J Neurophysiol 101: 1375–1385, 2009.
First published December 24, 2008; doi:10.1152/jn.90643.2008.

Mechanisms of Group I mGluR-Dependent Long-Term Depression of NMDA Receptor–Mediated Transmission at Schaffer Collateral–CA1 Synapses

David R. Ireland and Wickliffe C. Abraham

Department of Psychology and the Brain Health and Repair Research Centre, University of Otago, Dunedin, New Zealand

Submitted 5 June 2008; accepted in final form 17 December 2008

Ireland DR, Abraham WC. Mechanisms of group I mGluR-dependent long-term depression of NMDA receptor–mediated transmission at Schaffer collateral–CA1 synapses. *J Neurophysiol* 101: 1375–1385, 2009. First published December 24, 2008; doi:10.1152/jn.90643.2008. The mechanisms underlying group I metabotropic glutamate receptor (mGluR)-dependent long-term depression (LTD) of *N*-methyl-D-aspartate receptor (NMDAR)-mediated synaptic currents ($EPSCs_{NMDAR}$) are poorly understood. Here we investigated the effects of (*R,S*)-3,5-dihydroxyphenylglycine (DHPG), a selective agonist of group I mGluRs, on the $EPSCs_{NMDAR}$ in area CA1 of acute hippocampal slices from 6- to 8-wk Sprague-Dawley rats. DHPG acutely and persistently depressed the isolated $EPSC_{NMDAR}$ and transiently slowed its decay rate. Combined antagonism of mGluR1 and mGluR5 blocked the effects of DHPG. Strong calcium buffering with intracellular BAPTA did not reduce the acute depression or LTD, making the involvement of elevated postsynaptic calcium unlikely. The acute depression and LTD were not mediated by activation of tyrosine kinases or phosphatases, nor were they dependent on protein synthesis. However, the LTD was prevented by the intracellular actin-stabilizer jasplakinolide, raising the possibility that it was associated with a lateral movement of NMDARs. Supporting this hypothesis, when the effective spatial spread of synaptically released glutamate was increased using the glutamate transporter inhibitor TBOA, the resultant $EPSC_{NMDAR}$ did not undergo LTD in response to DHPG. Importantly, isolation of the extrasynaptic $EPSC_{NMDAR}$ by blockade of synaptic NMDARs with MK-801 showed that this was not due to a potentiation of the preexisting extrasynaptic component. These findings indicate that LTD of NMDAR-mediated synaptic transmission occurs via lateral movement of receptors away from the synapse.

INTRODUCTION

Ionotropic glutamate receptors such as AMPA receptors (AMPARs) and *N*-methyl-D-aspartate receptors (NMDARs) form ion channels that mediate direct excitatory synaptic transmission. Metabotropic glutamate receptors (mGluRs) in contrast do not form ion channels but instead regulate, among other things, the ionotropic receptors through activation of G protein–coupled receptors. The effects of mGluR activity on synaptic transmission, particularly AMPAR-mediated, has been extensively studied in hippocampal and other neurons. Activation of group I mGluRs consistently induces long-term depression (LTD) of AMPAR responses in hippocampal neurons (Camodeca et al. 1999; Palmer et al. 1997) via mechanisms that are increasingly well understood. In contrast, NMDAR-mediated currents can be either potentiated or depressed by group I mGluR activation depending on whether the currents are elicited by exogenous NMDA application or synaptic stimulation.

Exogenous NMDA-evoked currents are typically transiently potentiated by group I mGluR activation (Benquet et al. 2002; Grishin et al. 2004; Heidinger et al. 2002; Mannaioni et al. 2001; Skeberdis et al. 2001; Snyder et al. 2001). This involves G protein–mediated activation of phospholipase C (PLC) (Skeberdis et al. 2001), release of calcium from inositol triphosphate (IP3)-sensitive calcium stores, and activation of protein kinase C (PKC) (Benquet et al. 2002; Skeberdis et al. 2001) and src family tyrosine kinases (Benquet et al. 2002; Heidinger et al. 2002). It is not clear whether the potentiation of the NMDA current results from an increase in the open probability of the NMDAR channels (Lu et al. 1999; Xiong et al. 1998) or an increase in the number of channels available in the membrane (Lan et al. 2001). Conversely, synaptically elicited NMDAR-mediated excitatory postsynaptic currents ($EPSCs_{NMDAR}$) undergo depression in response to group I mGluR activation (Baskys and Malenka 1991; Snyder et al. 2001; Watabe et al. 2002). The reasons for this disparity and the processes responsible for the mGluR-induced depression of the $EPSC_{NMDAR}$ are not known.

Here we examined group I mGluR-mediated rapid depression and LTD of the synaptically evoked $EPSC_{NMDAR}$ and determined some of the underlying mechanisms. We found that both the acute depression and LTD are initiated by activation of mGluR1 and mGluR5, but are not dependent on tyrosine kinase or phosphatase activity, an increase in intracellular calcium, or protein synthesis. Although the mechanism underlying the transient acute depression is most likely presynaptic, the LTD appears to be mediated postsynaptically by lateral movement of synaptic NMDARs via actin depolymerization.

METHODS

Hippocampal slice preparation

Hippocampi were dissected from the brains of male Sprague-Dawley rats (6–8 wk) killed following deep anesthesia with ketamine (100 mg/kg, ip). Transverse slices (400 μm) were cut using a Vibroslicer (Campden Instruments) in oxygenated ice-cold artificial cerebrospinal fluid (ACSF) with sucrose substituted for NaCl (composition in mM: 210 sucrose, 26 $NaHCO_3$, 20 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 0.5 $CaCl_2$, 3 $MgCl_2$, 2 ascorbic acid, and 3 Na-pyruvate, pH 7.4, when gassed with 95% O_2-5% CO_2). Slices were held in interface conditions on a Millipore culture plate insert in an incubation chamber filled with oxygenated ASCF (composition in mM: 124 NaCl, 26 $NaHCO_3$, 20 glucose, 2.5 KCl, 1.25 NaH_2PO_4, 2.5 $CaCl_2$, 1.3 $MgCl_2$, 2 ascorbic acid, and 3 Na-pyruvate) at ~34°C for 30 min, after which they were held in the same chamber at room temperature until needed. During each experiment, a single slice was transferred to a glass coverslip glued to the bottom of a

Address for reprint requests and other correspondence: D. R. Ireland, Dept. of Psychology, Univ. of Otago, PO Box 56, Dunedin, New Zealand (E-mail: direland@psy.otago.ac.nz).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

0022-3077/09 $8.00 Copyright © 2009 The American Physiological Society

Downloaded from http://jn.physiology.org/ by 10.220.33.2 on October 25, 2016

tissue bath and superfused (2 ml/min) with oxygenated ACSF (lacking ascorbate and pyruvate) at 32°C.

Patch-clamp electrophysiology

Standard whole cell patch-clamp techniques were used to record $EPSCs_{NMDAR}$ from the somata of visualized CA1 pyramidal neurons near the slice surface. Patch electrodes were formed from soft borosilicate glass (WPI, Sarasota) and had resistances of 2–3 MΩ when filled with a Cs-based recording solution (composition in mM: 140 Cs-methanesulfonate, 10 HEPES, 4 Na_2ATP, 0.4 NaGTP, 4 $MgCl_2$, Na_2 phosphocreatine, 0.2 EGTA-4 Na, and 1 QX-314, pH 7.25, when adjusted with CsOH, ~310 mOsm when adjusted with water). Access resistance (<15 MΩ) was continuously monitored throughout each experiment, and experiments in which it differed from baseline values by >20% were discarded. At least 20 min were allowed after obtaining whole cell mode for the electrode solution to equilibrate with the cytosol before recordings were commenced. With the exception of experiments involving MK-801 application, $EPSCs_{NMDAR}$ were evoked at a rate of 1 per 30 s (unless specified otherwise) by electrical stimulation of the Schaffer collaterals via a broken-off patch electrode filled with ACSF. After 10 min of baseline recording, the group I mGluR agonist (*R,S*)-3,5-dihydroxyphenylglycine (DHPG; 20 μM) was applied for 10 min via the bathing solution and washed out for 20 min. These DHPG experiments were interleaved with the experiments involving antagonists and inhibitors described throughout the results. AMPA, $GABA_A$, and $GABA_B$ receptors were blocked with bath-applied CNQX (10 μM), picrotoxin (20 μM), and CGP55845 (1 μM), respectively, to reveal the $EPSC_{NMDAR}$. Voltage-dependent calcium currents due to L-type channels were inhibited with bath-applied nimodipine (10 μM). Single $EPSCs_{NMDAR}$ (200–400 pA) were recorded in voltage-clamp mode at a holding potential of −30 mV using an Axopatch 1D amplifier and pCLAMP 9 software (Molecular Devices, Union City, CA). They were sampled at 10 kHz and filtered at 2 kHz. Reagents were sourced from the following suppliers: Tocris (CNQX, picrotoxin, CGP55845, JNJ16259685, lavendustin A, LY367385, LY341495, MK-801, MPEP, nimodipine, RS-DHPG, QX-314, D-TBOA), Sigma (anisomycin, Cs-methanesulfonate, cycloheximide, Na_2ATP, NaGTP, phosphocreatine, EGTA-4Na), and Calbiochem (jasplakinolide). All other reagents were sourced from BDH. With the exception of BAPTA and jasplakinolide, which were added to the patch electrode solution, all inhibitors were bath applied. Jasplakinolide was dissolved in methanol before being added to the patch electrode solution.

Patch-clamp data analysis

Raw data were analyzed using pCLAMP 9 software. $EPSC_{NMDAR}$ peak amplitudes were measured relative to the prestimulus baseline and expressed as percentages of the baseline average. The $EPSC_{NMDAR}$ decay time-constant was measured by fitting a single exponential to the initial 100 ms of the decay phase. Acute changes induced by DHPG application were determined by averaging the responses obtained during the last 5 min of DHPG application, whereas the LTD was assessed by averaging the responses obtained during the last 5 min of recording, which was 20 min after DHPG washout, because by this time the LTD had largely stabilized. Differences between the datasets were analyzed with one-way ANOVA and Dunnett's post hoc tests at the $P < 0.05$ significance level. Dunnett's post hoc tests were one-sided unless otherwise specified. Data are reported as mean ± SE.

RESULTS

mGluR-induced changes in the $EPSC_{NMDAR}$

DHPG acutely depressed the peak amplitude of the $EPSC_{NMDAR}$ to 52 ± 3% of baseline ($n = 18$; Fig. 1, *A* and *B*). The peak amplitude recovered and stabilized at 69 ± 3% of baseline following DHPG washout ($n = 18$). The depression could persist for ≥60 min (Fig. 1*A*). Control cells not given DHPG treatment showed only a small run-down of the $EPSC_{NMDAR}$ (92 ± 3% of baseline, $n = 10$; Fig. 1*B*). The acute depression

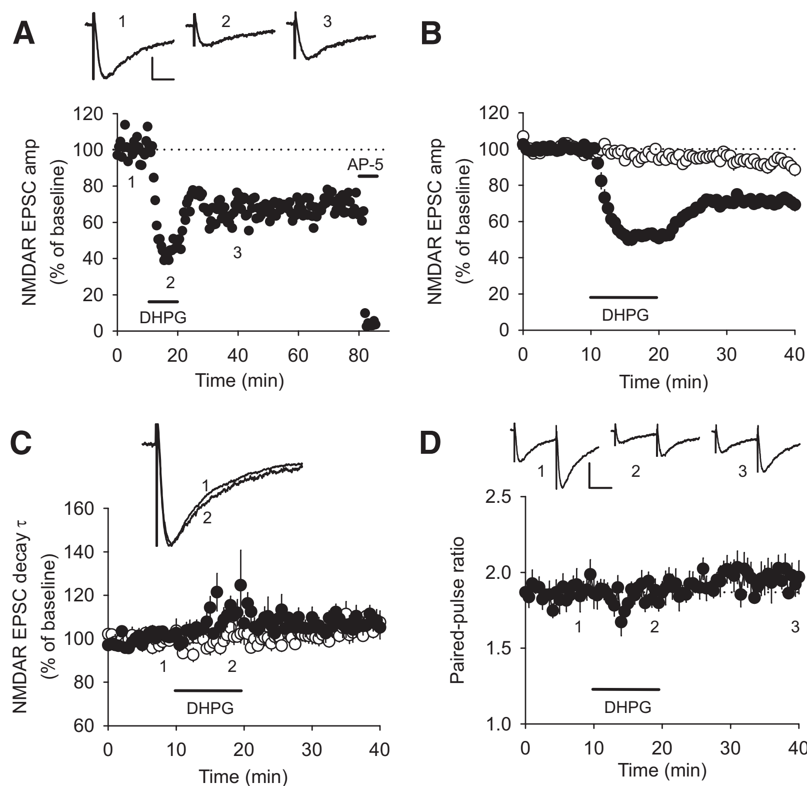

FIG. 1. (*R,S*)-3,5-dihydroxyphenylglycine (DHPG) changes the amplitude and decay time-course of the *N*-methyl-D-aspartate receptor (NMDAR)-mediated synaptic currents ($EPSCs_{NMDAR}$). *A*: single experiment showing the acute depression and long-term depression (LTD) of the $EPSC_{NMDAR}$ peak amplitude induced by 20 μM DHPG applied for 10 min. AP-5 (50 μM) applied at the end of the experiment completely blocked the $EPSC_{NMDAR}$. *Inset*: waveforms are single responses from this experiment at the times indicated. Calibration bars: 100 pA, 25 ms. Stimulus artifacts have been truncated. *B*: normalized average peak amplitude of the $EPSC_{NMDAR}$ ($n = 18$) in neurons exposed to DHPG (●) and control neurons (○). *C*: normalized average $EPSC_{NMDAR}$ decay time-constant in the same neurons as *B* is transiently increased by DHPG. *Inset*: waveforms are averages of 20 responses from a single experiment taken at the times indicated. Traces have been normalized to peak amplitude. Stimulus artifacts have been truncated. *D*: average paired-pulse ratio (pulse2/pulse1) in a subset of DHPG-treated neurons is not affected by DHPG. *Inset*: waveforms show paired-pulse responses from the same experiment as in *A* taken at the times indicated. Calibration bars: 200 pA, 50 ms.

Downloaded from http://jn.physiology.org/ by 10.220.33.2 on October 25, 2016

induced by DHPG was accompanied by a small increase in the $EPSC_{NMDAR}$ decay time-constant (112 ± 3% of baseline, from 31 ± 1 to 35 ± 2 ms, n = 18; Fig. 1*C*). Following drug washout, the decay time-constant decayed toward baseline and was no longer significantly larger than controls 20 min after DHPG washout (33 ± 2 ms). In some experiments, paired-pulse stimulation (100 ms interpulse interval) was used to detect changes in presynaptic release probability. The baseline paired-pulse ratio of the $EPSC_{NMDAR}$ (1.89 ± 0.06, n = 6; Fig. 1*D*) was not affected by DHPG either acutely (1.87 ± 0.04, n = 6) or following drug washout (1.96 ± 0.07), suggesting a lack of change in the probability of presynaptic glutamate release.

Both mGluR1 and mGluR5 can mediate the depression by DHPG

We used specific antagonists of mGluR1 and mGluR5, as well as a broad-spectrum mGluR antagonist, to determine which subtypes of group I mGluRs are responsible for the depression of the $EPSC_{NMDAR}$ by DHPG. Application of the mGluR5-specific noncompetitive antagonist MPEP (10 μM) for ≥20 min before DHPG application significantly reduced the acute depression caused by DHPG (66 ± 3%, n = 5, P = 0.034), but caused no significant reduction of the ensuing LTD (76 ± 3%, n = 5, P = 0.455; Fig. 2, *A* and *E*). Similarly, application of the mGluR1-specific antagonists LY367385 (100 μM) or JNJ16259685 (1 μM) significantly reduced the acute depression (69 ± 8%, n = 7, P = 0.003) without significantly reducing the LTD (76 ± 3%, n = 7, P = 0.330; Fig. 2, *B* and *E*). However, when mGluR5 and mGluR1 were simultaneously blocked using the subtype-specific antagonists together (MPEP + LY367385 or MPEP +10–100 μM JNJ16259685), both the acute depression (84 ± 3%, n = 15) and the LTD (82 ± 2%, n = 15) were significantly reduced (P < 0.001, P = 0.002; Fig. 2, *C* and *E*). Similar results were obtained with the broad-spectrum mGluR antagonist LY341495 (100 μM; acute depression, 89 ± 2%, n = 9, P < 0.001; LTD, 82 ± 3%, n = 9, P = 0.010; Fig. 2, *D* and *E*). However, given that blocking both mGluR1 and mGluR5 would be expected to completely prevent the actions of DHPG, it was surprising that the acute depression in MPEP + mGluR1 antagonist was still significantly greater than in control experiments (P = 0.025) and that the LTD approached being significantly greater than in control experiments (MPEP + mGluR1 antagonist vs. control, P = 0.054; LY341495 vs. control, P = 0.091). These observations could potentially be explained if tonic inhibition of mGluRs by itself induced a gradual depression of the $EPSC_{NMDAR}$, independently of DHPG. We tested this idea by repeating the experiments that used LY341495, but without

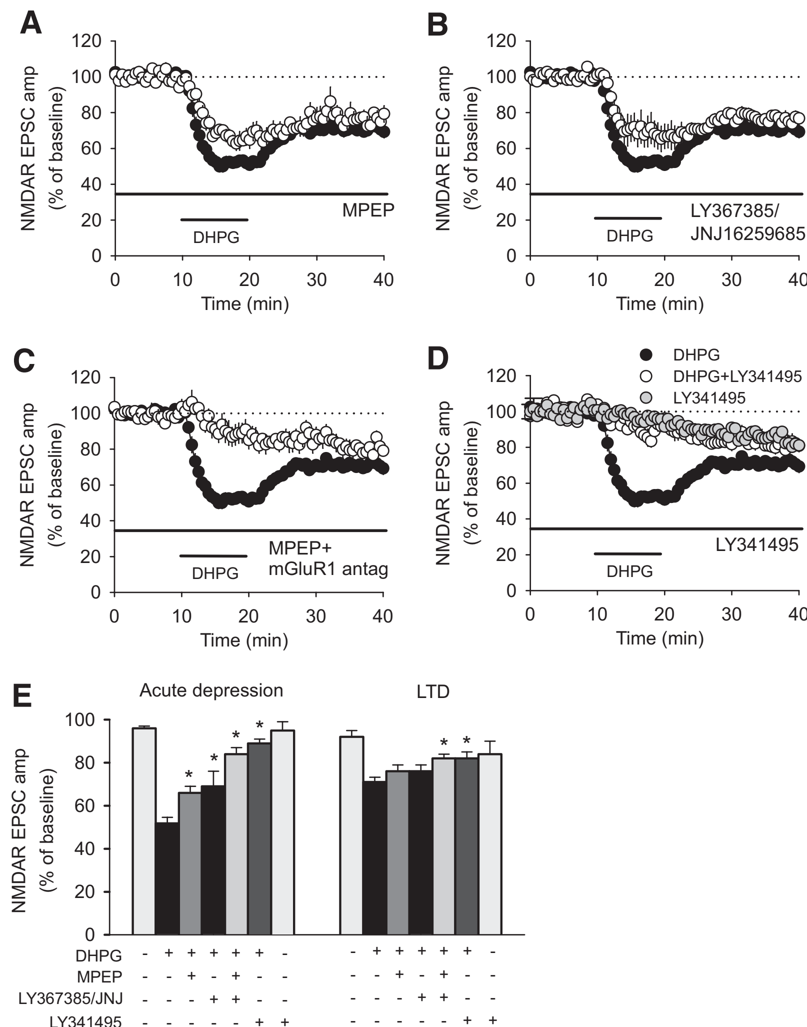

FIG. 2. Metabotropic glutamate receptor (mGluR)1 and mGluR5 participate in the DHPG-induced changes. *A–C*: normalized average peak amplitude of the $EPSC_{NMDAR}$ in neurons exposed to DHPG in the presence (○) or absence (●) of (*A*) mGluR5 antagonist MPEP (10 μM), (*B*) mGluR1 antagonists LY367385 (100 μM) or JNJ16259685 (1 μM), (*C*) MPEP + LY367385 or MPEP + JNJ16259685. *D*: normalized average peak amplitude of the $EPSC_{NMDAR}$ in neurons exposed to either DHPG, the broad-spectrum mGluR antagonist LY341495 (100 μM) + DHPG, or LY341495 without DHPG. *E*: summary of the effects of DHPG on the $EPSC_{NMDAR}$ peak amplitude in the presence of mGluR antagonists. Each bar represents the normalized average peak amplitude of the $EPSC_{NMDAR}$ during the DHPG-induced acute depression and LTD. ±, presence or absence of DHPG and antagonists; *, significant difference from the depression in neurons treated with DHPG alone. The columns corresponding to LY341495 alone represent the depression observed in neurons exposed to LY341495 in the absence of DHPG. Neither the acute depression nor LTD was significantly different to the corresponding values in LY341495 + DHPG.

Downloaded from http://jn.physiology.org/ by 10.220.33.2 on October 25, 2016

application of DHPG. Indeed, chronic application of LY341495 resulted in a gradually increasing response depression (acute depression, 95 ± 4%; chronic depression, 84 ± 6%, $n = 5$; Fig. 2, *D* and *E*), which was not significantly different from the depression generated by DHPG in the presence of LY341495 (Fig. 2, *D* and *E*). Thus mGluR inhibition by itself leads to a slowly developing depression of the $EPSC_{NMDAR}$, but nonetheless it fully prevents any further depression induced by DHPG.

It is apparent from Fig. 2*D* that the prolonged administration of group I mGluR antagonists by itself leads to a faster response depression of the $EPSC_{NMDAR}$ than under normal control conditions. To avoid this faster run-down over time, we repeated some of the above experiments but with only transient antagonist application that was terminated at the same time as the DHPG application. Using this protocol, both the acute depression and the LTD were completely blocked relative to untreated controls by either MPEP + mGluR1 antagonist (acute depression, 87 ± 3%, $n = 7$, $P = 0.208$; LTD, 90 ± 5%, $n = 7$, $P = 0.836$; Fig. 3, *A* and *C*) or LY341495 treatments (acute depression, 91 ± 4%, $n = 6$, $P = 0.541$; LTD, 89 ± 3%, $n = 6$, $P = 0.710$; Fig. 3, *B* and *C*).

The acute and transient increase in the decay time-constant of the $EPSC_{NMDAR}$ was not significantly reduced by any treatment save LY341495, when washed out with DHPG (100 ± 4%, $n = 6$, $P = 0.041$). Because of the small size and considerable variability of the effect of DHPG on the decay time-constant, the underlying mechanisms were not studied further.

Depression is not Ca^{2+} dependent

One well-documented outcome of group I mGluR activation is production of IP3 via activation of PLC, and subsequent release of calcium from IP3-sensitive calcium stores. Therefore we used a high intracellular concentration (30 mM) of the fast calcium buffer BAPTA in the internal patch solution to suppress mGluR-induced rises in internal calcium. Similar or lower concentrations of BAPTA completely prevent measurable mGluR-induced rises in intracellular calcium (Hirono et al. 1998; Nakamura et al. 2000; Rae and Irving 2004). Neither the acute depression (54 ± 7%, $n = 9$) nor the LTD (63 ± 3%, $n = 9$) was inhibited by BAPTA, indicating that they are unlikely to be mediated by an mGluR-triggered increase in postsynaptic calcium (Fig. 4, *A* and *E*).

Depression is independent of tyrosine kinase or phosphatase activity

Both NMDARs and the effects of group I mGluR activation are strongly regulated by protein tyrosine kinase (PTK) and phosphatase (PTP) activity (Coussens et al. 2000; Huang and Hsu 2006; Ireland et al. 2004; Kumar and Foster 2007; Moult et al. 2002, 2006; Salter and Kalia 2004). The PTK inhibitor lavendustin A was used to assess whether PTK activity is required for the mGluR-dependent depression of the $EPSC_{NMDAR.}$ Lavendustin A (10 μM), applied for ≥20 min before DHPG application, did not reduce either the acute depression (54 ± 7%, $n = 6$) or LTD (72 ± 5%, $n = 6$; Fig. 4, *B* and *E*). These data suggest that the DHPG-induced depression is not dependent on PTK activity. However, it is possible that the depression of the $EPSC_{NMDAR}$ relies on PTP activity, as shown for DHPG-induced depression of the $EPSC_{AMPAR}$ (Moult et al. 2002, 2006). We therefore used the PTP inhibitor orthovanadate (1 mM) to block PTPs before and during DHPG application. Since orthovanadate by itself depressed the $EPSC_{NMDAR}$ (to 44 ± 2% of baseline, $n = 3$), we allowed sufficient time for the $EPSC_{NMDAR}$ to stabilize before applying DHPG, at which point a new baseline average was calculated. In the presence of orthovandate, neither the acute depression (46 ± 4%, $n = 6$) nor the LTD (73 ± 2, $n = 6$) induced by DHPG was reduced (Fig. 4, *C* and *E*), showing that PTP activity is also not required for the mGluR-induced depression.

Protein synthesis is not necessary for depression

Both DHPG-induced LTD of AMPAR responses and the DHPG-induced internalization of AMPAR and NMDAR in hip-

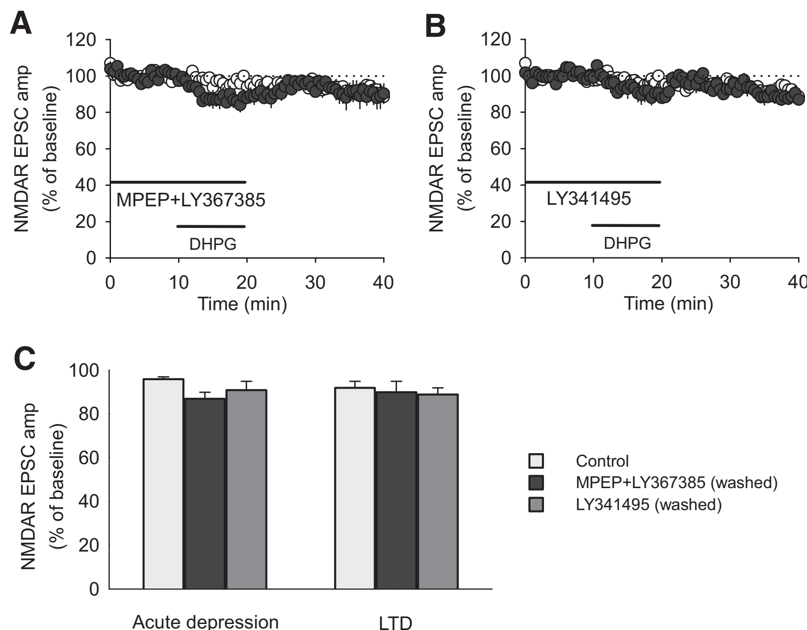

FIG. 3. Depression is completely blocked by inhibition of mGluR1 and mGluR5. *A* and *B*: normalized average peak amplitude of the $EPSC_{NMDAR}$ in neurons exposed to DHPG in the presence of (●) either (*A*) MPEP + LY367385 or (*B*) LY341495, compared with untreated control neurons (○). In these experiments, the antagonists were washed out at the same time as DHPG. *C*: summary of the effects of DHPG on the $EPSC_{NMDAR}$ peak amplitude in the presence of mGluR antagonists. Each bar represents the normalized average peak amplitude of the $EPSC_{NMDAR}$ during the DHPG-induced acute depression and LTD. Under these conditions, blocking mGluR1 and mGluR5, or all mGluRs, prevented the DHPG-induced depression relative to untreated controls.

Downloaded from http://jn.physiology.org/ by 10.220.33.2 on October 25, 2016

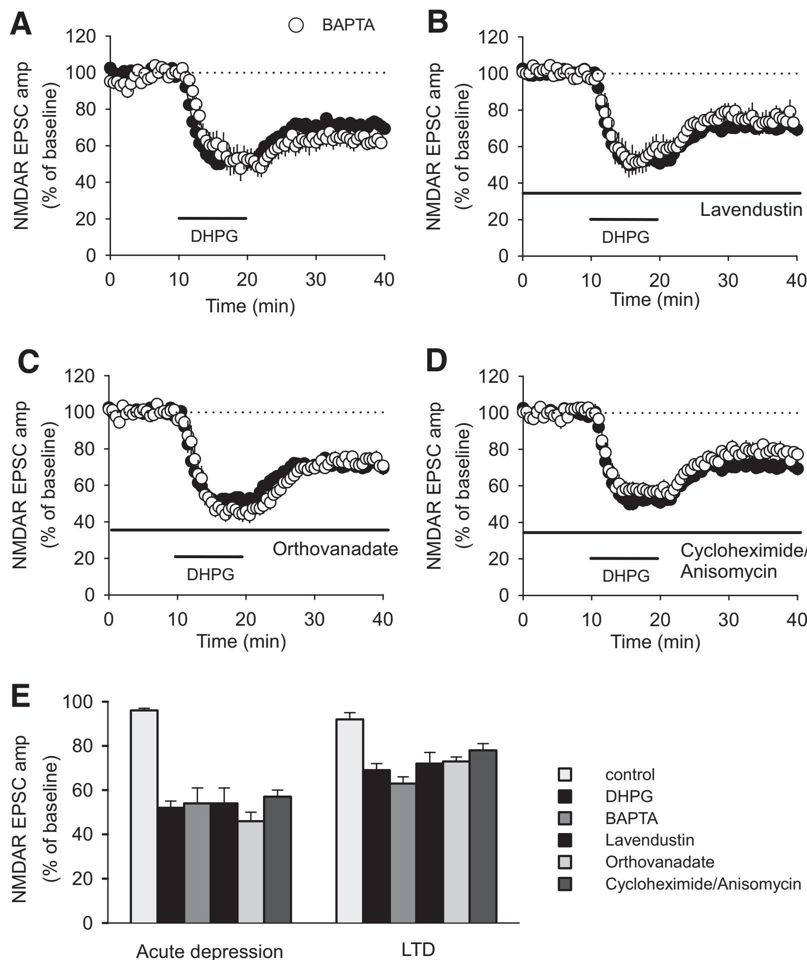

FIG. 4. Depression is not dependent on postsynaptic calcium, tyrosine kinases, tyrosine phosphatases, or protein synthesis. *A–D*: normalized average peak amplitude of the $EPSC_{NMDAR}$ in neurons exposed to DHPG in the presence (○) or absence (●) of (*A*) the fast calcium buffer BAPTA (30 mM, intracellular), (*B*) the protein tyrosine kinase (PTK) inhibitor lavendustin A (10 μM), (*C*) the protein tyrosine phosphatase (PTP) inhibitor orthovanadate (1 mM), or (*D*) protein synthesis inhibitors cycloheximide (60 μM) or anisomycin (25 μM). *E*: summary of the effects of DHPG on the $EPSC_{NMDAR}$ amplitude in the presence of signaling inhibitors. Each bar represents the normalized average peak amplitude of the $EPSC_{NMDAR}$ during the DHPG-induced acute depression and LTD.

pocampal neurons are dependent on protein synthesis under some conditions (Huber et al. 2000, 2001; Kumar and Foster 2007; Nosyreva and Huber 2005; Snyder et al. 2001). Therefore we predicted that the mGluR-induced LTD of the $EPSC_{NMDAR}$ would also be protein synthesis dependent. To test this, either cycloheximide (60 μM, $n = 4$) or anisomycin (25 μM, $n = 4$) was used to block protein synthesis for ≥30 min before DHPG application. These concentrations of protein synthesis inhibitors were used successfully in the preceding LTD studies. However, under our conditions, neither the rapid depression (57 ± 3%, $n = 8$) nor LTD (78 ± 3%, $n = 8$) was reduced by the protein synthesis inhibitors (Fig. 4, *D* and *E*).

LTD of the $EPSC_{NMDAR}$ is dependent on actin depolymerization

Both mGluR-dependent LTD of the $EPSC_{AMPAR}$ and non–mGluR-dependent LTD of the $EPSC_{NMDAR}$ can be prevented by inhibiting depolymerization of the actin cytoskeleton (Morishita et al. 2005; Xiao et al. 2001). Therefore we predicted that mGluR-dependent depression of the $EPSC_{NMDAR}$ would also require actin depolymerization. In confirmation of this hypothesis, adding the actin stabilizing agent jasplakinolide (2 μM) to the patch pipette solution reduced both the DHPG-induced acute depression (65 ± 3%, $n = 10$, $P < 0.001$) and LTD (85 ± 3%, $n = 10$; $P = 0.022$) compared with interleaved experiments performed with DHPG alone (rapid depression, 42 ± 5%, $n = 6$; LTD, 73 ± 5%, $n = 6$; Fig. 5). Because jasplakinolide did not seem to completely block LTD, we considered the possibility that, as for the mGluR antagonists, this was confounded by a slow run-down of the $EPSC_{NMDAR}$ caused by chronic application of the drug or its solvent methanol. Thus we compared these data to a control group in which jasplakinolide (with methanol solvent) was applied without DHPG and found that, whereas the DHPG-induced acute depression in the presence of jasplakinolide was still significantly greater ($P < 0.001$) than in these controls (control, 95 ± 2%, $n = 5$), the LTD was completely eliminated (control, 85 ± 3%, $n = 5$, $P = 0.590$ vs. jasplakinolide + DHPG; Fig. 5).

DHPG does not induce LTD when glutamate transport is inhibited

Since actin depolymerization can lead to dispersal of synaptic NMDARs (Allison et al. 1998; Sattler et al. 2000), we investigated whether the DHPG-induced depression could be due to lateral movement of synaptic NMDARs out of the synapse. First, we used the glutamate uptake inhibitor TBOA to increase the access of synaptically released glutamate to extrasynaptic areas of membrane, predicting that there would be no depression of the net synaptic current if glutamate had access to both the static synaptic receptors and those that had moved to nearby extrasynaptic locations. TBOA (20 μM) was applied for >20 min before DHPG to allow the $EPSC_{NMDAR}$ to

Downloaded from http://jn.physiology.org/ by 10.220.33.2 on October 25, 2016

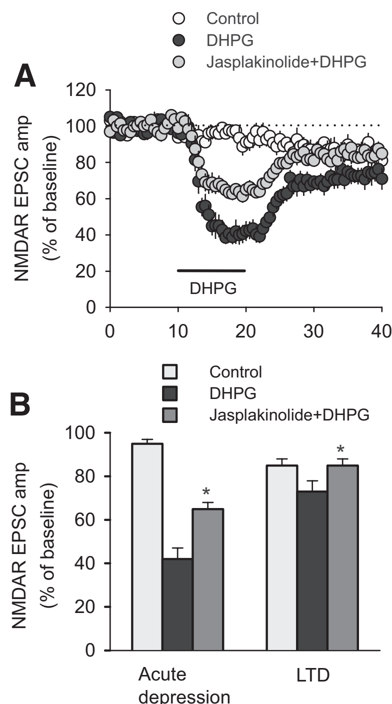

FIG. 5. LTD is dependent on actin depolymerization. *A*: normalized average peak amplitude of the $EPSC_{NMDAR}$ in neurons exposed to DHPG in the presence (○) or absence (●) of the actin-stabilizing agent jasplakinolide (2 μM, intracellular) compared with neurons exposed to jasplakinolide alone. *B*: summary of the effects of DHPG on the $EPSC_{NMDAR}$ amplitude in the presence or absence of jasplakinolide. Each bar represents the normalized average peak amplitude of the $EPSC_{NMDAR}$ during the DHPG-induced acute depression and LTD. *Significant difference between DHPG + jasplakinolide vs. DHPG alone. Note that all experiments for this dataset involved the solvent methanol (0.4%) in the patch pipette.

stabilize (peak amplitude 157 ± 9% of baseline; Fig. 6*A*). When DHPG was applied, the acute depression of the net $EPSC_{NMDAR}$ (39 ± 4%, $n = 7$) was in fact greater than the equivalent acute depression of the synaptic $EPSC_{NMDAR}$ ($P = 0.014$, 2-sided Dunnett's test; Fig. 6*B*). Critically, however, the LTD of the net $EPSC_{NMDAR}$ in the presence of TBOA (89 ± 5%, $n = 7$) was completely blocked ($P = 0.001$, 2-sided Dunnett's test relative to the LTD of the synaptic $EPSC_{NMDAR}$) and was not significantly different to the gradual response run-down seen in untreated synaptic controls ($P = 0.954$, 2-sided Dunnett's test; Fig. 6, *B* and *F*). Given the different temporal profiles of the net $EPSC_{NMDAR}$ in TBOA compared with the synaptic $EPSC_{NMDAR}$, we also measured the effects of DHPG on net $EPSC_{NMDAR}$ transferred charge and found that the acute depression (29 ± 4%, $n = 5$) and LTD (90 ± 6%, $n = 5$) were consistent with the effects of DHPG on peak amplitude. Overall, these data support the hypothesis that increasing the spread of synaptically released glutamate prevents LTD by permitting activation of NMDARs that have moved extrasynaptically.

An alternative explanation is that the lack of LTD in TBOA is caused by a potentiation of preexisting extrasynaptic NMDAR currents that offsets the depression of the synaptic current. We tested this possibility by using the irreversible use-dependent NMDAR antagonist MK-801 and TBOA to isolate an $EPSC_{NMDAR}$ mediated by extrasynaptic receptors. MK-801 (50 μM) was applied for 10 min, during which time the stimulation was increased to 1/5 s to increase the rate of blockade (Fig. 6*C*). After the synaptic $EPSC_{NMDAR}$ was blocked, stimulation was stopped and the MK-801 was washed from the slice for 20 min. Following MK-801 washout, the stimulation was resumed at 1/30 s. At this point, the synaptic $EPSC_{NMDAR}$ remained fully blocked (3 ± 3% of pre-MK-801, $n = 4$; Fig. 6*C*). TBOA (20 μM) was applied for >20 min to reveal an $EPSC_{NMDAR}$ mediated by extrasynaptic NMDARs (Fig. 6*C*). When DHPG was applied, the extrasynaptic $EPSC_{NMDAR}$ underwent both an acute depression (27 ± 4%, $n = 4$) and LTD (64 ± 7%, $n = 4$; Fig. 6, *D* and *F*). The acute depression was significantly greater than that of the synaptic $EPSC_{NMDAR}$ ($P < 0.001$, 2-sided Dunnett's test), whereas the LTD was not significantly different($P = 0.745$, 2-sided Dunnett's test). These results demonstrate that the failure of DHPG to induce LTD of the combined synaptic/extrasynaptic $EPSC_{NMDAR}$ evoked in the presence of TBOA (Fig. 6*B*) cannot be due to a potentiation of preexisting extrasynaptic NMDARs that offsets depression of the synaptic NMDARs. Note that, in this experiment, any synaptic NMDARs that move extrasynaptically in response to DHPG will already be irreversibly blocked by MK-801 and so will not contribute to the observed extrasynaptic $EPSC_{NMDAR}$.

Although a previous study has indicated that the synaptic $EPSC_{NMDAR}$ remains completely blocked for ≥20 min following MK-801 washout from the slice (Harris and Pettit 2007), other data suggest that extrasynaptic NMDARs have a basal mobility that allows them to move into the synapse immediately following blockade of synaptic NMDARs with MK-801 (Tovar and Westbrook 2002; Zhao et al. 2008). Our data show that this does not occur within the duration of the 20-min MK-801 washout period (Fig. 6*C*), but the length of our total experimental protocol means that either basal mobility of extrasynaptic NMDARs or recovery of synaptic NMDARs from MK-801 block later in the experiment could potentially confound our results. We tested for this in three experiments by briefly washing out TBOA at the end of the DHPG washout period (Fig. 6*E*) but found that the synaptic $EPSC_{NMDAR}$ still remained blocked by MK-801 at the end of the experiment.

DISCUSSION

Effect of mGluR activation on the $EPSC_{NMDAR}$

Previous studies that used exogenous NMDA to activate NMDARs have observed a transient potentiation by mGluR activation of the whole cell NMDA-evoked current (Benquet et al. 2002; Grishin et al. 2004; Heidinger et al. 2002; Mannaioni et al. 2001; Skeberdis et al. 2001; Snyder et al. 2001). In contrast, those studies using synaptic activation of NMDARs have shown depression of the $EPSC_{NMDAR}$ (Baskys and Malenka 1991; Snyder et al. 2001; Watabe et al. 2002). Possible explanations for this disparity are either that the depression of the $EPSC_{NMDAR}$ is a presynaptic phenomenon (Watabe et al. 2002) or that the potentiation observed in the former studies was mediated by extrasynaptic NMDARs that were activated by applied NMDA but not by synaptic stimulation. In this study, we were particularly interested in the effects of mGluR activation on synaptic NMDAR currents. In agreement with previous studies, we found that activation of group I mGluRs with DHPG causes both an acute depression of the $EPSC_{NMDAR}$ that quickly recovers following agonist washout and an LTD. We also found that mGluR activation

Downloaded from http://jn.physiology.org/ by 10.220.33.2 on October 25, 2016

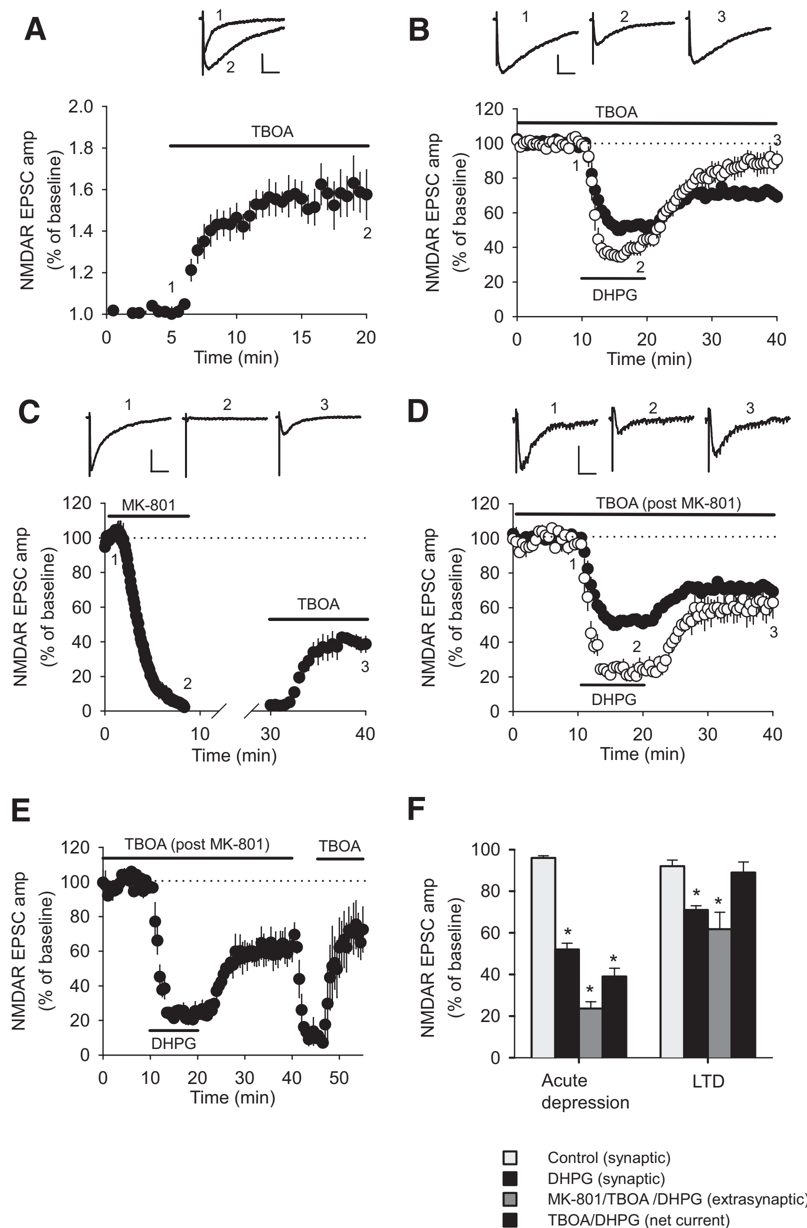

FIG. 6. Lateral movement of synaptic NMDAR underlies the mGluR-mediated LTD of the $EPSC_{NMDAR}$. *A*: normalized average peak amplitude of the $EPSC_{NMDAR}$ in neurons exposed to TBOA (20 μM). *Inset*: waveforms are single responses recorded at the times indicated. Calibration bars: 200 pA, 100 ms. *B*: normalized average peak amplitude of the $EPSC_{NMDAR}$ in neurons exposed to DHPG in the presence (○) or absence (●) of TBOA. *Inset*: waveforms are single responses recorded at the times indicated from the same neuron as *A*. Calibration bars: 200 pA, 100 ms. *C*: normalized average peak amplitude of the $EPSC_{NMDAR}$ showing the effects of progressive block of synaptic NMDAR by MK-801 (50 μM), washout of MK-801 from the slice, and application of TBOA. *Inset*: waveforms are single responses recorded at the times indicated. Calibration bars: 200 pA, 100 ms. *D*: normalized average peak amplitude of the extrasynaptic $EPSC_{NMDAR}$ in neurons exposed to DHPG (○) compared with the synaptic $EPSC_{NMDAR}$ (from different neurons) (●). TBOA application followed irreversible block of synaptic NMDAR by MK-801 and subsequent MK-801 washout as shown in *C*. *Inset*: waveforms are single responses recorded at the times indicated from the same neuron as *C*. Calibration bars: 50 pA, 100 ms. Note that these responses have been scaled vertically to match the amplitude of responses in *A*–*C*. *E*: normalized average peak amplitude of the $EPSC_{NMDAR}$ showing the effects of TBOA removal and reapplication on neurons previously exposed to MK-801. These data confirm that blockade of synaptic NMDAR by MK-801 is effectively irreversible within the time frame of our experiments. *F*: summary of the effects of DHPG on the synaptic $EPSC_{NMDAR,}$ the extrasynaptic $EPSC_{NMDAR}$ in the presence of TBOA following MK-801 application and washout, and the net $EPSC_{NMDAR}$ in the presence of TBOA. Each bar represents the normalized average $EPSC_{NMDAR}$ amplitude during the DHPG-induced acute depression and LTD. *Significant difference from the synaptic $EPSC_{NMDAR}$ in untreated control neurons.

transiently slows the decay rate of the $EPSC_{NMDAR}$ during the acute depression phase, a finding not previously reported. This latter effect, however, was too small and variable to warrant further studies of its underlying mechanisms.

Although the precise roles of mGluR subtypes in the DHPG-induced depression of AMPAR-mediated responses is controversial, both mGluR1 and mGluR5 have been found to contribute in a number of studies (Faas et al. 2002; Hou and Klann 2004; Huang and Hsu 2006; Kumar and Foster 2007; Mannaioni et al. 2001; Volk et al. 2006). Our data show that both mGluR1 and mGluR5 can individually mediate the DHPG-induced depression of NMDAR-mediated synaptic responses and that a block of both receptor subtypes is required to completely suppress the LTD.

Presynaptic locus of acute depression

Downstream of mGluR activation, either presynaptic or postsynaptic mechanisms could in theory account for the acute depression of the $EPSC_{NMDAR}$. Superficially, the lack of change in PPF during DHPG application would argue against a presynaptic mechanism for the acute depression, but a number of factors strongly counter this. First, several studies support the idea that a presynaptic change in glutamate release is not necessarily accompanied by a change in PPF (Barnes-Davies and Forsythe 1995; Hanse and Gustafsson 2001; Manabe et al. 1993; Watabe et al. 2002). Such a scenario could occur if, rather than a change in the average release probability, a proportion of synapses became presynaptically silent during DHPG application (Manabe et al. 1993), or there were other changes in release mechanisms not sensitive to paired-pulse stimulation. Second, postsynaptically applied jasplakinolide at most only partly inhibited the acute depression while eliminating the LTD and, depending on the amount of temporal overlap between the acute depression and LTD, may not have affected the acute depression at all (Fig. 4*A*). Third, because it seems relatively certain that the acute depression of the AMPAR-mediated exci-

Downloaded from http://jn.physiology.org/ by 10.220.33.2 on October 25, 2016

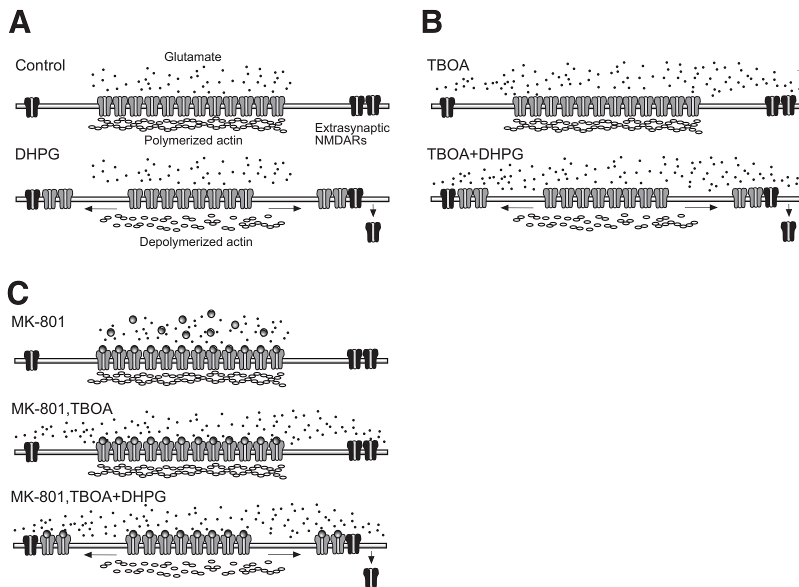

FIG. 7. Proposed mechanism of mGluR-dependent LTD of the $EPSC_{NMDAR}$. *A*: under control conditions, synaptically released glutamate activates only synaptic NMDARs. LTD of the synaptic $EPSC_{NMDAR}$ after washout of DHPG (mGluRs not shown) is due to actin depolymerization-dependent lateral movement of synaptic NMDARs into the extrasynaptic membrane and away from the released glutamate. *B*: inhibition of glutamate uptake with TBOA allows glutamate to access both synaptic and extrasynaptic receptors. This prevents the induction of significant LTD of the mixed $EPSC_{NMDAR}$ by DHPG. *C*: MK-801 (circles) applied during synaptic stimulation irreversibly blocks synaptic NMDARs. Following washout of MK-801 from the bath, TBOA application permits glutamate to activate only preexisting extrasynaptic NMDARs. The LTD of the extrasynaptic $EPSC_{NMDAR}$ that is present after DHPG washout is likely due to endocytosis of preexisting extrasynaptic NMDARs (Snyder et al. 2001). Although preexisting synaptic receptors are now also present extrasynaptically, they remain blocked by MK-801 and thus are electrophysiologically silent.

tatory postsynaptic current ($EPSC_{AMPAR}$) is due to an inhibition of glutamate release (Baskys and Malenka 1991; Faas et al. 2002; Nosyreva and Huber 2005; Rouach and Nicoll 2003; Watabe et al. 2002), it is extremely difficult to envisage how a presynaptic depression of the $EPSC_{AMPAR}$ could occur without an accompanying depression of the $EPSC_{NMDAR}$. A transient depression of glutamate release would also partly account for the disparity between the effects of mGluR activation on NMDA-evoked versus synaptically evoked currents since a presynaptic depression would only be present in experiments testing synaptically evoked currents. Therefore we believe that the explanation most consistent with both our data and what is known about the mGluR-induced depression of the $EPSC_{AMPAR}$ is that the acute depression is caused by a transient reduction in presynaptic glutamate release.

Interestingly, the acute depression of the extrasynaptic $EPSC_{NMDAR}$ was much larger than that of the synaptic $EPSC_{NMDAR}$. This is also consistent with a presynaptic locus for the acute depression, given previous evidence suggesting that multiple hippocampal synapses can cooperate in "pooling" their glutamate to achieve a sufficient concentration to activate extrasynaptic NMDARs (Arnth-Jensen et al. 2002; Scimemi et al. 2004). Therefore an ~50% reduction in the number of synapses that release glutamate during the acute depression, while only reducing the synaptic $EPSC_{NMDAR}$ by ~50%, might be expected to reduce the extrasynaptic $EPSC_{NMDAR}$ to a greater extent through reduction of glutamate levels to below that needed to activate many of the extrasynaptic NMDARs.

Postsynaptic locus of LTD

In contrast to the acute depression, there is a lack of consensus as to the locus of the mGluR-induced LTD of the $EPSC_{AMPAR}$ in CA1. This is likely due in part to developmental regulation (Nosyreva and Huber 2005) and to the possibility of presynaptic changes initiated by postsynaptic mGluRs (Watabe et al. 2002). Taken together, our observations that PPF of the $EPSC_{NMDAR}$ was not changed by DHPG and that the LTD was completely blocked by postsynaptic jasplakinolide mean that, although we could not completely rule out a presynaptic mechanism, a postsynaptic process seemed more likely to account for the LTD of the $EPSC_{NMDAR}$ in our young adult animals. Therefore we hypothesized that one potential mechanism was the group I mGluR-triggered rise in postsynaptic calcium that is mediated by IP3-sensitive calcium stores (Nakamura et al. 1999, 2000, 2002; Rae and Irving 2004), possibly amplified by store-operated calcium channels in the plasma membrane (Mellentin et al. 2006) and acting synergistically with voltage-dependent calcium channels (Nakamura et al. 2000) and NMDARs (Nakamura et al. 2002; Rae et al. 2000). Ryanodine receptor–operated calcium stores have also been shown to mediate mGluR actions in hippocampal neurons (Mellentin et al. 2006; Wu et al. 2008). However, the fact that a high concentration of intracellular BAPTA did not block the LTD makes it unlikely that the underlying processes involve an elevation of intracellular calcium, although it is possible that NMDARs located very close to sources of calcium may be exposed to calcium microdomains that are insensitive to BAPTA.

Alternative mechanisms considered were tyrosine phosphorylation and dephosphorylation, which exert a powerful and multilayered influence on NMDAR function. For example, NMDAR activity and trafficking are regulated by both src family PTKs (Kohr and Seeburg 1996; Yu and Salter 1999) and STEP (striatal enriched tyrosine phosphatase) (Braithwaite et al. 2006; Pelkey et al. 2002; Snyder et al. 2005). Src PTKs are in turn regulated by tyrosine dephosphorylation by PTPα (Le et al. 2006; Lei et al. 2002). In this study, neither PTK nor PTP activity was required for the LTD of the $EPSC_{NMDAR}$. This means that the mGluR LTD is not the same process as the tyrosine kinase–dependent LTD of NMDAR responses that we have previously described (Coussens et al. 2000). Our results also contrast with other mGluR-induced processes such as the depression of the slow afterhyperpolarization (Ireland et al. 2004) and LTD of the $EPSC_{AMPAR}$ (Huang and Hsu 2006; Kumar and Foster 2007; Moult et al. 2006) that rely on PTP activity.

Because postsynaptically applied jasplakinolide completely blocked the LTD, it seems that actin depolymerization is a critical step in the DHPG-triggered pathway. One

Downloaded from http://jn.physiology.org/ by 10.220.33.2 on October 25, 2016

way in which the actin cytoskeleton could influence the $EPSC_{NMDAR}$ is via alterations in the biophysical properties of NMDARs, such as single-channel conductance and open probability. In cultured hippocampal neurons, rundown of whole cell NMDAR currents as a consequence of reduced channel open probability (Rosenmund and Westbrook 1993b) is dependent on actin depolymerization (Rosenmund and Westbrook 1993a) and can be prevented by stabilizing actin with jasplakinolide (Li et al. 2002). However, it seems unlikely that the same mechanism is at work here given that the NMDAR current rundown is calcium dependent and inhibited by intracellular BAPTA (Rosenmund and Westbrook 1993b), whereas we showed that the mGluR-induced LTD is resistant to BAPTA. A different mechanism involving actin depolymerization that could underlie the LTD of the $EPSC_{NMDAR}$ is a physical movement of NMDARs away from the postsynapse. This could occur via direct endocytosis because there is evidence that mGluR-dependent LTD of the $EPSC_{AMPAR}$ may be a result of AMPAR endocytosis (Moult et al. 2006; Snyder et al. 2001; Xiao et al. 2001) requiring actin depolymerization (Xiao et al. 2001). Further internalization of NMDARs in response to group I mGluR activation has been shown in cultured hippocampal neurons (Snyder et al. 2001). However, several factors point away from direct endocytosis of synaptic NMDARs during our experiments. First, synaptic NMDARs seem to be immune to endocytosis compared with extrasynaptic NMDARs (Li et al. 2002; Roche et al. 2001). Second, the group I mGluR-induced endocytosis of NMDARs requires protein synthesis (Snyder et al. 2001), and our results showed that the depression of the $EPSC_{NMDAR}$ is not protein synthesis dependent. Third, the NMDAR-dependent LTD of the synaptic $EPSC_{NMDAR}$ in hippocampal slices involves actin depolymerization but not endocytosis (Morishita et al. 2005).

Since synaptic NMDARs are capable of lateral travel (Fong et al. 2002; Tovar and Westbrook 2002; Zhao et al. 2008) under conditions of actin depolymerization (Allison et al. 1998; Sattler et al. 2000), any mGluR-induced movement of synaptic NMDARs would be predicted to be a lateral one into the extrasynaptic membrane. We directly addressed this hypothesis by inhibiting glutamate uptake, thus allowing synaptically released glutamate to spread outside the synapse and activate both synaptic and extrasynaptic NMDARs. Consistent with our prediction, DHPG did not induce LTD of the resultant combined synaptic/extrasynaptic $EPSC_{NMDAR}$. Alternatively, this result could be explained if mGluR activation simultaneously upregulated the biophysical properties of existing extrasynaptic NMDARs or increased the number of these receptors (via exocytosis or decreased endocytosis), while at the same time inhibiting synaptic NMDAR function. Such differential regulation would be consistent with the opposing roles frequently attributed to synaptic and extrasynaptic NMDARs (Hardingham and Bading 2003; Hardingham et al. 2002; Ivanov et al. 2006; Massey et al. 2004; Papadia et al. 2005). This explanation could also resolve the disparity between the effects of mGluR activation on synaptically evoked and NMDA-evoked NMDAR responses because NMDA-evoked currents are generated by both synaptic and extrasynaptic NMDARs, in contrast to specifically synaptic currents evoked by single pulse afferent stimulation. However, our finding that the extrasynaptic $EPSC_{NMDAR}$, isolated by blockade of synaptic NMDARs with MK-801, was strongly depressed rather than potentiated by DHPG rules this out. Therefore the explanation most consistent with our data is that the mGluR-induced LTD of the synaptic $EPSC_{NMDAR}$ results from actin depolymerization-dependent movement of NMDARs away from the synapse.

Superficially it may appear difficult to reconcile the LTD of the extrasynaptic $EPSC_{NMDAR}$ with the lack of LTD of the net $EPSC_{NMDAR}$ in TBOA. However, since we estimate that, under our conditions of partial glutamate transporter blockade, the extrasynaptic $EPSC_{NMDAR}$ makes a relatively small contribution to the net $EPSC_{NMDAR}$ (~15–30%), the LTD of the extrasynaptic component would not be expected to have a significant impact on the net $EPSC_{NMDAR}$. Furthermore, we speculate that movement of a significant fraction of the larger synaptic NMDAR complement to the extrasynaptic region may lead to an accumulation of extrasynaptic receptors, despite any endocytosis of the smaller preexisting complement that may occur. Thus it is possible that mGluR-mediated potentiation of NMDA-evoked responses is caused by preferential activation by exogenous NMDA of the extrasynaptic pool of NMDARs, which has been enhanced by lateral movement of receptors from synaptic sites. Whether the translocated NMDARs and/or existing extrasynaptic NMDARs are subsequently endocytosed is not known, but previous data (Snyder et al. 2001), as well as our own finding of a depression of the isolated extrasynaptic NMDAR current by DHPG, suggest that this is likely to occur.

ACKNOWLEDGMENTS

We thank Dr. Clarke Raymond for technical advice and Dr. John Wagner for useful discussions and comments on the manuscript.

GRANTS

This research was supported by a grant from the New Zealand Health Research Council.

REFERENCES

Allison DW, Gelfand VI, Spector I, Craig AM. Role of actin in anchoring postsynaptic receptors in cultured hippocampal neurons: differential attachment of NMDA versus AMPA receptors. *J Neurosci* 18: 2423–2436, 1998.

Arnth-Jensen N, Jabaudon D, Scanziani M. Cooperation between independent hippocampal synapses is controlled by glutamate uptake. *Nat Neurosci* 5: 325–331, 2002.

Barnes-Davies M, Forsythe ID. Pre- and postsynaptic glutamate receptors at a giant excitatory synapse in rat auditory brainstem slices. *J Physiol* 488: 387–406, 1995.

Baskys A, Malenka RC. Agonists at metabotropic glutamate receptors presynaptically inhibit EPSCs in neonatal rat hippocampus. *J Physiol* 444: 687–701, 1991.

Benquet P, Gee CE, Gerber U. Two distinct signaling pathways upregulate NMDA receptor responses via two distinct metabotropic glutamate receptor subtypes. *J Neurosci* 22: 9679–9686, 2002.

Braithwaite SP, Adkisson M, Leung J, Nava A, Masterson B, Urfer R, Oksenberg D, Nikolich K. Regulation of NMDA receptor trafficking and function by striatal-enriched tyrosine phosphatase (STEP). *Eur J Neurosci* 23: 2847–2856, 2006.

Camodeca N, Breakwell NA, Rowan MJ, Anwyl R. Induction of LTD by activation of group I mGluR in the dentate gyrus in vitro. *Neuropharmacology* 38: 1597–1606, 1999.

Coussens CM, Williams JM, Ireland DR, Abraham WC. Tyrosine phosphorylation-dependent inhibition of hippocampal synaptic plasticity. *Neuropharmacology* 39: 2267–2277, 2000.

Faas GC, Adwanikar H, Gereau RWt, Saggau P. Modulation of presynaptic calcium transients by metabotropic glutamate receptor activation: a differ-

Downloaded from http://jn.physiology.org/ by 10.220.33.2 on October 25, 2016

ential role in acute depression of synaptic transmission and long-term depression. *J Neurosci* 22: 6885–6890, 2002.

Fong DK, Rao A, Crump FT, Craig AM. Rapid synaptic remodeling by protein kinase C: reciprocal translocation of NMDA receptors and calcium/calmodulin-dependent kinase II. *J Neurosci* 22: 2153–2164, 2002.

Grishin AA, Gee CE, Gerber U, Benquet P. Differential calcium-dependent modulation of NMDA currents in CA1 and CA3 hippocampal pyramidal cells. *J Neurosci* 24: 350–355, 2004.

Hanse E, Gustafsson B. Paired-pulse plasticity at the single release site level: an experimental and computational study. *J Neurosci* 21: 8362–8369, 2001.

Hardingham GE, Bading H. The yin and yang of NMDA receptor signalling. *Trends Neurosci* 26: 81–89, 2003.

Hardingham GE, Fukunaga Y, Bading H. Extrasynaptic NMDARs oppose synaptic NMDARs by triggering CREB shut-off and cell death pathways. *Nat Neurosci* 5: 405–414, 2002.

Harris AZ, Pettit DL. Extrasynaptic and synaptic NMDA receptors form stable and uniform pools in rat hippocampal slices. *J Physiol* 584: 509–519, 2007.

Heidinger V, Manzerra P, Wang XQ, Strasser U, Yu SP, Choi DW, Behrens MM. Metabotropic glutamate receptor 1-induced upregulation of NMDA receptor current: mediation through the Pyk2/Src-family kinase pathway in cortical neurons. *J Neurosci* 22: 5452–5461, 2002.

Hirono M, Konishi S, Yoshioka T. Phospholipase C-independent group I metabotropic glutamate receptor-mediated inward current in mouse purkinje cells. *Biochem Biophys Res Commun* 251: 753–758, 1998.

Hou L, Klann E. Activation of the phosphoinositide 3-kinase-Akt-mammalian target of rapamycin signaling pathway is required for metabotropic glutamate receptor-dependent long-term depression. *J Neurosci* 24: 6352–6361, 2004.

Huang CC, Hsu KS. Sustained activation of metabotropic glutamate receptor 5 and protein tyrosine phosphatases mediate the expression of (S)-3,5-dihydroxyphenylglycine-induced long-term depression in the hippocampal CA1 region. *J Neurochem* 96: 179–194, 2006.

Huber KM, Kayser MS, Bear MF. Role for rapid dendritic protein synthesis in hippocampal mGluR-dependent long-term depression. *Science* 288: 1254–1257, 2000.

Huber KM, Roder JC, Bear MF. Chemical induction of mGluR5- and protein synthesis-dependent long-term depression in hippocampal area CA1. *J Neurophysiol* 86: 321–325, 2001.

Ireland DR, Guevremont D, Williams JM, Abraham WC. Metabotropic glutamate receptor-mediated depression of the slow afterhyperpolarization is gated by tyrosine phosphatases in hippocampal CA1 pyramidal neurons. *J Neurophysiol* 92: 2811–2819, 2004.

Ivanov A, Pellegrino C, Rama S, Dumalska I, Salyha Y, Ben-Ari Y, Medina I. Opposing role of synaptic and extrasynaptic NMDA receptors in regulation of the extracellular signal-regulated kinases (ERK) activity in cultured rat hippocampal neurons. *J Physiol* 572: 789–798, 2006.

Kohr G, Seeburg PH. Subtype-specific regulation of recombinant NMDA receptor-channels by protein tyrosine kinases of the src family. *J Physiol* 492: 445–452, 1996.

Kumar A, Foster TC. Shift in induction mechanisms underlies an age-dependent increase in DHPG-induced synaptic depression at CA3-CA1 synapses. *J Neurophysiol* 98: 2729–2736, 2007.

Lan JY, Skeberdis VA, Jover T, Grooms SY, Lin Y, Araneda RC, Zheng X, Bennett MV, Zukin RS. Protein kinase C modulates NMDA receptor trafficking and gating. *Nat Neurosci* 4: 382–390, 2001.

Le HT, Maksumova L, Wang J, Pallen CJ. Reduced NMDA receptor tyrosine phosphorylation in PTPα-deficient mouse synaptosomes is accompanied by inhibition of four src family kinases and Pyk2: an upstream role for PTPα in NMDA receptor regulation. *J Neurochem* 98: 1798–1809, 2006.

Lei G, Xue S, Chery N, Liu Q, Xu J, Kwan CL, Fu YP, Lu YM, Liu M, Harder KW, Yu XM. Gain control of N-methyl-D-aspartate receptor activity by receptor-like protein tyrosine phosphatase alpha. *EMBO J* 21: 2977–2989, 2002.

Li B, Chen N, Luo T, Otsu Y, Murphy TH, Raymond LA. Differential regulation of synaptic and extra-synaptic NMDA receptors. *Nat Neurosci* 5: 833–834, 2002.

Lu WY, Xiong ZG, Lei S, Orser BA, Dudek E, Browning MD, MacDonald JF. G-protein-coupled receptors act via protein kinase C and Src to regulate NMDA receptors. *Nat Neurosci* 2: 331–338, 1999.

Manabe T, Wyllie DJ, Perkel DJ, Nicoll RA. Modulation of synaptic transmission and long-term potentiation: effects on paired pulse facilitation and EPSC variance in the CA1 region of the hippocampus. *J Neurophysiol* 70: 1451–1459, 1993.

Mannaioni G, Marino MJ, Valenti O, Traynelis SF, Conn PJ. Metabotropic glutamate receptors 1 and 5 differentially regulate CA1 pyramidal cell function. *J Neurosci* 21: 5925–5934, 2001.

Massey PV, Johnson BE, Moult PR, Auberson YP, Brown MW, Molnar E, Collingridge GL, Bashir ZI. Differential roles of NR2A and NR2B-containing NMDA receptors in cortical long-term potentiation and long-term depression. *J Neurosci* 24: 7821–7828, 2004.

Mellentin C, Jahnsen H, Abraham WC. Priming of long-term potentiation mediated by ryanodine receptor activation in rat hippocampal slices. *Neuropharmacology* 52: 118–125, 2007.

Morishita W, Marie H, Malenka RC. Distinct triggering and expression mechanisms underlie LTD of AMPA and NMDA synaptic responses. *Nat Neurosci* 8: 1043–1050, 2005.

Moult PR, Gladding CM, Sanderson TM, Fitzjohn SM, Bashir ZI, Molnar E, Collingridge GL. Tyrosine phosphatases regulate AMPA receptor trafficking during metabotropic glutamate receptor-mediated long-term depression. *J Neurosci* 26: 2544–2554, 2006.

Moult PR, Schnabel R, Kilpatrick IC, Bashir ZI, Collingridge GL. Tyrosine dephosphorylation underlies DHPG-induced LTD. *Neuropharmacology* 43: 175–180, 2002.

Nakamura T, Barbara JG, Nakamura K, Ross WN. Synergistic release of Ca^{2+} from IP3-sensitive stores evoked by synaptic activation of mGluRs paired with backpropagating action potentials. *Neuron* 24: 727–737, 1999.

Nakamura T, Lasser-Ross N, Nakamura K, Ross WN. Spatial segregation and interaction of calcium signalling mechanisms in rat hippocampal CA1 pyramidal neurons. *J Physiol* 543: 465–480, 2002.

Nakamura T, Nakamura K, Lasser-Ross N, Barbara JG, Sandler VM, Ross WN. Inositol 1,4,5-trisphosphate (IP3)-mediated Ca^{2+} release evoked by metabotropic agonists and backpropagating action potentials in hippocampal CA1 pyramidal neurons. *J Neurosci* 20: 8365–8376, 2000.

Nosyreva ED, Huber KM. Developmental switch in synaptic mechanisms of hippocampal metabotropic glutamate receptor-dependent long-term depression. *J Neurosci* 25: 2992–3001, 2005.

Palmer MJ, Irving AJ, Seabrook GR, Jane DE, Collingridge GL. The group I mGlu receptor agonist DHPG induces a novel form of LTD in the CA1 region of the hippocampus. *Neuropharmacology* 36: 1517–1532, 1997.

Papadia S, Stevenson P, Hardingham NR, Bading H, Hardingham GE. Nuclear Ca^{2+} and the cAMP response element-binding protein family mediate a late phase of activity-dependent neuroprotection. *J Neurosci* 25: 4279–4287, 2005.

Pelkey KA, Askalan R, Paul S, Kalia LV, Nguyen TH, Pitcher GM, Salter MW, Lombroso PJ. Tyrosine phosphatase STEP is a tonic brake on induction of long-term potentiation. *Neuron* 34: 127–138, 2002.

Rae MG, Irving AJ. Both mGluR1 and mGluR5 mediate Ca^{2+} release and inward currents in hippocampal CA1 pyramidal neurons. *Neuropharmacology* 46: 1057–1069, 2004.

Rae MG, Martin DJ, Collingridge GL, Irving AJ. Role of Ca^{2+} stores in metabotropic L-glutamate receptor-mediated supralinear Ca^{2+} signaling in rat hippocampal neurons. *J Neurosci* 20: 8628–8636, 2000.

Roche KW, Standley S, McCallum J, Dune Ly C, Ehlers MD, Wenthold RJ. Molecular determinants of NMDA receptor internalization. *Nat Neurosci* 4: 794–802, 2001.

Rosenmund C, Westbrook GL. Calcium-induced actin depolymerization reduces NMDA channel activity. *Neuron* 10: 805–814, 1993a.

Rosenmund C, Westbrook GL. Rundown of N-methyl-D-aspartate channels during whole-cell recording in rat hippocampal neurons: role of Ca^{2+} and ATP. *J Physiol* 470: 705–729, 1993b.

Rouach N, Nicoll RA. Endocannabinoids contribute to short-term but not long-term mGluR-induced depression in the hippocampus. *Eur J Neurosci* 18: 1017–1020, 2003.

Salter MW, Kalia LV. Src kinases: a hub for NMDA receptor regulation. *Nat Rev* 5: 317–328, 2004.

Sattler R, Xiong Z, Lu WY, MacDonald JF, Tymianski M. Distinct roles of synaptic and extrasynaptic NMDA receptors in excitotoxicity. *J Neurosci* 20: 22–33, 2000.

Scimemi A, Fine A, Kullmann DM, Rusakov DA. NR2B-containing receptors mediate cross talk among hippocampal synapses. *J Neurosci* 24: 4767–4777, 2004.

Skeberdis VA, Lan J, Opitz T, Zheng X, Bennett MV, Zukin RS. mGluR1-mediated potentiation of NMDA receptors involves a rise in intracellular

Downloaded from http://jn.physiology.org/ by 10.220.33.2 on October 25, 2016

calcium and activation of protein kinase C. *Neuropharmacology* 40: 856–865, 2001.

Snyder EM, Nong Y, Almeida CG, Paul S, Moran T, Choi EY, Nairn AC, Salter MW, Lombroso PJ, Gouras GK, Greengard P. Regulation of NMDA receptor trafficking by amyloid-beta. *Nat Neurosci* 8: 1051–1058, 2005.

Snyder EM, Philpot BD, Huber KM, Dong X, Fallon JR, Bear MF. Internalization of ionotropic glutamate receptors in response to mGluR activation. *Nat Neurosci* 4: 1079–1085, 2001.

Tovar KR, Westbrook GL. Mobile NMDA receptors at hippocampal synapses. *Neuron* 34: 255–264, 2002.

Volk LJ, Daly CA, Huber KM. Differential roles for group 1 mGluR subtypes in induction and expression of chemically induced hippocampal long-term depression. *J Neurophysiol* 95: 2427–2438, 2006.

Watabe AM, Carlisle HJ, O'Dell TJ. Postsynaptic induction and presynaptic expression of group 1 mGluR-dependent LTD in the hippocampal CA1 region. *J Neurophysiol* 87: 1395–1403, 2002.

Wu J, Harney S, Rowan MJ, Anwyl R. Involvement of group I mGluRs in LTP induced by strong high frequency stimulation in the dentate gyrus in vitro. *Neurosci Lett* 436: 235–238, 2008.

Xiao MY, Zhou Q, Nicoll RA. Metabotropic glutamate receptor activation causes a rapid redistribution of AMPA receptors. *Neuropharmacology* 41: 664–671, 2001.

Xiong ZG, Raouf R, Lu WY, Wang LY, Orser BA, Dudek EM, Browning MD, MacDonald JF. Regulation of N-methyl-D-aspartate receptor function by constitutively active protein kinase C. *Mol Pharmacol* 54: 1055–1063, 1998.

Yu XM, Salter MW. Src, a molecular switch governing gain control of synaptic transmission mediated by N-methyl-D-aspartate receptors. *Proc Natl Acad Sci USA* 96: 7697–7704, 1999.

Zhao J, Peng Y, Xu Z, Chen RQ, Gu QH, Chen Z, Lu W. Synaptic metaplasticity through NMDA receptor lateral diffusion. *J Neurosci* 28: 3060–3070, 2008.

Downloaded from http://jn.physiology.org/ by 10.220.33.2 on October 25, 2016