AP5. A-F. Plotted are the average (± SEM) initial slope values of field potentials (FP) normalized to the pre-DHPG or pre-PPLFS baseline. LTD was induced with paired pulse low frequency stimulation, PP-LFS, (2 pulses with 50ms inter-stimulus interval at 1Hz) for 15 min. (D-F) or 20 min. (A, B1, C) or with 20 min. of 100 μM DHPG (B2). A, Synaptically-induced LTD is normal in mgluR1 KO mice. B, Synaptically-induced LTD is normal in mgluR5 KO mice (B1). In contrast to synaptically-induced LTD, LTD induced with 20 min. of DHPG is drastically reduced in the mGluR5 KO mouse (B2). C, Synaptically-induced LTD in mGluR5 KO mice is not affected by the addition of the mGluR1 antagonist LY367385 (100μM). D, Pharmacological blockade of group I mGluRs with LY367385 (100μM) and MPEP (10μM) has no effect on LTD in Long Evans rats. E,
Pharmacological inhibition of all mGluRs with the broad range mGluR antagonist LY341495 (100µM) blocks synaptically-induced LTD in Long Evans rats. F, Inhibition of groups II and III mGluRs with 20µM LY341495 or inhibition of groups I, II and III mGluRs with 20µM LY341495 + 100µM LY367385 + 10µM MPEP has no effect on synaptically-induced LTD.
Figure 1 Volk et al.
Figure 2 Volk et al.,
Figure 3 Volk et al.,
Figure 4 Volk et al.,

A. Graph showing FP slope normalized with time from DHPG onset (minutes).

B. Table showing GluR1 levels with different conditions.

C. Bar graph showing surface/total GluR1 (% condition control) for control, ACSF, MPEP, LY367385, and LY367385 + MPEP conditions.

D. Bar graph showing surface/total GluR1 (% condition control) for control, ACSF, LY367385 + DHPG conditions.
Figure 5 Volk et al.