A characterisation of long-term depression induced by metabotropic glutamate receptor activation in the rat hippocampus *in vitro*

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1. In the CA1 region of hippocampal slices prepared from juvenile (12- to 18-day-old) rats, activation of group I metabotropic L-glutamate (mGlu) receptors by the specific agonist (*RS*)-3,5-dihydroxyphenylglycine (DHPG) induces a form of long-term depression (LTD) of excitatory synaptic transmission.

2. We have used a variety of electrophysiological techniques applied to CA1 neurones in hippocampal slices and from pyramidal cells in dissociated hippocampal cultures to investigate the Ca^{2+} dependence and locus of expression of DHPG-induced LTD.

3. In patch-clamp experiments from hippocampal slices, bath application of DHPG induced a depression of synaptically evoked responses that persisted for the duration of the recording (up to 2 h after commencing washout of DHPG) in 27 of 29 neurones investigated.

4. DHPG-induced LTD was associated with an increase in both the paired-pulse facilitation ratio and the coefficient of variation of EPSCs.

5. Using dendritic recording, there was a decrease in EPSC success rate (number of trials that elicited a detectable response) but no change in potency (mean EPSC amplitude excluding failures) associated with DHPG-induced LTD.

6. In experiments using dissociated hippocampal cultures, application of DHPG elicited a persistent decrease in the frequency of tetrodotoxin-resistant miniature EPSCs but no change in the amplitude of such events.

7. DHPG-induced LTD was not blocked by intracellular application of the calcium chelator BAPTA. It was also unaffected when intracellular calcium stores were depleted by perfusion with thapsigargin. Furthermore, when synaptic transmission was blocked by perfusing with Ca^{2+}-free medium, DHPG application reliably induced LTD.

8. These data suggest that DHPG-induced LTD is Ca^{2+} independent and is expressed presynaptically.

The metabotropic L-glutamate (mGlu) receptors are G-protein coupled receptors that have been implicated in a variety of brain functions and pathological conditions (Conn & Pin, 1997; Anwyl, 1999). There are currently eight known subtypes of mGlu receptors, which are divided into three groups based on sequence homology, pharmacology and signal transduction mechanisms. There is evidence that mGlu receptors have a role in the induction of various forms of synaptic plasticity such as long-term potentiation and long-term depression (LTD) (Anwyl, 1999; Bortolotto *et al.* 1999).

Synaptic activation of mGlu receptors has been shown to be involved in LTD induction under a variety of experimental conditions (Stanton *et al.* 1991; Bashir *et al.* 1993; Bolshakov & Siegelbaum, 1994; Oliet *et al.* 1997; Fitzjohn *et al.* 1998*a*; Kemp & Bashir, 1999; Huber *et al.* 2000). However, mGlu receptor-dependent LTD often co-exists at the same synapses with NMDA receptor-dependent LTD (e.g. Oliet *et al.* 1997). One strategy to selectively activate mGlu receptor-dependent LTD is to apply a specific mGlu receptor agonist. The broad spectrum mGlu receptor agonist 1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) has been shown to induce LTD in both the dentate gyrus (O'Mara *et al.* 1995) and CA1 region of the hippocampus (Bolshakov & Siegelbaum, 1994; Overstreet *et al.* 1997). Although several studies have failed to observe LTD induced by ACPD (Baskys &

Malenka, 1991; Vignes *et al.* 1995; Harvey *et al.* 1996; Palmer *et al.* 1997), a more reproducible form of LTD induced by the group I-specific mGlu receptor agonist (*RS*)-3,5-dihydroxyphenylglycine (DHPG) has been reported recently (e.g. Palmer *et al.* 1997; Fitzjohn *et al.* 1998*a*, 1999; Camodeca *et al.* 1999; Schnabel *et al.* 1999; Tan *et al.* 1999; Wetzel *et al.* 1999; Huber *et al.* 2000). The ability to chemically induce LTD should help elucidate the molecular basis of this form of synaptic plasticity, as has been the case for NMDA receptor-dependent LTD (Lee *et al.* 1998).

The mechanism of expression of DHPG-induced LTD is unknown. Although electrically induced mGlu receptor-dependent LTD has been shown to be expressed presynaptically (Oliet *et al.* 1997), a recent report suggested that DHPG-induced LTD is expressed postsynaptically (Snyder *et al.* 2000). In the present study we have utilised electrophysiological recording of synaptic transmission in the hippocampus to investigate the locus of expression of this form of synaptic plasticity. Some of this work has been presented previously in abstract form (Fitzjohn *et al.* 1996, 1998*b*; Palmer & Collingridge, 1999).

METHODS

Hippocampal slices

Young (12- to 18-day-old) Wistar rats were killed by decapitation and 400 μm transverse hippocampal slices prepared. Slices were maintained in artificial cerebrospinal fluid (aCSF) that comprised (mM): 124 NaCl, 3 KCl, 26 $NaHCO_3$, 1.4 NaH_2PO_4, 1 $MgSO_4$, 2 $CaCl_2$, 10 D-glucose, bubbled with 95% O_2–5% CO_2, pH 7.4.

Somatic recording

Slices were maintained in a submerged recording chamber at room temperature (20–24 °C) perfused with aCSF at 2 ml min^{-1}. Whole-cell recordings were made blind from stratum pyramidale in area CA1. Patch electrodes had a resistance of 4–7 MΩ and the intracellular solution comprised (mM): 130 caesium methane sulphonate, 5 Hepes, 5 QX-314, 1 NaCl, 1 $MgCl_2$, 4 Mg-ATP, 0.3 Na_2-GTP, 0.05 EGTA, 0.035 $CaCl_2$, pH 7.35. In experiments using the calcium chelator BAPTA, EGTA was replaced with 10 mM BAPTA free acid and the caesium methane sulphonate concentration was reduced to maintain osmolarity. Cells were voltage clamped at −80 mV and EPSCs evoked by electrical stimulation of the Schaffer collateral–commissural pathway at a frequency of 0.066 Hz. Inhibitory postsynaptic potentials were blocked by inclusion of 50 μM picrotoxin in the aCSF (to block $GABA_A$ receptors) and the use of Cs^+ and QX314 in the intracellular solution (to block $GABA_B$ receptor-mediated activation of K^+ channels). Recordings were made using an Axopatch 200B amplifier. Data were filtered at 5 kHz, digitised at 10 kHz, and collected and analysed both on- and off-line using the LTP program (Anderson & Collingridge, 2001; http://www.ltp-program.com). Two forms of analysis, paired-pulse facilitation and coefficient of variation, were performed to investigate the locus of expression of DHPG-induced LTD.

Extracellular recording

Extracellular recordings of field EPSPs (fEPSPs) were performed from the stratum radiatum of area CA1 of hippocampal slices using a glass microelectrode filled with 4 M NaCl (resistance 1–3 MΩ). Slices were maintained in a submerged recording chamber and perfused at a rate of 2 ml min^{-1} with aCSF at room temperature. Responses were evoked by stimulation of the Schaffer collateral–commissural pathway at a frequency of 0.033 Hz. Stimulation strength was set so as to give a slope value 50% of that where a population spike was first observed. Recordings were made using an Axopatch 200B amplifier. Data were filtered at 5 kHz, digitised at 10 kHz and recorded and analysed both on- and off-line using LTP software. The slope of the rising phase of fEPSPs (20–80% of peak response amplitude) was used as a measure of synaptic transmission.

Dendritic recording

Dendritic recordings were used for a failures analysis since there is less dendritic filtering and hence small EPSCs are more readily distinguished from true failures (Isaac *et al.* 1998). Slices were maintained at room temperature in a submerged recording chamber, perfused at a rate of 2 ml min^{-1} with aCSF of composition as above with the addition of ascorbic acid (2 mM) and increased glucose (15 mM). Whole-cell recordings were obtained from the proximal apical dendrites of CA1 pyramidal cells under visual control using infrared illumination and differential interference contrast optics, as described previously (Isaac *et al.* 1998). Patch electrodes of resistance 7–9 MΩ were filled with an intracellular solution of composition (mM): 130 caesium methane sulphonate, 10 Hepes, 5 QX-314, 8 NaCl, 0.5 EGTA, 4 Mg-ATP, 0.3 Na_2-GTP, pH 7.25. Recordings were obtained from apical dendrites between 30 and 50 μm from soma. Cells were voltage clamped at −70 mV and the Schaffer collateral–commissural pathway stimulated at a frequency of 0.5 Hz using a fine monopolar electrode, placed 30–50 μm from the recording site. All experiments were carried out in the presence of 50 μM picrotoxin. Minimal stimulation was used to evoke a mixture of EPSCs and failures of synaptic transmission, with the stimulus intensity set to produce a success rate of around 70%. Under the conditions of the present experiments, fibre failures do not contribute significantly to the synaptic failures (Isaac *et al.* 1998). Recordings were made using an Axopatch-1B amplifier. Data were filtered at 5 kHz, digitised at 10 kHz, and collected and analysed using the LTP program. A failures analysis was performed to investigate the locus of DHPG-induced LTD.

Paired-pulse facilitation

To measure paired-pulse facilitation (PPF), two stimuli were delivered with an inter-stimulus interval of 50 ms in place of single stimuli for two 1 min periods during the baseline, for 1 min during DHPG application, and for 1 min every 5 min following washout of DHPG. The paired-pulse ratio (PPR) was calculated as the ratio of the amplitude of the second EPSC to the first EPSC. To ensure that the measurement of the peak amplitude of the second EPSC was not contaminated by residual current from the first EPSC, this residual component was first removed by subtracting the average response to a single stimulation obtained in the minute prior to recording PPF from the average response to two stimuli.

Coefficient of variation

The coefficient of variation (CV) was calculated as described by Kullmann (1994). The mean and standard deviation were calculated for the EPSC amplitudes recorded during successive 5 min epochs ($S.D._{EPSC}$ and $Mean_{EPSC}$). The standard deviation of the background noise was also calculated for each 5 min epoch using a period immediately prior to electrical stimulation ($S.D._{Noise}$). The CV for each epoch was calculated as $(S.D._{EPSC} - S.D._{Noise})/Mean_{EPSC}$.

Failures analysis

EPSC amplitude was estimated by measuring the average current over two time windows (3 ms wide), one positioned immediately before the stimulus artefact and the other over the peak of the EPSC. Failures were visually identified and average EPSC amplitude

(average of successes and failures), potency (average EPSC amplitude excluding failures) and success rate calculated for successive epochs throughout each experiment.

Hippocampal cultures

Cultures of dissociated hippocampal neurones were prepared from P3–P5 rats (Richmond *et al.* 1996). Rats were killed by decapitation and the brain rapidly removed. The CA1–CA3 region of the hippocampus was isolated and cells dissociated by trypsin digestion and mechanical dissociation before subsequent plating on 22 mm glass coverslips coated with poly-L-ornithine (25 μg ml^{-1}; Sigma, UK) and matrigel (1:50 dilution; Becton Dickinson, UK). Cultures were maintained at 37 °C in a humidified atmosphere of 95 % O_2–5 % CO_2 in medium comprising minimal essential medium, 38.9 mM glucose, 2 mM glutamine, 15 mM Hepes, 100 μg ml^{-1} bovine transferrin, 30 μg ml^{-1} insulin, 0.1 μg ml^{-1} biotin, 1.5 μg ml^{-1} vitamin B_{12}, 2 μg ml^{-1} gentamicin, 10 % fetal calf serum. After 24 h, medium was supplemented with cytosine arabinofuranoside (2.5 μM) to prevent glial cell proliferation. Culture medium was changed three times per week and after 10 days the concentration of fetal calf serum was reduced to 5 %. Cultures were used 6–14 days after plating. For experiments, cells were perfused continuously with Hepes buffered saline (HBS) at approximately 2 ml min^{-1} at room temperature. HBS comprised (mM): 119 NaCl, 5 KCl, 25 Hepes, 33 glucose, 2 $CaCl_2$, 2 $MgCl_2$, 0.0005 tetrodotoxin, 0.1 picrotoxin, pH 7.4, 305 mosmol l^{-1}. Whole-cell recordings were made from the soma of pyramidal neurones voltage clamped at −70 mV using an Axopatch-1D amplifier. Intracellular solution comprised (mM): 110 caesium methane sulfonate, 40 Hepes, 0.6 EGTA, 10 NaCl, 4 Mg-ATP, 0.3 Na_2-GTP, pH 7.2, 290 mosmol l^{-1}. After obtaining a stable 10 min baseline period, cells were perfused locally with DHPG-containing HBS (100 μM for 5 min) via a glass capillary tube positioned near to the recorded cell. Application of DHPG was preceded and succeeded by 5 min local perfusion with HBS. Access resistance was monitored throughout using LTP software.

Data were filtered at 5 kHz and collected continuously on videotape for off-line analysis and subsequently digitised at 10 kHz and converted to Axon binary files using Axoscope software (Axon Instruments, USA). mEPSC detection was performed using MiniAnalysis software (Synaptosoft Inc., USA). Events were detected by setting the threshold value for detection at three times the level of the root mean squares noise, followed by visual confirmation of mEPSC detection. For construction of cumulative probability plots, 100 successive events were used from the period immediately preceding application of DHPG and from the period 30 min after commencing washout of DHPG. For pooled cumulative probability plots, data were normalised to the median value in the pre-DHPG period. Statistical significance was tested using the Kolmorogov-Smirnov test.

Drugs

QX-134 and BAPTA were obtained from Sigma, UK. RS-DHPG (Tocris, UK) was dissolved in H_2O at a stock concentration of 100 times test concentration. Thapsigargin (Calbiochem, UK) was dissolved in DMSO at a stock concentration of 10 mM. Picrotoxin (Sigma, UK) was prepared as a stock solution of 5 mM in equimolar NaOH, except for use in hippocampal culture experiments where picrotoxin was dissolved directly in HBS. All stock solutions were stored as frozen aliquots and diluted to test concentration in aCSF. Neither DMSO (0.001 %) nor NaOH (50 μM) applied alone affected synaptic transmission when tested (data not shown).

Data analysis

All data are presented as means ± S.E.M. Unless stated otherwise, statistical significance was assessed using ANOVA.

RESULTS

Somatic recording from slices

Application of DHPG (30 μM for 20 min) induced LTD of synaptic transmission in 13/13 cells voltage clamped at −80 mV (Fig. 1). During application of DHPG, responses were depressed to 40 ± 4 % of control ($n = 13$) and after washout of DHPG the depression persisted for the time that the recording was maintained (minimum time 30 min, maximum time 120 min after commencing washout). Thirty minutes after washout of DHPG, responses were depressed to 56 ± 11 % of control ($n = 13$; $P < 0.05$). The change in EPSC amplitude was not associated with a change in access resistance, cell input resistance or holding current (Fig. 1). In three experiments, cells were voltage clamped at −30 mV for the period of DHPG application only. In these three cells the level of mGlu-LTD observed was similar to that seen when cells were voltage clamped at −80 mV throughout (responses depressed to 60 ± 10 % of control 30 min after commencing washout of DHPG; $n = 3$; data not shown).

PPF and CV were analysed in seven of the cells voltage clamped at −80 mV. LTD induced by DHPG was associated with an increase in PPF (Fig. 2*A*–*C*). During application of DHPG, responses decreased to 37 ± 7 % of control ($P < 0.05$) whilst the PPR increased from 2.0 ± 0.1 to 2.6 ± 0.2 ($P < 0.05$). After 30 min of DHPG washout, responses were 56 ± 13 % of control ($P < 0.05$) and the PPR remained elevated at 2.8 ± 0.4 ($P < 0.05$ compared to baseline). In these seven cells, CV also increased with application of DHPG (Fig. 2*D*). Baseline CV was 0.22 ± 0.03, which increased to 0.35 ± 0.04 during DHPG application and remained elevated at 0.29 ± 0.03 30 min after commencing washout of DHPG ($P < 0.05$).

Dendritic recording from slices

We used dendritic recording for a failures analysis since this provides an enhanced signal to noise ratio and thus an unambiguous identification of failures (Isaac *et al.* 1998). Using dendritic whole-cell recording with minimal stimulation, application of DHPG (100 μM for 10 min) elicited mGlu-LTD in six of eight cells tested (Fig. 3). The DHPG-induced LTD was associated with a significant decrease in success rate but with no significant change in potency. During application of DHPG, average EPSC amplitude was depressed to 35 ± 10 % of control ($P < 0.05$; $n = 6$). This depression was associated with a decrease in success rate from 66 ± 6 % to 27 ± 5 % ($P < 0.05$) but no significant change in potency (−14 ± 4 to −10 ± 2 pA). Fifteen minutes after commencing washout of DHPG, average EPSC amplitude remained depressed at 38 ± 13 % of control ($P < 0.05$), success rate was 27 ± 7 % ($P < 0.05$ compared with baseline), but potency was not significantly changed (−14 ± 4 pA).

For the duration of each experiment, access resistance remained constant (baseline 40 ± 3 MΩ; DHPG washout

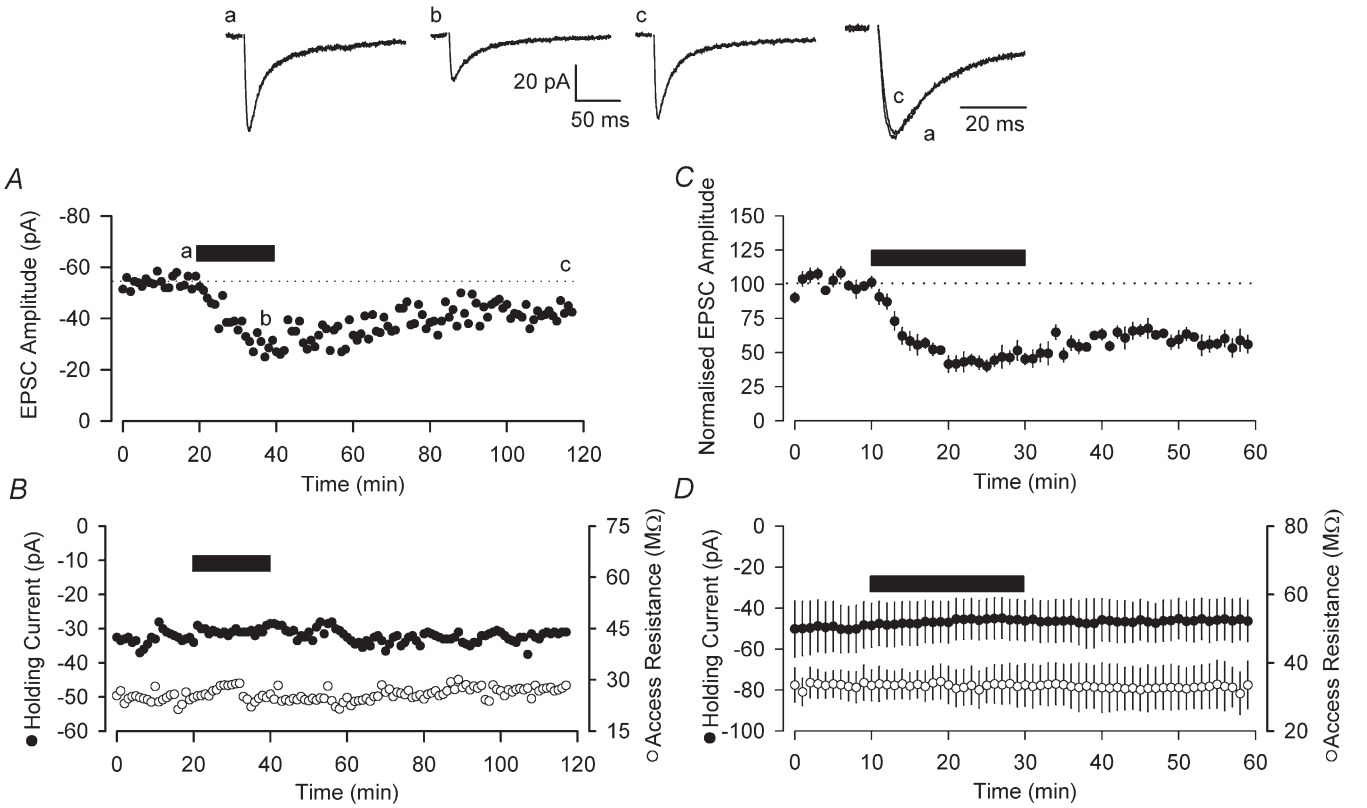

Figure 1. DHPG induces LTD

A, a representative experiment using somatic recording in which DHPG (30 μM) application induced LTD. In this and all subsequent figures DHPG application is indicated by a black bar. Example traces are taken from the time points indicated. Each point represents the mean of four successive EPSCs. *B*, holding current (●) and access resistance (○) remained unchanged during this experiment. *C* and *D*, pooled data from 13 cells. Data are presented as means ± S.E.M.

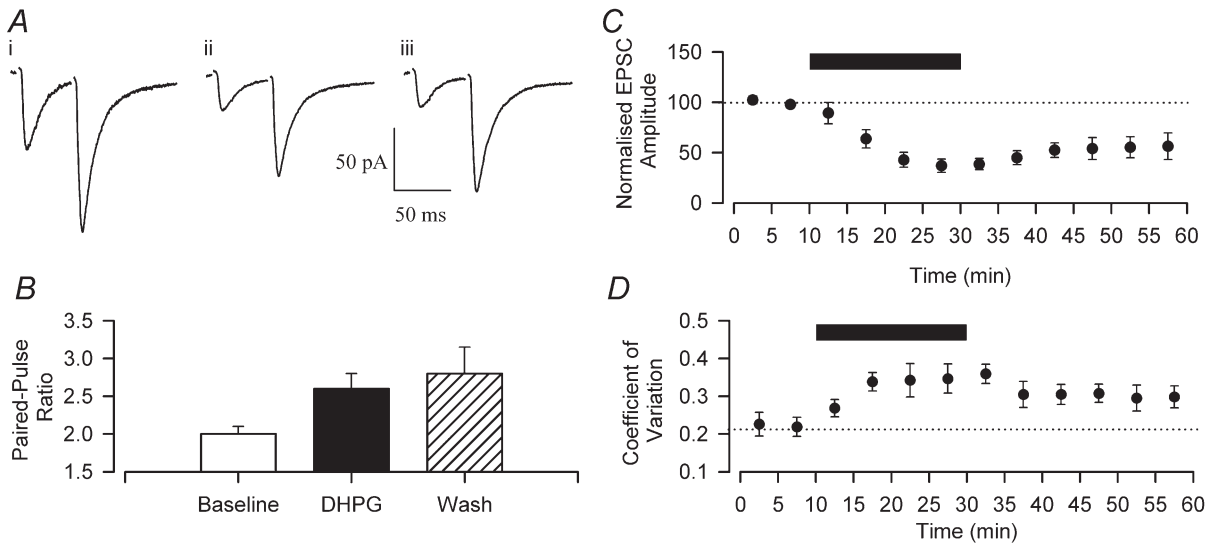

Figure 2. DHPG induces an increase in PPF and CV

A, examples of EPSCs induced by paired pulses taken from a representative experiment (i) during baseline, (ii) during DHPG application and (iii) following DHPG washout. *B–D*, pooled data for seven cells showing changes in paired-pulse ratio, EPSC amplitude and coefficient of variation induced by DHPG application (30 μM for 20 min).

44 ± 4 MΩ). The rise and decay phases of mean EPSCs were fitted with single exponential functions to give time constants (τ) which were not significantly changed by DHPG application (baseline $\tau_{rise} = 1.4 \pm 0.2$ ms, $\tau_{decay} = 8.5 \pm 0.6$ ms; LTD $\tau_{rise} = 1.5 \pm 0.3$ ms, $\tau_{decay} = 9.1 \pm 0.8$ ms; $n = 6$).

The observation that potency did not decrease despite large decreases in success rate was surprising given the likelihood that multiple synapses were being activated (Hessler *et al.* 1993; Rosenmund *et al.* 1993; Allen & Stevens, 1994). When the change in potency was compared with that predicted from the Poisson equation for the change in success rate for each cell (Isaac *et al.* 1998) it was found that in the majority of cases (5/6) the actual potency ratio (LTD/baseline) was greater than the predicted ratio. One possible explanation for this observation is that DHPG induces not only a decrease in success rate, which causes LTD, but also a small increase in potency via another mechanism.

Hippocampal cultures

Application of DHPG (100 μM for 10 min) resulted in a decrease in the frequency of tetrodotoxin-resistant mEPSCs (Fig. 4). Thirty minutes after commencing washout of DHPG, mEPSC frequency was reduced to 61 ± 8% of that recorded prior to application of DHPG ($n = 5$; $P < 0.05$). mEPSC amplitude was, however, not affected by application of DHPG; amplitude immediately prior to, and 30 min after commencing washout of,

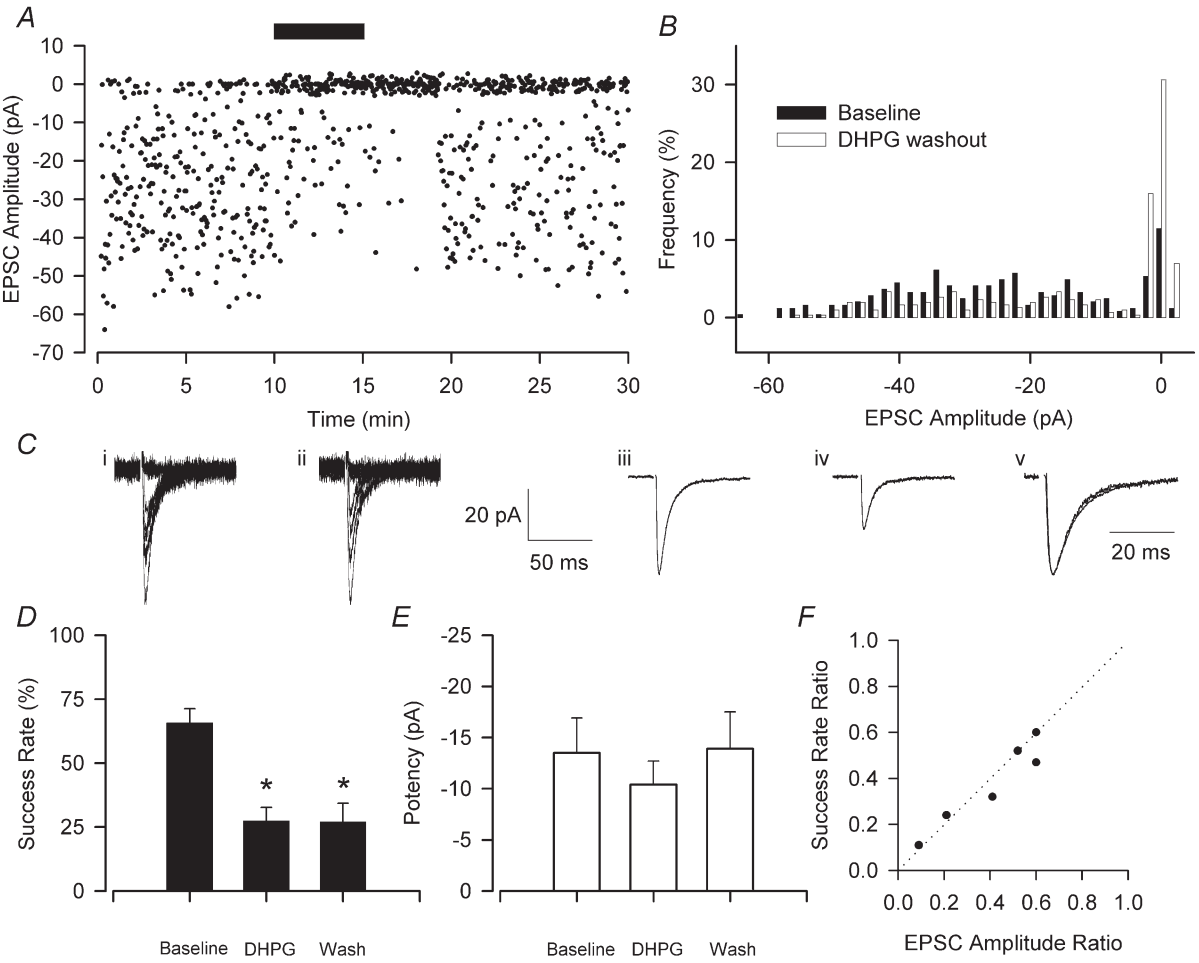

Figure 3. DHPG induces an increase in failure rate

A, a representative experiment using dendritic recording and minimal stimulation, in which DHPG (100 μM) induced an increase in the failure rate of EPSCs but had little effect on potency. *B*, EPSC amplitude histogram for the experiment shown in *A*. Points around 0 mV represent failures. Bin width = 2 pA. *C*, 10 consecutive EPSCs (i and ii) and mean EPSCs (responses and failures; iii and iv) taken from baseline (i and iii) and DHPG washout periods (ii and iv) for a different cell. The mean EPSCs are scaled in v to show the lack of change in response kinetics. *D* and *E*, pooled data ($n = 6$) for cells showing success rate and potency changes during DHPG application and 15 min after DHPG washout (* $P < 0.05$ compared with baseline). *F*, plot of average EPSC amplitude ratio (DHPG washout/baseline) *versus* success rate ratio (DHPG washout/baseline) for cells in which LTD was induced, showing that the LTD can be fully accounted for by the decrease in success rate.

DHPG was 45 ± 8 and 49 ± 10 pA, respectively ($n = 5$; $P > 0.05$). These changes were accompanied by a shift in the cumulative probability plot for inter-event interval ($P < 0.05$, Kolmorogov-Smirnov test; Fig. 4*E* and *F*) but no change in the corresponding plots for mEPSC amplitude ($P > 0.05$).

Role of calcium in DHPG-induced LTD

To investigate the role of postsynaptic Ca^{2+} in DHPG-induced LTD, somatic recordings were made from CA1 neurones in hippocampal slices using an intracellular solution containing 10 mM of the calcium chelator BAPTA (experiments were interleaved with experiments using non-BAPTA containing solution, Fig. 1). Cells were dialysed for at least 1 h to ensure complete dialysis of BAPTA into the cell. Application of DHPG (30 μM for 20 min) induced LTD of synaptic transmission in cells voltage clamped at −80 mV. Thus, 30 min after commencing washout of DHPG, responses were 53 ± 15 % of control ($n = 5$; Fig. 5*A*).

As group I mGlu receptors couple to release of Ca^{2+} from intracellular stores that may occur at the presynaptic site, we then investigated whether depletion of these stores by thapsigargin blocked DHPG-induced LTD using extracellular recordings (Fig. 5*B*). In these experiments, 40 min after application of DHPG (50 μM for 20 min) in the presence of thapsigargin (10 μM), responses were depressed to 67 ± 10 % of control ($n = 5$; $P < 0.05$). Finally, we performed experiments where DHPG was applied along with removal of extracellular Ca^{2+}. Application of Ca^{2+}-free aCSF, which completely abolished synaptic transmission, failed to prevent the induction of LTD by DHPG (50 μM for 20 min; Fig. 5*C* and *D*). Forty minutes after washout of DHPG (35 min after reintroduction of Ca^{2+}) responses were 69 ± 6 % of control ($n = 5$; $P < 0.05$). In each experiment, additional applications of Ca^{2+}-free media for similar periods (40 min) without DHPG application, both prior to and following the application of DHPG, did not lead to any long-term effects on synaptic transmission (Fig. 5*C* and

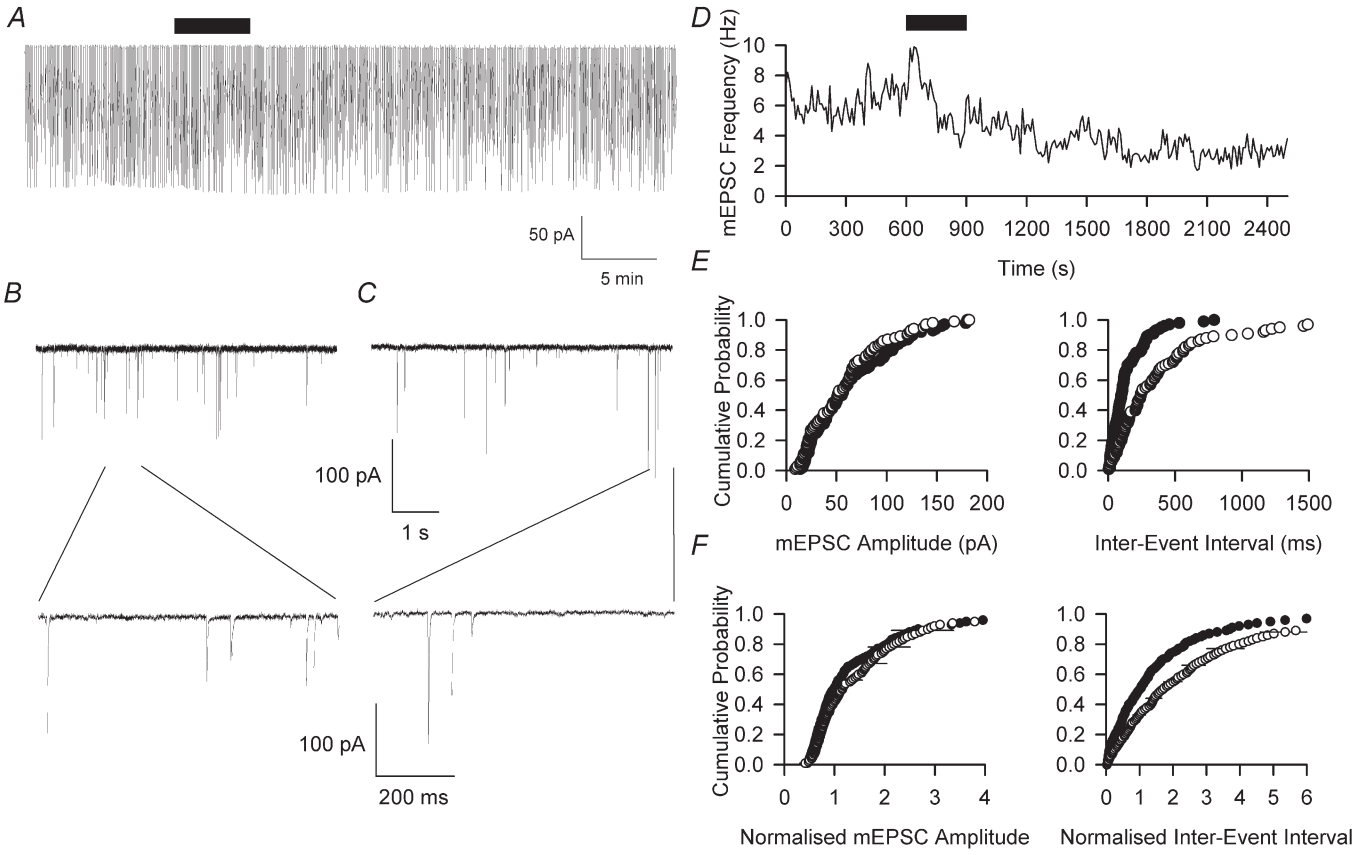

Figure 4. DHPG application elicits a decrease in mEPSC frequency but no change in amplitude

A, trace showing experiment where DHPG (100 μM for 10 min) was applied to cultured hippocampal neurones. Downward deflections indicate mEPSCs. Voltage steps used to measure access resistance have been blanked. *B* and *C*, traces from experiment shown in *A* on expanded time base taken from the period immediately prior to (*B*) and 30 min after (*C*) application of DHPG. Lower panel shows region of trace expanded from area indicated. *D*, plot showing decrease in the frequency of mEPSCs after application of DHPG in experiment shown in *A*. Data were divided into 10 s bins. *E*, cumulative probability plots for the experiment in *A*, showing no change in mEPSC amplitude (left) but a shift in inter-event interval (right; baseline period ●, post-DHPG ○). *F*, pooled cumulative probability plots for five such cells.

E). Thirty-five minutes after reintroduction of Ca^{2+} (without DHPG application) responses were 98 ± 6 % of control (both applications of Ca^{2+}-free medium from five experiments, $P > 0.05$).

DISCUSSION

The principal findings of the present study are that DHPG-induced LTD is induced via a Ca^{2+}-independent mechanism and is expressed presynaptically.

On the mechanism of induction of DHPG-induced LTD

It has been shown previously that DHPG-induced LTD does not require electrical stimulation of afferent pathways for its induction (Fitzjohn *et al.* 1999). We now show that the induction is independent of alterations in postsynaptic Ca^{2+}, since extensive loading of neurones with BAPTA failed to inhibit the effect. Indeed it is unlikely that Ca^{2+} is necessary for the effect since perfusion with Ca^{2+}-free medium, which abolished synaptic transmission, or treatment with thapsigargin, which completely blocks mGlu receptor-mediated Ca^{2+} mobilisation in hippocampal neurones in culture (Rae *et al.* 2000) and is active in hippocampal slices (Harvey & Collingridge, 1992), also failed to prevent DHPG-induced LTD. These findings do not, however, distinguish between Ca^{2+}-independent induction mechanisms in the pre- or postsynaptic neurone.

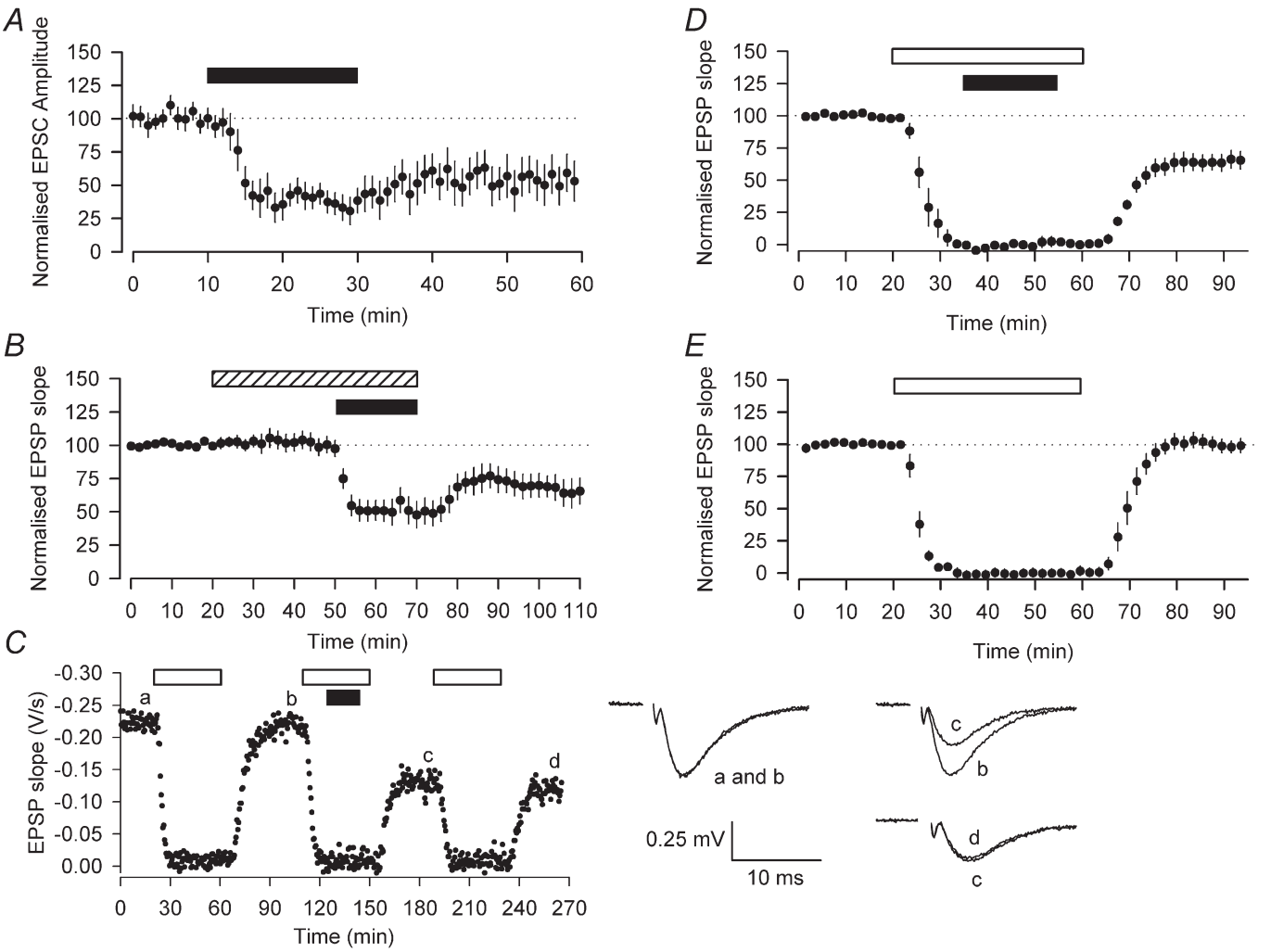

Figure 5. DHPG-induced LTD is independent of calcium

A, pooled data ($n = 5$) showing the effects of DHPG application (30 μM) in single cells dialysed with BAPTA-containing intracellular solution voltage clamped at −80 mV. *B*, pooled data ($n = 5$) for extracellular experiments where DHPG (50 μM; black bar) was applied in the presence of thapsigargin (10 μM; hatched bar). *C*, single extracellular experiment where DHPG (50 μM; black bar) was applied in the presence of Ca^{2+}-free medium (open bars). Additional applications of Ca^{2+}-free medium were performed without DHPG application prior to and after DHPG application. Traces to the right are taken from the time points indicated. *D*, pooled data showing the effects of DHPG application in the absence of extracellular Ca^{2+} ($n = 5$). *E*, pooled data from experiments as in *D* showing lack of long-term effect of Ca^{2+}-free application ($n = 5$).

On the mechanism of expression of DHPG-induced LTD

Collectively, the findings that DHPG-induced LTD is associated with (1) an increase in paired-pulse facilitation, (2) an increase in the coefficient of variation of EPSC amplitude, (3) a decrease in success rate but not potency and (4) a decrease in mEPSC frequency but not amplitude, suggest strongly that this form of plasticity is due to a decrease in release probability (Bekkers & Stevens, 1990; Manabe *et al.* 1993; Kullman, 1994; Isaac *et al.* 1998). Recently, it has been suggested that DHPG-induced LTD involves the internalisation of AMPA receptors (Snyder *et al.* 2000). Changes in the coefficient of variation of EPSC amplitude, failure rate and mEPSC frequency could be explained by a postsynaptic mechanism if DHPG resulted in the silencing of synapses by causing internalisation of the entire AMPA receptor complement. In this context, it has been shown that NMDA receptor-dependent LTD involves the silencing of synapses (Lüthi *et al.* 1999) and internalisation of AMPA receptors (Carroll *et al.* 1999). However, postsynaptic silencing of synapses is unlikely to explain DHPG-induced LTD since the effect was associated with an increase in the level of paired-pulse facilitation. Thus a decrease in the number of AMPA receptor-containing synapses should not affect the level of paired-pulse facilitation, unless AMPA receptors are preferentially removed from sites where the probability of glutamate release is higher than average. This situation might arise if DHPG-induced LTD required both activation of mGlu receptors and synaptic activation of AMPA receptors. However, the finding that DHPG-induced LTD does not require either electrical stimulation of afferent pathways (Fitzjohn *et al.* 1999) or Ca^{2+}-dependent synaptic transmission does not support this explanation. Alternatively, other Ca^{2+}-independent methods for removing AMPA receptors from high probability synapses may exist. However, our results are most simply explained by a presynaptic mechanism of expression. This does not exclude the possibility that a form of DHPG-induced LTD that is expressed postsynaptically can co-exist at these synapses under certain conditions.

From induction to expression of DHPG-induced LTD

How is this form of presynaptic LTD induced? If the induction is postsynaptic then the process requires a Ca^{2+}-independent, retrograde signalling mechanism. Alternatively, the entire process may occur presynaptically. Thus although group I mGlu receptors are predominantly located postsynaptically (Lujan *et al.* 1997) they have also been observed presynaptically (Romano *et al.* 1995). Furthermore, activation of group I mGlu receptors has been shown to modulate glutamate release from hippocampal synaptoneurosomes (Rodriguez-Moreno *et al.* 1998) and produces an acute depression of synaptic transmission that is also most readily explained by a presynaptic locus (Manzoni & Bockaert, 1995; Gereau & Conn, 1995).

The physiological significance of DHPG-induced LTD

There are intense investigations into the mechanisms of LTD in relation to synaptic plastic mechanisms involved in development and learning and memory. The most intensely studied is an NMDA receptor-dependent form of LTD (Dudek & Bear, 1992; Mulkey & Malenka, 1992), which is expressed postsynaptically (e.g. Oliet *et al.* 1997; Lüthi *et al.* 1999; Carroll *et al.* 1999). The ability to induce this form of LTD with a specific NMDA receptor agonist is enabling the molecular basis of this form of plasticity to be elucidated (Lee *et al.* 1998). Likewise, the ability to induce mGlu receptor-dependent LTD using a specific mGlu receptor agonist should similarly facilitate the understanding of its molecular basis. The physiological importance of DHPG-induced LTD is indicated by the findings that mGlu receptor-dependent LTD can be induced synaptically (Stanton *et al.* 1991; Bashir *et al.* 1993; Bolshakov & Siegelbaum, 1994; Oliet *et al.* 1997; Fitzjohn *et al.* 1998*a*; Kemp & Bashir, 1999; Huber *et al.* 2000). It is likely, however, that there is more than one form of mGlu receptor-dependent LTD. For example the form investigated here differs significantly from that studied by Oliet *et al.* (1997). The latter was input specific and dependent on postsynaptic Ca^{2+}, and could not be induced when $GABA_A$ receptor-mediated synaptic inhibition was blocked. It also required artificially high concentrations of divalent cations (4 mM Ca^{2+} plus 4 mM Mg^{2+}). Furthermore, it was blocked by a protein kinase C (PKC) inhibitor whereas DHPG-induced LTD at CA1 synapses is resistant to a variety of PKC inhibitors (Schnabel *et al.* 1999, 2001). Further work is necessary to determine the conditions required to induce synaptically the form of LTD that can be selectively induced by DHPG.

The presence of pre- and postsynaptic forms of LTD at the same synapses confers a greater flexibility in the long-term modulation of synaptic strength. NMDA receptor-dependent LTD senses both pre- and postsynaptic activity in a Hebbian manner whereas DHPG-induced LTD does not require active participation of the postsynaptic cell.

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