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Spike-timing-dependent plasticity at resting and conditioned lateral perforant path synapses on granule cells in the dentate gyrus: different roles of *N*-methyl-D-aspartate and group I metabotropic glutamate receptors

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Abstract

We examined the mechanisms underlying spike-timing-dependent plasticity induction at resting and conditioned lateral perforant pathway (LPP) synapses in the rat dentate gyrus. Two stimulating electrodes were placed in the outer third of the molecular layer and in the granule cell layer in hippocampal slices to evoke field excitatory postsynaptic potentials (fEPSPs) and antidromic field somatic spikes (afSSs), respectively. Long-term potentiation (LTP) of LPP synapses was induced by paired stimulation with fEPSP preceding afSS. Reversal of the temporal order of fEPSP and afSS stimulation resulted in long-term depression (LTD). Induction of LTP or LTD was blocked by D,L-2-amino-5-phosphonopentanoic acid (AP5), showing that both effects were *N*-methyl-D-aspartate receptor (NMDAR)-dependent. Induction of LTP was also blocked by inhibitors of calcium–calmodulin kinase II, protein kinase C or mitogen-activated/extracellular-signal regulated kinase, suggesting that these are downstream effectors of NMDAR activation, whereas induction of LTD was blocked by inhibitors of protein kinase C and protein phosphatase 2B. At LPP synapses previously potentiated by high-frequency stimulation or depressed by low-frequency stimulation, paired fEPSP–afSS stimulation resulted in 'de-depression' at depressed LPP synapses but had no effect on potentiated synapses, whereas reversal of the temporal order of fEPSP–afSS stimulation resulted in 'de-potentiation' at potentiated synapses but had no effect on depressed synapses. Induction of de-depression and de-potentiation was unaffected by AP5 but was blocked by 2-methyl-6-(phenylethynyl) pyridine hydrochloride, a group I metabotropic glutamate receptor blocker, showing that both were NMDAR-independent but group I metabotropic glutamate receptor-dependent. In conclusion, our results show that spike-timing-dependent plasticity can occur at both resting and conditioned LPP synapses, its induction in the former case being NMDAR-dependent and, in the latter, group I metabotropic glutamate receptor-dependent.

Introduction

The strength of synaptic transmission in the central nervous system (CNS) can undergo marked use-dependent changes. The best known phenomena are long-term potentiation (LTP) (Bliss & Lomo, 1973; Bliss & Collingridge, 1993; Malenka & Nicoll, 1999) and long-term depression (LTD) (Dudek & Bear, 1992; Bear & Malenka, 1994; Kirkwood & Bear, 1994) of synaptic efficacy, which are induced, respectively, by presynaptic high-frequency stimulation (HFS) or low-frequency stimulation (LFS). LTP and LTD can also be induced by presynaptic LFS in conjunction with postsynaptic spiking at synapses in different regions of the CNS, including the hippocampus (Magee & Johnston, 1997; Markram *et al.*, 1997; Lin *et al.*, 2003), visual cortex (Froemke & Dan, 2002), sensory cortex (Feldman, 2000) and in cultured neurons (Bi & Poo, 1998; Debanne *et al.*, 1998); this phenomenon is referred to as spike-timing-dependent plasticity (STDP) (for review see Bi & Poo, 2001; Dan & Poo, 2004). STDP is bidirectional, i.e. synaptic efficacy can be either potentiated or depressed by paired pre and postsynaptic spiking, depending both on the timing interval and the temporal order of the pre and postsynaptic spiking. In general, to induce STDP, the timing interval between paired pre and postsynaptic spiking has to be less than ~25 ms, whereas a significantly wider time window (up to ~100 ms) has been suggested for LTD induction (Debanne *et al.*, 1998; Feldman, 2000). As regards the temporal order of pre and post spiking, repeated paired pre and post spiking results in LTP if presynaptic stimulation precedes postsynaptic stimulation but results in LTD if the temporal order of pre and post spiking is reversed. STDP provides a good explanation for synaptic modifications that are dependent on the simultaneous activity of pre- and postsynaptic neurons, and is considered to be the cellular mechanism underlying learning and memory and the activity-driven

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refinement of developing circuits in the brain (Song *et al.*, 2000; Bi & Poo, 2001; Song & Abbott, 2001; Dan & Poo, 2004).

Despite the important role of STDP in many brain functions, the detailed mechanisms of its induction and expression are not fully understood. For example, although it has been reported that the induction of spike-timing-dependent LTP and LTD requires activation of the *N*-methyl-D-aspartate receptor (NMDAR) (Bi & Poo, 1998; Magee & Johnston, 1997; Markram *et al.*, 1997; Feldman, 2000; Lin *et al.*, 2003), some reports have suggested that, in LTD induction, voltage-dependent calcium channels (VDCCs) (Bi & Poo, 1998; Normann *et al.*, 2000) or internal calcium sources (Nishiyama *et al.*, 2000) are also involved. Furthermore, it remains unclear whether signal effectors acting downstream of the NMDAR, including calcium–calmodulin kinase II (CaMKII), protein kinase C (PKC) and extracellular signal-regulated kinase (ERK) of mitogen activated protein kinase (MAP/ERK), which have been reported to be involved in induction of homosynaptic LTP and LTD (Roberson *et al.*, 1996; Impey *et al.*, 1999; Otmakhova *et al.*, 2000; Yang *et al.*, 2002), are also involved in spike-timing-dependent LTP and LTD. In the present study, we set out to address this issue by using, as a model, the synapses between the lateral perforant pathway (LPP) and granule cells in the dentate gyrus. This region was chosen because it is the area in which temporal specificity of associative synaptic modification was first reported *in vivo* (Levy & Steward, 1983), the cellular mechanisms for induction of homosynaptic LTP and LTD in this area have been well established (Roberson *et al.*, 1996) and it is one of the areas known to be important in memory formation.

Materials and methods

The use of animals in this study was in accordance with the guidelines of the Ethical Committee for Animal Research of the National Taiwan University. Male Sprague-Dawley rats aged 25–35 days were anesthetized with halothane and decapitated, and the brains rapidly removed and placed in ice-cold artificial cerebral spinal fluid (aCSF) containing (mM): NaCl, 119; KCl, 2.5; $NaHCO_3$, 26.2; NaH_2PO_4, 1; $MgSO_4$, 1.3; $CaCl_2$, 2.5; glucose, 11, pH adjusted to 7.4 by gassing with 5% CO_2/95% O_2. Transverse hippocampal slices (450 μm thick) were cut using a vibrating tissue slicer (Campden Instruments, Loughborough, UK) and placed in an interface-type holding chamber at room temperature (26 °C). For extracellular field potential recording, slices were transferred to an immersion-type recording chamber and perfused at a rate of 2 mL/min with aCSF containing 0.1 mM picrotoxin at 26 °C. A glass pipette filled with 3 M NaCl was positioned in the outer third of the molecular layer of the dentate gyrus to record field neuronal activity. Two bipolar stainless steel electrodes (FHC, Bowdoinham, ME, USA) were placed in the outer third of the molecular layer, a few hundred micrometers from the recording pipette, and in the inferior border of the granule cell layer (Fig. 1, A1). The first stimulating electrode was used to elicit field excitatory postsynaptic potentials (fEPSPs); only activity that showed paired-pulsed (50-ms interpulse interval) facilitation was considered as fEPSP of LPP synapses (Colino & Malenka, 1993; Min *et al.*, 1998). The second stimulating electrode was used to elicit antidromic field somatic spikes (afSSs) in granule cells. Stable baseline fEPSP activity of the LPP was elicited by stimulation every 30 s for at least 10 min. Bidirectional plasticity of the LPP was then induced by pairing fEPSP and afSS stimulation at 6-s intervals for 10 min. During the pairing protocol, the delivery of fEPSP stimulation preceded, or followed, that of afSS stimulation by ∼3–100 ms. Baseline fEPSP activity of the LPP was again elicited every 30 s for another 30 min. All signals were filtered at 2 kHz by a low pass Bessel filter provided by the amplifier (Axopatch-1D, Axon Instruments, Foster City, CA, USA), and digitized at 5 kHz using a CED micro 1401 interface running SIGNAL software (Cambridge Electronic Design, Cambridge, UK). All drugs were purchased from Sigma (St Louis, MO, USA), except for D,L-2-amino-5-phosphonopentanoic acid (AP5), chelerythrine, (RS)-2-chloro-5-hydroxyphenylglycine (CHPG), (S)-3,5-dihydroxyphenylglycine (DHPG), H-7, 2-methyl-6-(phenylethynyl) pyridine hydrochloride (MPEP), nimodipine, PD98059 and U0126, which were from Tocris-Cookson (Bristol, UK). All drugs were bath applied in aCSF, except for H-7, PD98059 and U0125; in these cases, the slices were preincubated with these drugs in aCSF for 1 h and then transferred to the recording chamber. The initial slope of the fEPSP was measured for data analysis. Synaptic responses were normalized to the average values measured during the baseline period. The average size of the slope of fEPSPs recorded between 25 and 30 min after the pairing protocol was used for statistical comparison. All data are presented as the mean ± SE and were statistically compared using either the paired *t*-test or one-way ANOVA test. The criterion for significance was $P < 0.05$.

Results

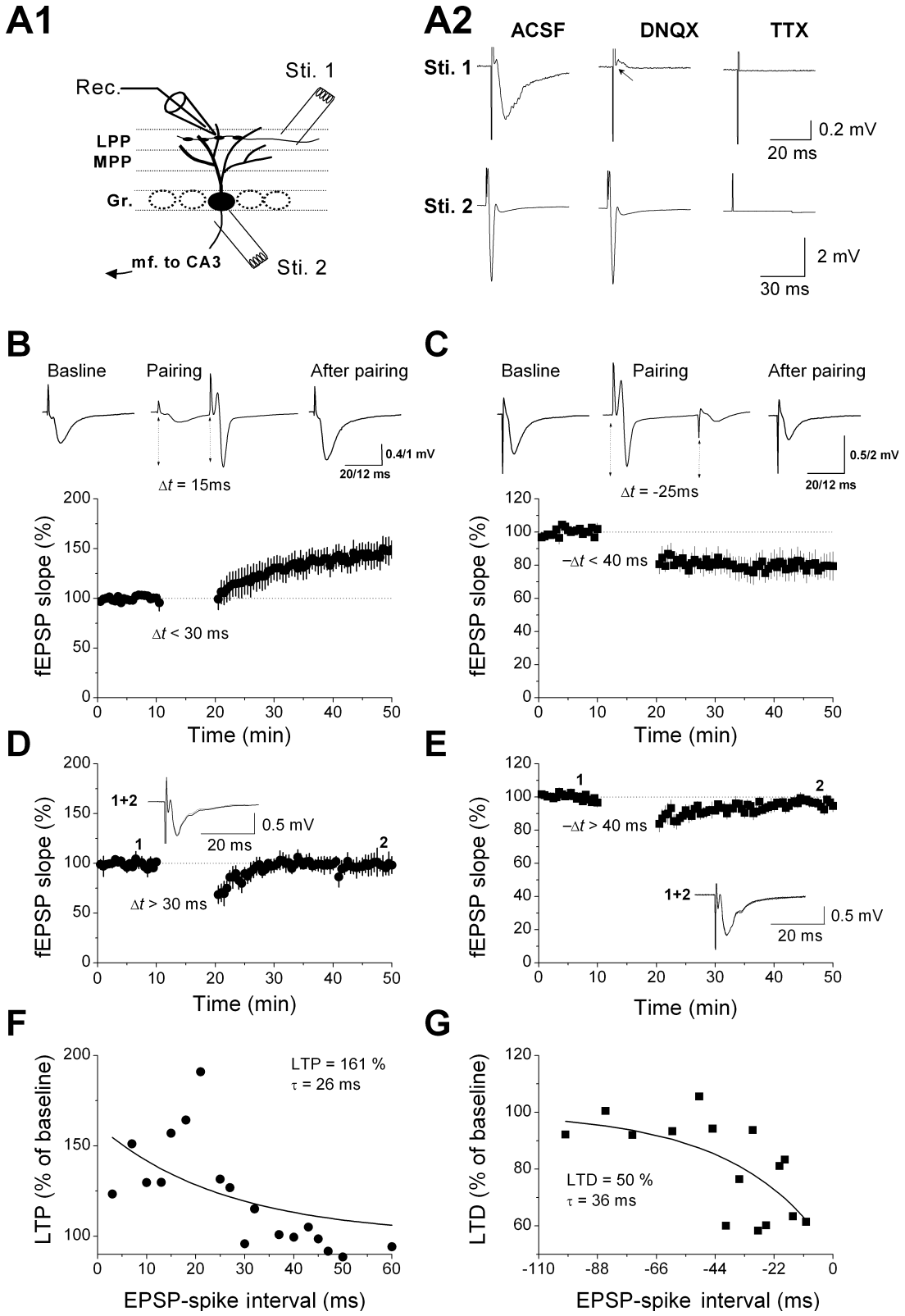
Using the arrangement of recording and stimulating electrodes shown in Fig. 1 (A1), a synaptic response (Fig. 1, A2, upper left trace), confirmed by its sensitivity to bath application of 10 μM DNQX, was elicited by the stimulating electrode in the outer third of the molecular layer of the dentate gyrus. In the presence of DNQX, a small, negative activity was seen immediately after the stimulus artifact (see arrow in Fig. 1, A2, upper middle trace) and this was completely blocked by 1 μM TTX (Fig. 1, A2, upper right trace). These features suggest that the evoked response was fEPSP activity preceded by a presynaptic spike volley. The activity elicited by the stimulating electrode in the inferior border of the granule cell layer in the same slices showed a biphasic waveform and was DNQX-resistant but TTX-sensitive (Fig. 1, A2, lower traces), suggesting that it was a pure field somatic spike that was antidromically elicited and recorded extracellularly.

After characterizing the evoked neuronal activity, in particular the confirmation of the purely somatic spike activity elicited by stimulating the cell body of granule cells (Fig. 1A), we were able to induce STDP by pairing pre (perforant path) and post (granule cell body) stimulation and to record it using an extracellular field potential recording technique. Following recording of a stable fEPSP by stimulation every 30 s for at least 10 min, LTP of fEPSP could be induced by paired fEPSP–afSS stimulation, repeated 100 times at 6-s intervals with the fEPSP preceding the afSS (see insets in Fig. 1B). The success rate for LTP induction depended on the timing interval (Δt) between the evoking of the fEPSP and the afSS. Paired fEPSP–afSS stimulation, with afSS following the fEPSP (i.e. with $0 < \Delta t < 30$ ms) resulted in LTP in all nine slices tested, the average magnitude of the LTP 25–30 min later being 144 ± 4% of baseline (Fig. 1B). However, paired fEPSP–afSS stimulation with a $\Delta t \geq 30$ ms resulted in no significant change in synaptic efficacy in eight out of nine slices tested (LTP = 98 ± 9% of baseline; Fig. 1D). The STDP-inducing protocol in which afSS stimulation preceded fEPSP stimulation resulted in LTD of LPP synapses (Fig. 1C and E); in the text hereafter, when $-\Delta t$ is used to clarify inequalities, it indicates the (positive) interval between afSS and the fEPSP for the protocol in which afSS stimulation preceded fEPSP stimulation. Again, the success rate for LTD induction depended on the value of $-\Delta t$. Significant synaptic depression was induced in eight out of nine slices

tested by paired afSS–fEPSP stimulation with $-\Delta t \leq 40$ ms. The average magnitude of the LTD was 78 ± 10% of baseline (Fig. 1C). However, paired afSS–fEPSP stimulation with $-\Delta t > 40$ ms resulted in no significant change in synaptic strength in six out of six slices tested (LTD = 96 ± 3% of baseline; Fig. 1E). Figure 1F and G shows the relationship between the change in synaptic efficacy and the Δt for all experiments, the solid line being the fit of the data to an equation modified from Song *et al.* (2000), in terms of percentage:

$$F(\Delta t) = 100 \pm \mathrm{A} \times \mathrm{e}^{-\Delta t/\tau}$$

where $F(\Delta t)$ is the synaptic potentiation (or depression) induced by paired fEPSP–afSS (or afSS–fEPSP) stimulation, Δt the timing



interval, A the scaling factor and τ the time constant. The parameter A determines the maximum synaptic potentiation (or depression), whereas τ determines the range of the fEPSP–afSS (or afSS–fEPSP) timing interval over which synaptic potentiation (or depression) occurs. The estimated A and τ for the potentiation induced by paired fEPSP–afSS stimulation at LPP synapses were 61% and 26 ms, respectively (Fig. 1F), the corresponding values for the depression induced by paired afSS–fEPSP stimulation being 50% and 36 ms (Fig. 1G).

We next examined the mechanisms underlying the changes in synaptic efficacy shown in Fig. 1. To examine the LTP, the paired fEPSP–afSS stimulation timing interval was kept as 10 ms in this and subsequent experiments. As seen in Fig. 2A, LTP was successfully induced in seven out of seven slices tested using this pairing protocol (LTP = 148 ± 5% of baseline) but no induction of LTP was seen in eight out of eight slices tested in the presence of 50 μM AP5, an NMDAR antagonist (LTP = 105 ± 6% of baseline). Induction of LTP also failed in six out of six slices tested in the presence of 20 μM KN-93, a CaMKII inhibitor (LTP = 100 ± 2% of baseline; Fig. 2B), but was seen in six out of six slices tested in the presence of 20 μM KN-92, an inactive analog of KN-93 (LTP = 135 ± 6% of baseline; Fig. 2B). LTP induction was also blocked in six out of six slices preincubated with 10 μM H-7, a protein kinase inhibitor (LTP = 104 ± 4% of baseline; Fig. 2C), and in seven out of seven slices perfused with 3 μM chelerythrine, a PKC inhibitor (LTP = 105 ± 7% of baseline; Fig. 2D). Finally, LTP induction also failed in six out of six slices preincubated with either 50 μM PD98059, a MAPK inhibitor (LTP = 103 ± 3% of baseline; Fig. 2E), or 20 μM U0126, an ERK inhibitor (LTP = 100 ± 3% of baseline; Fig. 2F), suggesting that ERK and/or other MAPK kinases are downstream effectors of PKC activation, caused by activation of NMDAR/CaMKII during paired fEPSP–afSS stimulation.

To examine LTD induction, we used paired afSS–fEPSP stimulation with $\Delta t = -10$ ms. As shown in Fig. 3A, this stimulation protocol resulted in LTD at LPP synapses in seven out of seven slices tested (LTD = 71 ± 4% of baseline). In the presence of 50 μM AP5, LTD induction failed in seven out of seven slices (LTD = 98 ± 2% of baseline; Fig. 3A). Inhibition of PKC activity by perfusion of slices with chelerythrine blocked LTD induction in six out of six slices tested. In addition, perfusion with aCSF containing 10 μM cypermethrin, an inhibitor of protein phosphatase 2B, also blocked LTD induction in seven out of seven slices. Taken together, the above results show that LTD induction using the pairing protocol in this study is NMDAR-dependent and involves the PKC and protein phosphatase 2B signaling pathway.

As it is well known that the CaMKII/PKC/MAPK/ERK and PKC/protein phosphatase 2B pathways are involved, respectively, in induction of homosynaptic LTP and LTD (Roberson *et al.*, 1996; Impey *et al.*, 1999; Otmakhova *et al.*, 2000; Kemp & Bashir, 2001; Yang *et al.*, 2002), our results suggested that the LTP and LTD induced by paired pre-post and post-pre stimulation, respectively, might share some cellular mechanisms with homosynaptic LTP and LTD induced by HFS and LFS, respectively. To determine if this was the case, we tested whether induction of LTP and LTD by HFS and LFS could occlude the induction of LTP and LTD using paired pre and post stimulation. We first confirmed that a constant and long-lasting homosynaptic LTP or LTD could be induced at LPP synapses by, respectively, delivery of three trains of HFS repeated three times at 5-min intervals with each train containing 100 pulses at 100 Hz (Fig. 4A, LTP = 130 ± 5% of baseline) or LFS consisting of 900 pulses at 1 Hz (Fig. 4B, LTD = 68 ± 8% of baseline). Following potentiation of LPP synapses by HFS, no further potentiation was induced by paired fEPSP–afSS stimulation with $\Delta t = 10$ ms (Fig. 4C; LTP = 135 ± 6% of baseline). Similarly, following depression of LPP by LFS, no further synaptic depression was induced by paired afSS–fEPSP stimulation with $\Delta t = -10$ ms (Fig. 4D; LTD = 67 ± 5% of baseline). These results are therefore consistent with our argument that the LTP and LTD induced by paired pre and post stimulation shares common cellular mechanisms with the homosynaptic LTP and LTD induced by HFS and LFS.

In addition to inducing LTP and LTD at resting synapses, paired pre and post stimulation also induced 'de-potentiation' (reversal of LTP) and 'de-depression' (reversal of LTD) at conditioned synapses. As shown in Fig. 5A, paired afSS–fEPSP stimulation with $\Delta t = -10$ ms reversed the homosynaptic LTP induced by HFS almost to baseline levels in six out of six slices tested (de-potentiation = 106 ± 8% of baseline). In addition, paired fEPSP–afSS stimulation with $\Delta t = 10$ ms reversed the LTD induced by prolonged LFS to almost baseline levels in six out of six slices tested (de-depression = 106 ± 10% of baseline; Fig. 5B). Paired afSS–fEPSP stimulation with $-\Delta t > 40$ ms resulted in no de-potentiation of homosynaptic LTP in seven out seven slices tested (de-potentiation = 130 ± 6% of baseline; Fig. 5C), and paired fEPSP–afSS stimulation with $\Delta t > 30$ ms resulted in no de-depression of homosynaptic LTD in six out of six slices tested (de-depression = 68 ± 4% of baseline; Fig. 5D). These results suggest that the time windows for the fEPSP–afSS (or afSS–fEPSP) stimulation interval for induction of de-depression (or de-potentiation) are the same as those for induction of LTP and LTD. De-potentiation of LTP using prolonged LFS is reported to be time-dependent (Fujii *et al.*, 1991; Bashir & Collingridge, 1992; O'Dell & Kandel, 1994; Staubli & Chun, 1996; Huang *et al.*, 1999; Staubli & Scafidi, 1999; Chen *et al.*, 2001). We therefore tested whether the property was seen in our system. As shown in Fig. 5E, paired afSS–fEPSP stimulation with $\Delta t = -10$ ms applied ~1 h after induction of homosynaptic LTP by HFS did not result in de-potentiation (133 ± 9% of baseline), and similar results were obtained for de-depression (Fig. 5F; de-depression = 61 ± 9% of baseline). These results show that there is

FIG. 1. Long-term potentiation (LTP) and long-term depression (LTD) induced by paired field excitatory postsynaptic potential (fEPSP)–antidromic field somatic spike (afSS) and afSS–fEPSP stimulation, respectively, at lateral perforant pathway (LPP) synapses. Arrangement of the recording (Rec.) and stimulating (Sti. 1 and 2) electrodes (A1) and the evoked neuronal activity (A2). (B) Induction of LTP by paired fEPSP–afSS stimulation. The upper traces show baseline fEPSP activity (left), fEPSP and afSS during pairing (middle) and fEPSP activity after pairing (right) for one experiment. Note the potentiation of fEPSP activity after paired fEPSP–afSS stimulation with $\Delta t = 15$ ms. The lower plot shows the summarized results for nine experiments, in which Δt for the paired fEPSP–afSS stimulation was < 30 ms (LTP = 144 ± 4%; $P < 0.01$, paired *t*-test). (C) Induction of LTD by paired afSS–fEPSP stimulation. Note the depression of fEPSP activity (cf. the left and right insets) after paired afSS–fEPSP stimulation with $\Delta t = -25$ ms (see middle inset). The lower plot shows the summarized results for eight experiments, in which the positive time interval ($-\Delta t$) between the afSS and fEPSP stimuli was < 40 ms (LTD = 78 ± 10%; $P < 0.01$, paired *t*-test). (D and E) No significant LTP or LTD was induced by paired fEPSP–afSS/afSS–fEPSP stimulation when $\Delta t > 30$ ms or when $-\Delta t > 40$ ms, respectively (LTP = 98 ± 9%, $n = 9$; $P > 0.3$, paired *t*-test; LTD = 96 ± 3%, $n = 7$; $P > 0.8$, paired *t*-test). Plots showing the relationship between the fEPSP–afSS stimulation interval and the resulting LTP (F) or LTD (G). Each point represents an individual experiment. The solid lines show the fitting of the data to a single exponential decay. aCSF, artificial cerebral spinal fluid; EPSP, excitatory postsynaptic potential; Gr, granule cell layer; mf, mossy fiber; MPP, medial peforant pathway.

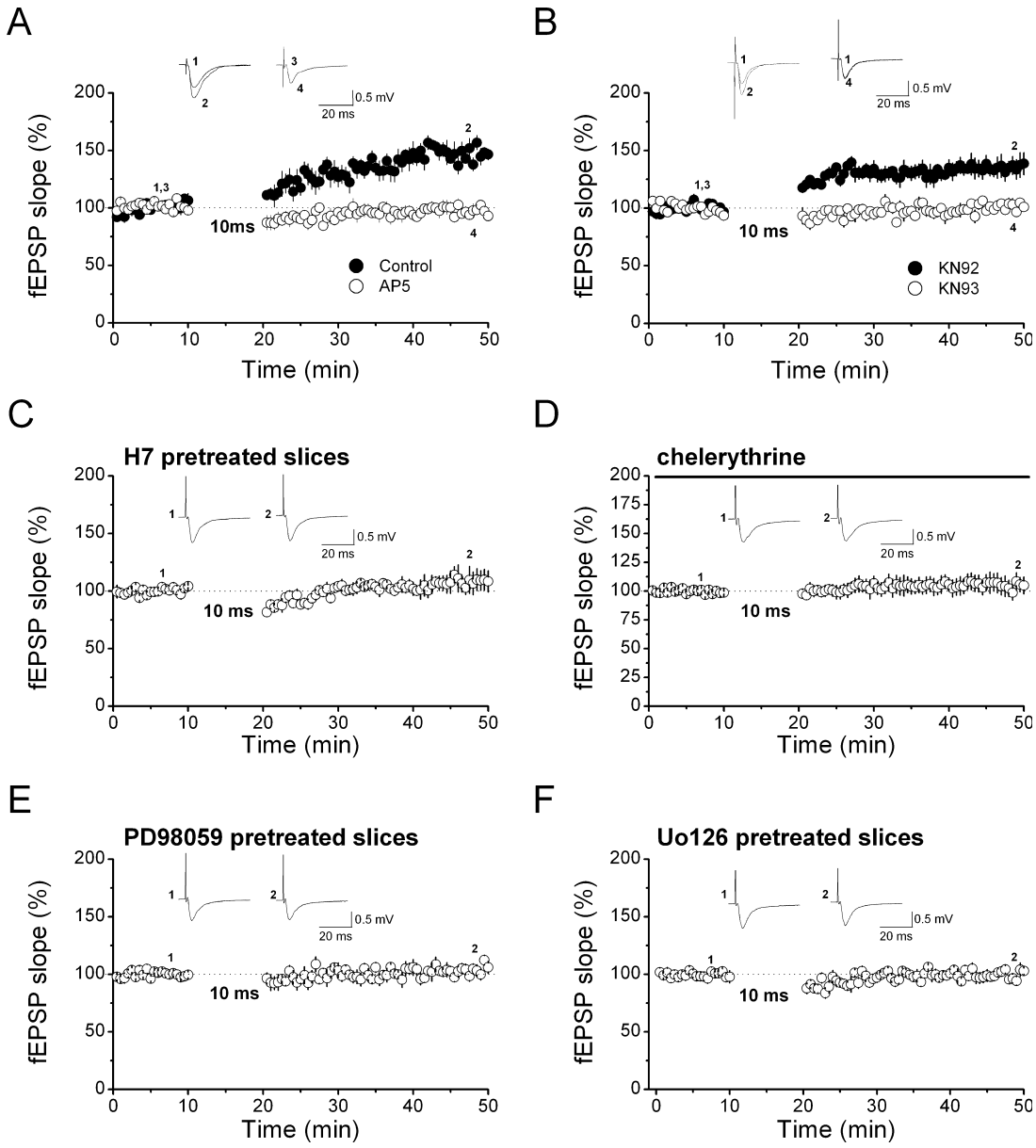


FIG. 2. Cellular mechanisms underlying long-term potentiation (LTP) induction. (A) Plot showing the LTP induced using paired field excitatory postsynaptic potential (fEPSP)–antidromic field somatic spike stimulation with $\Delta t = 10$ ms under control conditions (●) (LTP = 148 ± 5%, $n = 7$; $P < 0.01$, paired *t*-test) or after bath application of 50 μM AP5 (○) (LTP = 105 ± 6%, $n = 8$; $P > 0.5$, paired *t*-test). The insets show averaged fEPSP activity at baseline (10 sweeps) or at 30 min after the end of the pairing protocol (10 sweeps). (B) Induction of LTP is blocked by the calcium–calmodulin kinase II inhibitor KN-93 (○) (LTP = 100 ± 2%, $n = 6$; $P > 0.8$, paired *t*-test) but not by KN-92, the inactive analog of KN-93 (●) (LTP = 135 ± 6%, $n = 6$; $P < 0.01$, paired *t*-test). The insets show averaged fEPSP activity at baseline (10 sweeps) and at 30 min after the end of the pairing protocol (10 sweeps). (C) Induction of LTP is blocked by preincubation with the protein kinase inhibitor H-7 (LTP = 104 ± 4%, $n = 6$; $P > 0.3$, paired *t*-test). (D) Induction of LTP is blocked by the protein kinase C inhibitor chelerythrine (LTP = 105 ± 7%, $n = 7$; $P > 0.5$, paired *t*-test). (E) Induction of LTP is blocked by preincubation with the MAPK inhibitor PD98059 (LTP = 103 ± 3%, $n = 6$; $P > 0.4$, paired *t*-test). (F) Induction of LTP is blocked by preincubation with the extracellular signal-regulated kinase inhibitor U0126 (LTP = 100 ± 3%, $n = 6$; $P > 0.9$, paired *t*-test). The insets in A–F show averaged fEPSP activity at baseline (10 sweeps) and at 30 min after the end of the pairing protocol (10 sweeps).

a time window for de-potentiation/depression induced by paired pre/post stimulation.

We then examined whether induction of de-depression and de-potentiation shared the same cellular mechanism as induction of spike-timing-dependent LTP and LTD. As shown in Fig. 6A, the LTP of 156 ± 6% of baseline induced by HFS was de-potentiated to 116 ± 4% of baseline by paired afSS–fEPSP stimulation with $\Delta t = -10$ ms ($P < 0.001$, paired *t*-test, $n = 6$) in aCSF containing AP5, an NMDAR antagonist; similarly, the LTD of 74 ± 4% of baseline induced by LFS was de-depressed to 106 ± 2% of baseline by paired fEPSP–afSS stimulation with $\Delta t = 10$ ms (Fig. 6B; $P < 0.001$, paired *t*-test, $n = 6$) in the presence of AP5, showing that both processes were NMDAR-independent. Interestedly, paired afSS–fEPSP stimulation with $\Delta t = -10$ ms only resulted in de-potentiation of the LTP of 159 ± 7% of baseline to 135 ± 6% of baseline ($n = 6$) in aCSF containing MPEP, a group I metabotropic glutamate receptor (mGluR) antagonist (Fig. 6C). This LTP was not significantly different from that induced by HFS in control conditions (shown in Fig. 4A)

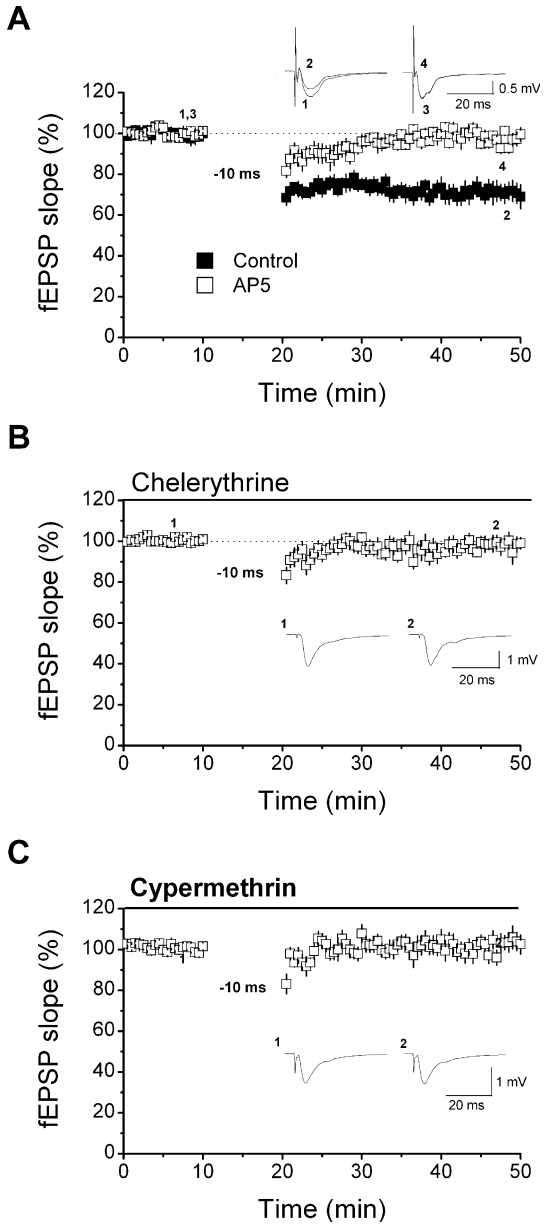


FIG. 3. Cellular mechanisms underlying induction of long-term depression (LTD). (A) Induction of LTD by paired antidromic field somatic spike–field excitatory postsynaptic potential (fEPSP) stimulation with $\Delta t = -10$ ms (■ LTD = 71 ± 4%, $n = 7$; $P < 0.01$, paired t-test) and its blockade by AP5 (□ long-term potentiation = 98 ± 2%, $n = 7$; $P > 0.3$, paired t-test). (B) Induction of LTD is blocked by chelerythrine (LTD = 97 ± 3%, $n = 6$; $P > 0.3$, paired t-test). (C) Induction of LTD is blocked by cypermethrin (LTD = 106 ± 4%, $n = 7$; $P > 0.7$, paired t-test). The insets in A–C show averaged fEPSP activity at baseline (10 sweeps) and at 30 min after the end of the pairing protocol (10 sweeps).

($P > 0.1$, one-way ANOVA test) but was significantly different from that in the presence of AP5 (Fig. 6C; $P < 0.05$, ANOVA test). Paired fEPSP–afSS stimulation with $\Delta t = 10$ ms only resulted in de-depression of the LTD of 70 ± 5% of baseline to 75 ± 5% of baseline when the mGluR5 receptor was blocked by MPEP ($n = 6$). Again, this LTD was not significantly different from that induced by LFS in control conditions (shown in Fig. 4B) ($P > 0.1$ one-way ANOVA test) but was significantly different from that in the presence of AP5 (Fig. 6D; $P < 0.05$, ANOVA test). These results strongly suggest that, at the LPP synapse, activation of the group I mGluR can cause a previously potentiated synapse to undergo de-potentiation and a depressed synapse to undergo de-depression. As Normann *et al.* (2000) reported that coactivation of mGluR5 receptors and N- and L-type VDCCs results in a spiking-timing-dependent LTD at CA1 synapses, we therefore tested whether the L-type VDCC was involved in the de-potentiation and de-depression reported here. As shown in Fig. 6E, paired afSS–fEPSP stimulation with $\Delta t = -10$ ms only resulted in de-potentiation of the LTP of 153 ± 8% of baseline induced by HFS to 150 ± 12% ($n = 5$) in the presence of 20 μM nimodipine, an L-type VDCC blocker. Similarly, paired fEPSP–afSS stimulation with $\Delta t = 10$ ms only resulted in de-depression of the LTD of 69 ± 6% of baseline induced by LFS to 71 ± 5% in the same conditions (Fig. 6F, $n = 5$). These results therefore support a role for L-type VDCCs in the de-potentiation and de-depression at LPP synapses.

At CA1 synapses, an NMDAR-independent form of LTD and de-potentiation can be induced by bath application of DHPG, a group I mGluR agonist (Huang & Hsu, 2001). We therefore compared the responses of CA1 and LLP synapses to short exposure to DHPG in both the native and depressed conditions. Consistent with previous reports (Faas *et al.*, 2002), we found that synaptic activity in the CA1 region was dramatically depressed immediately after bath application of DHPG, and only partially recovered following 30 min washout of DHPG, resulting in LTD of 56 ± 7% of baseline (Fig. 7A). In the case of the responses of LPP synapses to bath application of DHPG, no dramatic decrease in synaptic activity was seen during DHPG application but a gradually developing LTD was induced following 30 min washout of DHPG in six out of seven slices tested (Fig. 7A). The magnitude of the induced LTD was highly significant (91 ± 2% of baseline, $P < 0.01$, paired t-test), although much smaller than that induced at CA1 synapses. This DHPG-induced LTD could not be ascribed to the running down of fEPSP activity, as it was blocked by addition of MPEP (Fig. 7A). At CA1 synapses, the DHPG-induced LTD was independent of the NMDAR-dependent LTD induced by LFS in six out of six slices tested (Fig. 7B). However, application of DHPG resulted in de-depression of the LTD of 66 ± 4% of baseline induced by LFS to 87 ± 3% ($n = 6$, $P < 0.001$, paired t-test), or in de-depression of the LTD of 76 ± 3% of baseline induced by paired fEPSP–afSS stimulation with $\Delta t = -10$ ms to 93 ± 3% ($n = 6$, $P < 0.0005$, paired t-test). To examine whether the mGluR5 was involved in de-depression, we repeated the above experiments using a selective mGluR5 agonist, CHPG. Bath application of 1 mM CHPG resulted in de-depression of the LTDs of 74 ± 6 and 73 ± 5% of baseline induced, respectively, by LFS and paired afSS–fEPSP stimulation with $\Delta t = -10$ ms to 94 ± 6% ($n = 5$) and 95 ± 6% ($n = 5$) of baseline, showing that DHPG and CHPG had the same effect on de-depression. The pooled results for DHPG and CHPG are shown in Fig. 7C and D for LTD induced, respectively, by LFS or paired stimulation. These results are therefore consistent with de-depression of LPP synapses being dependent on mGluR5 activation. As a role of the mGluR5 in mediating reversal of LTP (or de-potentiation) has been reported at other synapses (Zho *et al.*, 2002), we tested whether it played a similar role at LPP synapses. Testing three slices using DHPG and four using CHPG, we again found no significant difference in the results obtained using these two drugs. The pooled results are shown in Fig. 7E and F for, respectively, the LTPs of 167 ± 13 and 138 ± 6% induced by HFS or paired stimulation; in both cases, activation of the mGluR5 by DHPG and CHPG resulted in de-potentiation of LTP to 117 ± 8% (Fig. 7E, paired t-test, $P < 0.01$) and 103 ± 7% (Fig. 7F, paired t-test, $P < 0.01$) of baseline, respectively.

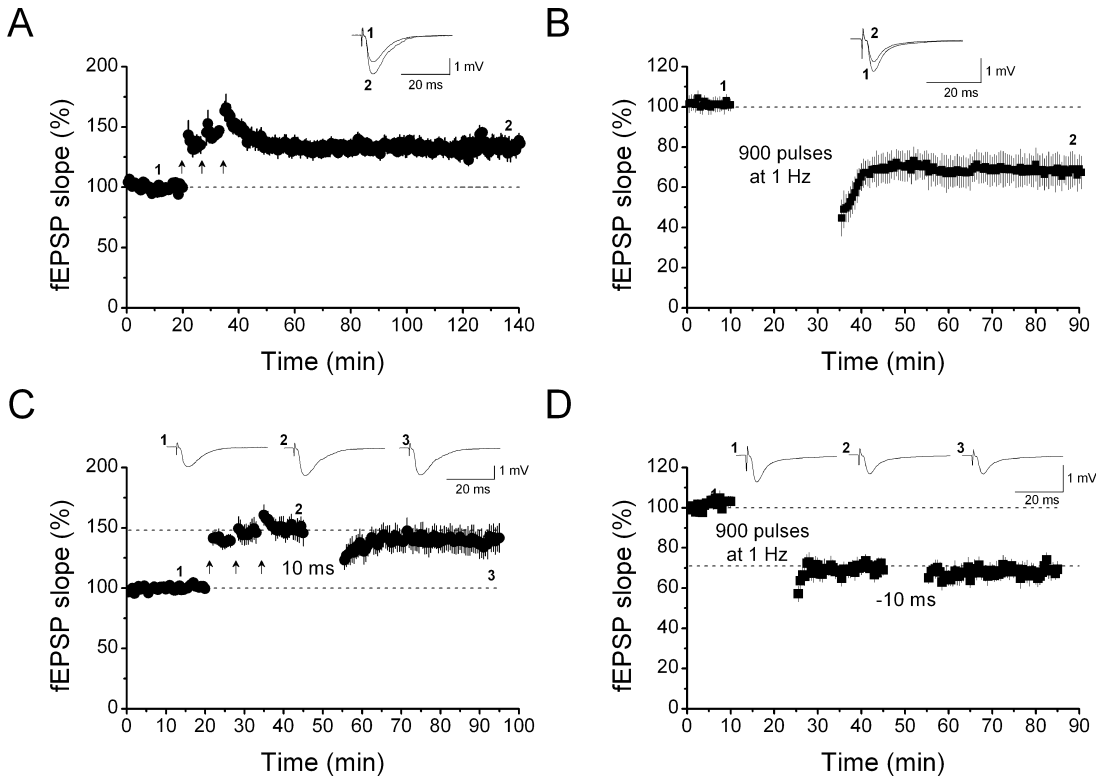


FIG. 4. Occlusion of spike-timing-dependent plasticity by saturated homosynaptic long-term potentiation (LTP) and long-term depression (LTD). (A) LTP induced by three trains of 100 pulses at 100 Hz (intertrain interval = 30 s), repeated three times at intervals of 5 min (see arrows) (LTP = 130 ± 5%, $n = 7$; $P < 0.005$, paired *t*-test). (B) LTD induced by stimulation with 900 pulses at 1 Hz (LTD = 68 ± 8%, $n = 7$; $P < 0.005$, paired *t*-test). (C) LTP induction by field excitatory postsynaptic potential (fEPSP)–antidromic field somatic spike (afSS) stimulation with $\Delta t = 10$ ms is occluded by induction of homosynaptic LTP (135 ± 6%, $n = 7$; $P < 0.001$, paired *t*-test). (D) LTD induction by afSS–fEPSP stimulation with $\Delta t = -10$ ms is occluded by homosynaptic LTD (67 ± 5%, $n = 7$; $P < 0.01$, paired *t*-test). The insets in A–D are raw synaptic responses averaged (10 sweeps) at the time epochs shown by the numbers on the recording.

Discussion

In the present study, we found that STDP could be induced at the LPP synapse, the estimated time constant for the timing interval between pre and post stimulation for induction of LTP and LTD being, respectively, 26 and 36 ms, both compatible with data for other synapses in the CNS (for review, see Bi & Poo, 2001). We also demonstrated that, at LPP synapses, induction of bidirectional STDP could be occluded when the synaptic strength was previously potentiated or depressed by HFS or LFS, respectively, suggesting that, at LPP synapses, induction of STDP and homosynaptic plasticity share some common molecular mechanisms (Roberson *et al.*, 1996; Impey *et al.*, 1999; Otmakhova *et al.*, 2000; Kemp & Bashir, 2001; Yang *et al.*, 2002). This argument was further supported by the finding that inhibitors of the NMDAR, CaMKII, PKC or MAP/ERK blocked STDP induction at LPP synapses (see also Lin *et al.*, 2003 for CA1 synapses).

In addition to inducing LTP and LTD at resting LPP synapses, paired fEPSP–afSS stimulation induced potentiation at synapses previously depressed by LFS, whereas paired afSS–fEPSP stimulation induced depression at synapses previously potentiated by HFS. The reversal of LTP by prolonged LFS, commonly referred to as de-potentiation, has been seen in many CNS synapses (O'Dell & Kandel, 1994; Wagner & Alger, 1996; Huang *et al.* 1999; Huang & Hsu, 2001). This phenomenon has been suggested to provide the mechanism for prevention of saturation of storage capacity in the neuronal network. Furthermore, it has recently been suggested to play an important role in refining newly formed neuronal connections in the developing visual cortex of *Xenopus* (Zhou *et al.*, 2003; Zhou & Poo, 2004) and in the hippocampus of animals subjected to a learning test (Xu *et al.*, 1998). As shown in these experiments using whole animal preparations, the most important factor for triggering de-potentiation at activated synapses is the spiking patterns of their postsynaptic neurons. As we have demonstrated that induction of homosynaptic plasticity and STDP share the same cellular mechanisms, our results also suggest that new potentiation (or depression) of synapses can be erased if the original temporal order of pre and post stimulation is reversed within, or is not maintained for, a short period of ~10 min after its induction. These results thus imply that STDP induced at conditioned synapses might provide another mechanism for refining newly formed neuronal circuits. De-potentiation and de-depression could not be induced when paired pre and post stimulation was applied ~1 h after induction of LTP and LTD (Fig. 5E and F), showing that both are time-dependent. This time-dependent nature of de-potentiation and de-depression is consistent with previous results for CA1 and CA3 synapses in hippocampal slices (Fujii *et al.*, 1991; Bashir & Collingridge, 1992; O'Dell & Kandel, 1994; Staubli & Chun, 1996; Huang *et al.*, 1999; Staubli & Scafidi, 1999; Chen *et al.*, 2001), synapses of retinal ganglion cells on tectal neurons in *Xenopus* (Zhou *et al.*, 2003) and at CA1 synapses in awake animals (Xu *et al.*, 1998); these studies defined more precisely a critical period of approximately 15 min during which activated synapses can be de-potentiated and after which the potentiated synaptic strength becomes stabilized.

Unlike bidirectional STDP induced at resting LPP synapses, which was found to be NMDAR-dependent (see Fig. 2A and B), spike-timing-dependent de-potentiation and de-depression are NMDAR-

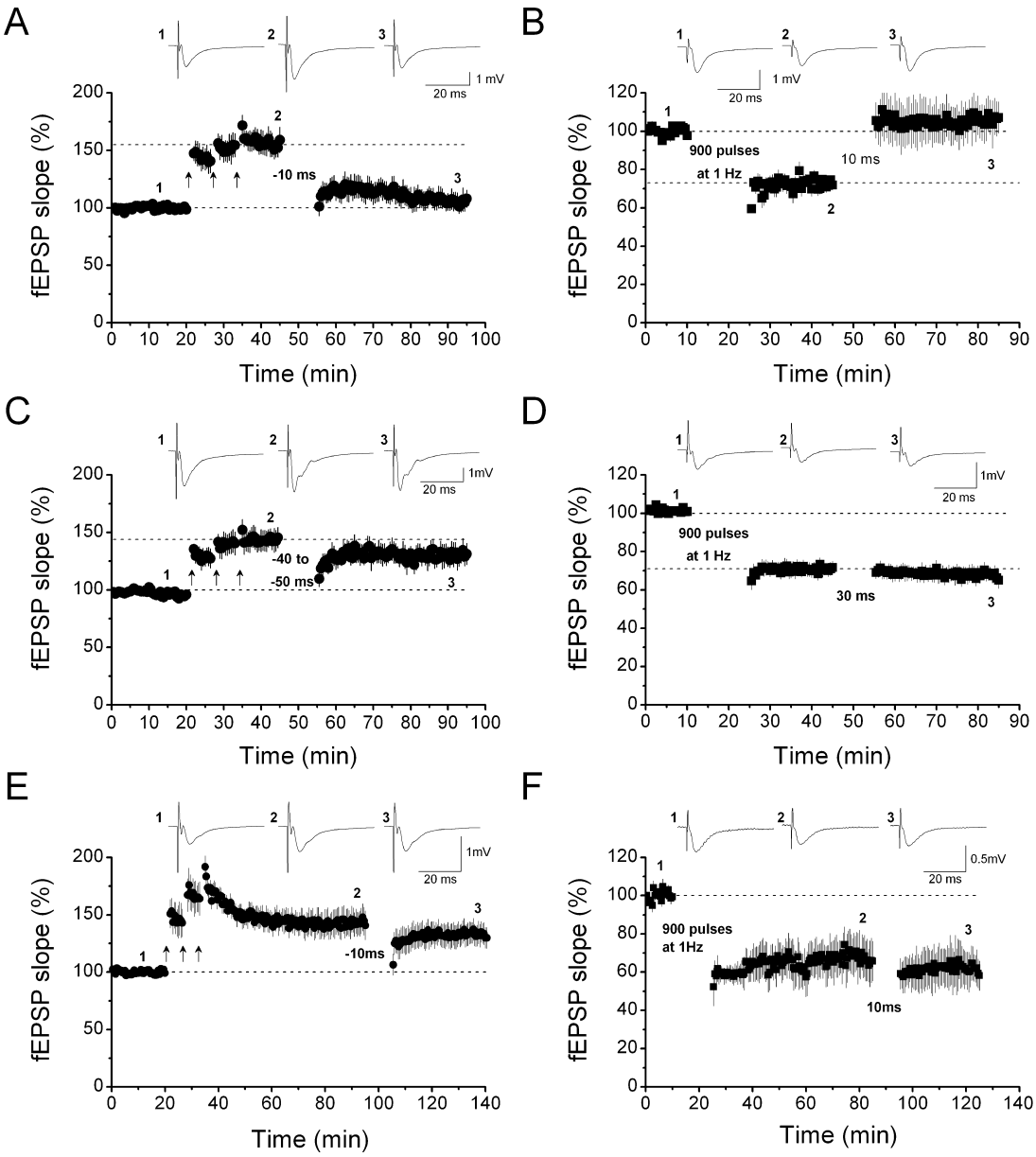


FIG. 5. Induction of de-potentiation and de-depression by paired pre/post stimulation and its time-dependent nature. (A) The homosynaptic long-term potentiation (LTP) of 152 ± 6% ($P < 0.005$, paired *t*-test) induced by high-frequency stimulation (HFS) and measured 10 min after application of the last train of HFS is reversed to 106 ± 8% of baseline 40 min after application of paired antidromic field somatic spike (afSS)–field excitatory postsynaptic potential (fEPSP) stimulation with $\Delta t = -10$ ms. The data are averaged from seven slices. (B) The homosynaptic long-term depression (LTD) of 74 ± 2% ($P < 0.005$, paired *t*-test) induced by low-frequency stimulation (LFS) and measured 20 min after application of LFS is reversed to 106 ± 10% of baseline 40 min after application of paired fEPSP–afSS stimulation with $\Delta t = 10$ ms. The data are averaged from six slices. (C) Reversal of homosynaptic LTP cannot be induced by paired afSS–fEPSP stimulation with $-\Delta t \geq 40$ ms (here $\Delta t = -50$ and the plot summarizes results from 7 experiments with $-\Delta t$ varying from 40 to ∼50 ms). The LTP before and after application of paired afSS–fEPSP stimulation was 138 ± 8 and 130 ± 6%, respectively ($P > 0.3$; paired *t*-test). The data are averaged from seven slices. (D) Reversal of homosynaptic LTD cannot be induced by paired fEPSP–afSS stimulation with $\Delta t > 30$ ms. The LTD before and after application of paired afSS–fEPSP stimulation was 72 ± 3 and 68 ± 4%, respectively ($n = 6$; $P > 0.4$ paired-*t*-test). (E) Reversal of homosynaptic LTP also failed when paired afSS–fEPSP stimulation with $\Delta t = -10$ ms was applied ∼1 h after LTP induction. The LTP before and after application of paired afSS–fEPSP stimulation was 143 ± 11 and 133 ± 9%, respectively ($P > 0.3$; paired *t*-test). The data are averaged from six slices. (F) Reversal of homosynaptic LTD fails when paired fEPSP–afSS stimulation with $\Delta t = 10$ ms was applied ∼1 h after LTD induction. The LTD before and after application of paired afSS–fEPSP stimulation was 66 ± 10 and 61 ± 9%, respectively ($P > 0.3$; paired *t*-test). The insets in A–F are raw synaptic responses averaged (10 sweeps) at the time epochs shown by the numbers on the recording.

independent but dependent on group I mGluR activation (see Fig. 6; also O'Mara *et al.*, 1995). Like the LTD induced by LFS at resting synapses, de-potentiation induced at CA1 synapses by prolonged LFS is also reported to be NMDAR-dependent (Fujii *et al.*, 1991; Holland & Wagner, 1998; Huang *et al.*, 2001; but see Bashir & Collingridge, 1994; Staubli & Chun, 1996). However, recent studies have reported a group I mGluR-mediated novel form of de-potentiation induced by LFS at several CNS synapses, including mossy fiber–CA3 synapses (Chen *et al.*, 2001), CA1 synapses (Bashir & Collingridge, 1994; Staubli & Chun, 1996) and perforant synapses in the dentate gyrus (O'Mara *et al.*, 1995). Furthermore, this form of de-potentiation of LTP can be pharmacologically induced by directly activating group I mGluRs using bath application of DHPG (Zho *et al.*, 2002). In fact, even at resting (or naive) CA1 synapses, activation of group I mGluRs

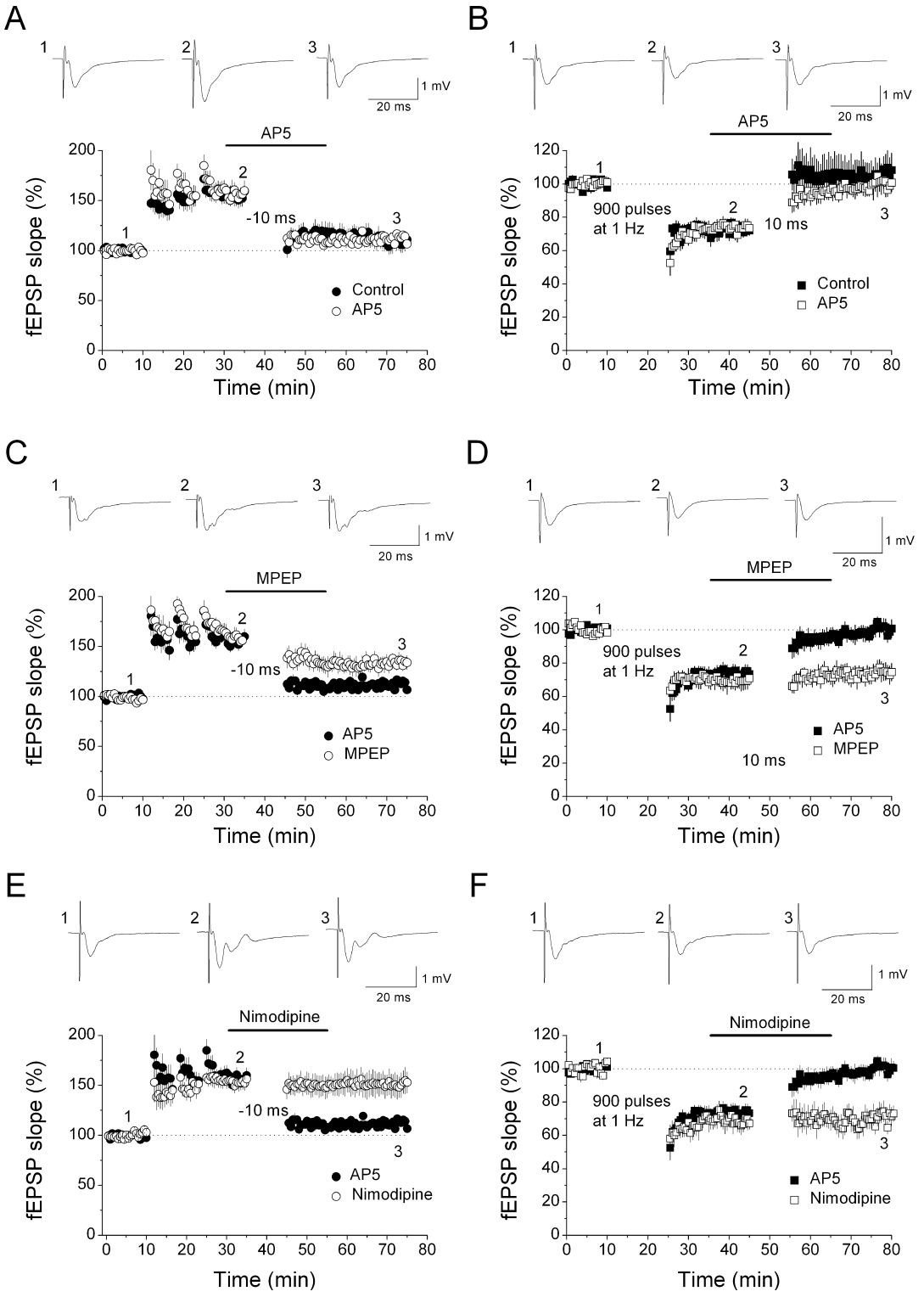


FIG. 6. De-potentiation and de-depression of long-term potentiation (LTP) and long-term depression (LTD) by paired pre and post stimulation is *N*-methyl-D-aspartate receptor-independent but group I metabotropic glutamate receptor- and L-type voltage-dependent calcium channel-dependent. (A and B) Reversal of LTP and LTD by paired antidromic field somatic spike (afSS)–field excitatory postsynaptic potential (fEPSP) and fEPSP–afSS stimulation with $\Delta t = -10$ ms and $\Delta t = 10$ ms, respectively, is unaffected by application of AP5 (○, □). The data are averaged from six slices. For comparison, the data from Fig. 5A and B are superimposed as the controls (●, ■). No significant difference was found between the two groups of data ($P > 0.3$ and 0.5, one-way ANOVA). (C and D) Reversal of LTP and LTD by paired afSS–fEPSP and fEPSP–afSS stimulation with $\Delta t = -10$ ms and $\Delta t = 10$ ms, respectively, is blocked by application of 2-methyl-6-(phenylethynyl) pyridine hydrochloride (MPEP) (○, □). The data are averaged from six slices. For comparison, the data from A and B are superimposed as controls (●, ■). A significant difference was found between the two groups of data ($P < 0.01$ for both, one-way ANOVA). (E and F) Reversal of LTP and LTD by paired afSS–fEPSP and fEPSP–afSS stimulation with $\Delta t = -10$ ms and $\Delta t = 10$ ms, respectively, is blocked by application of nimodipine (○, □). The data are averaged from five slices. For comparison, the data from A and B are superimposed as controls (●, ■). A significant difference was found between the two groups of data ($P < 0.01$ for both, one-way ANOVA). The insets in A–F are raw synaptic responses averaged at the points shown by the numbers on the recording.

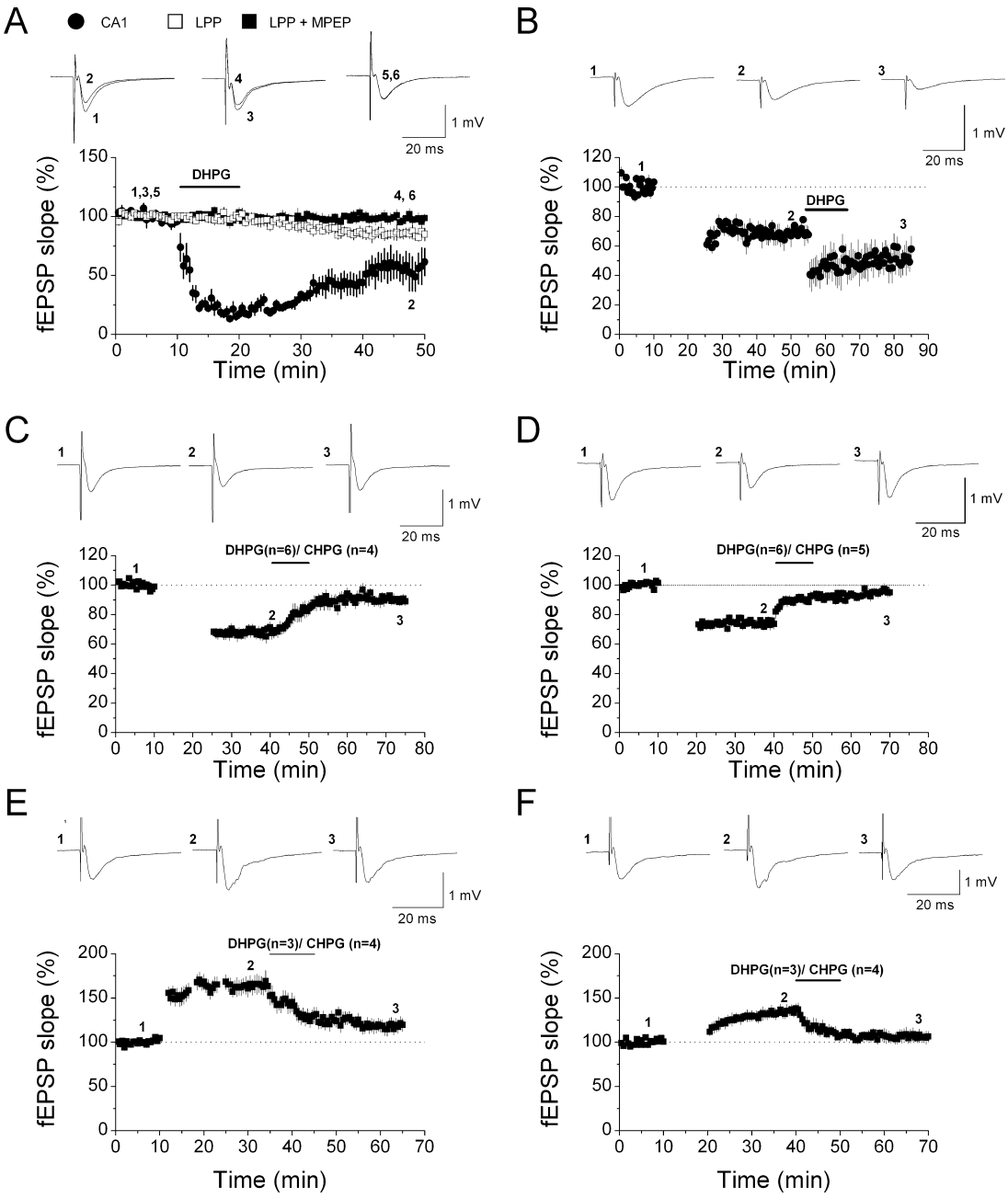


FIG. 7. Comparison of the responses at lateral perforant pathway (LPP) and CA1 synapses to short exposure to (S)-3,5-dihydroxy-phenylglycine (DHPG) and role of the metabotropic glutamate receptor 5 in de-depression and de-potentiation at LPP synapses. (A) The long-term depression (LTD) induced by bath application of 50 μM DHPG at LPP synapses (□) and CA1 synapses (●) is 91 ± 2% ($P < 0.01$, paired t-test) and 56 ± 7% ($P < 0.003$; paired t-test) of baseline, respectively. At LPP synapses, the DHPG-induced LTD was blocked by 5 μM 2-methyl-6-(phenylethynyl) pyridine hydrochloride (MPEP) [long-term potentiation (LTP) = 98 ± 3%, $n = 5$; ■ $P < 0.05$, one-way ANOVA test]. The data are the average of seven slices for control LPP synapses, five for MPEP-treated LPP synapses and six for CA1 synapses. Also note the dramatic difference in the time-course of the response to DHPG at the two different synapses. (B) Application of DHPG produces further LTD (51 ± 6% of baseline, as indicated by '1') at CA1 synapses after previous induction of homosynaptic LTD (71 ± 4% of baseline 1). The LTDs before and after DHPG application were significantly different ($P < 0.05$, paired t-test). Pooled DHPG and (RS)-2-chloro-5-hydroxyphenylglycine (CHPG) results showing that application of either drug reverses the LTD (i.e. produces de-depression) induced at LPP synapses either (C) by low-frequency stimulation (LTD = 69 ± 4% of baseline, $n = 6$ for DHPG and $n = 5$ for CHPG, $P < 0.001$, paired t-test) to 90 ± 3% of baseline or (D) by paired field excitatory postsynaptic potential (fEPSP)–antidromic field somatic spike (afSS) stimulation with $\Delta t = -10$ ms (LTD = 74 ± 2% of baseline, $n = 6$ for DHPG and $n = 6$ for CHPG, $P < 0.001$, paired t-test) to 96 ± 3% of baseline. The reversal was significant in both cases (paired t-test, $P < 0.01$ in both). Pooled DHPG and CHPG results showing that application of either drug reverses the LTP (i.e. produces de-potentiation) induced at LPP synapses either (E) by high-frequency stimulation (LTP = 167 ± 13% of baseline, $n = 3$ for DHPG and $n = 4$ for CHPG, $P < 0.001$, paired t-test) to 117 ± 8% of baseline or (F) by paired fEPSP–afSS stimulation with $\Delta t = -10$ ms (LTP = 138 ± 6% of baseline, $n = 3$ for DHPG and $n = 4$ for CHPG, $P < 0.001$, paired t-test) to 103 ± 7% of baseline. The reversal was significant in both cases (paired t-test, $P < 0.01$ for both). The insets in A–F are raw synaptic responses averaged at the time epochs shown by the numbers on the recording.

by bath application of DHPG results in a persistent decrease in synaptic transmission, i.e. DHPG-LTD (Bolshakov & Siegelbaum, 1994; Olieat *et al.*, 1997; Palmer *et al.*, 1997; Huber *et al.*, 2000). A detailed pharmacological study revealed that the DHPG-induced decrease in synaptic transmission consists of two distinct components (Faas *et al.*, 2002). The first is a dramatic decrease in synaptic transmission upon drug application; this effect can be quickly washed out, suggesting that it is completely drug-dependent. This component has been shown to be mediated by the mGluR1 (Faas *et al.*, 2002). The second component, which is mediated by the mGluR5, as its expression is blocked by MPEP, is a persistent decrease in synaptic efficacy even hours after drug wash out (Faas *et al.*, 2002). DHPG-LTD is independent of the NMDAR-dependent LTD induced by prolonged LFS, as application of DHPG can cause a further depression of synaptic strength after saturation of NMDAR-dependent LTD (Huber *et al.*, 2001). Furthermore, Huber *et al.* (2001) demonstrated that LTD analogous to DHPG-LTD can be electrically induced by prolonged paired-pulse stimulation at low frequency.

We confirmed the above-mentioned effect of group I mGluRs on synaptic transmission and plasticity at CA1 synapses in our recording system (Fig. 7A and B), and found some distinct features of LPP synapses in terms of the role of group I mGluRs in synaptic plasticity. Firstly, at resting LPP synapses, in response to bath application of DHPG, no significant decrease in synaptic transmission was seen, i.e. the first component of the DHPG-induced decrease in synaptic transmission, which is mediated by the mGluR1, was observed at CA1 synapses but not at LPP synapses. However, a small (compared with the CA1 synapse) but highly significant LTD could be induced after DHPG wash out (but see O'Leary & O'Connor, 1997, 1999 for comparison with medial perforant synapses). These results might suggest the absence of the mGluR1 and the presence of the mGluR5 in this area (see also Romano *et al.*, 1995; Shigemoto *et al.*, 1997; Rush *et al.*, 2002). Secondly, instead of causing further expression of DHPG-LTD, as reported at CA1 synapses (Huber *et al.*, 2001), activation of group I mGluRs at LPP synapses previously depressed by prolonged LFS resulted in potentiation (i.e. de-depression; see Fig. 7C and D). Interestingly, potentiation of synaptic transmission by (S)-DHPG has also been observed at resting perforant synapses in the dentate gyrus of immature rats (O'Leary & O'Connor, 1997, 1999). On the other hand, for de-potentiation of LTP, activation of group I mGluRs was required at both LPP and CA1 synapses. Moreover, we found no significant difference between the effect of DHPG and that of CHPG, a selective mGluR5 agonist, in mediating the de-potentiation and de-depression at LPP synapses, suggesting that both DHPG and CHPG mainly act on the mGluR5. Taken together, the above results suggest that activation of group I mGluRs plays different roles in modulating synaptic plasticity at LPP and CA1 synapses, although a previous study suggested that LPP synapses are more similar than medial perforant synapses to CA1 synapses in terms of many other physiological functions and in plasticity (Min *et al.*, 1998).

Group I mGluRs are coupled to phospholipase C (Conn & Pin, 1997; Schnabel *et al.*, 1999), so activation of the receptor results in an increase in the intracellular calcium concentration (Mannaioni *et al.*, 2001), which then activates the Ca^{2+}-dependent signaling pathway, leading to de-potentiation (or de-depression). It is therefore very likely that, at LPP synapses, the group I mGluR functions as a detector of correlated pre/postsynaptic activity, which triggers biochemical mechanisms resulting in de-potentiation (or de-depression) in potentiated (or depressed) conditions, similar to the role of the NMDAR in plasticity in the resting condition (Bear & Kirkwood, 1996; Bear, 1996). The fact that activation of the NMDAR is voltage-dependent makes it an ideal detector of coincidence of pre and postsynaptic activity. However, the mGluR lacks this property and therefore must cooperate with other voltage sensors to play a similar role to the NMDAR. The depolarization of the dendritic membrane caused by back propagating spikes is sufficient to activate N- and L-type VDCCs and cause influx of calcium into the cytoplasm (Normann *et al.*, 2000). It is therefore very likely that some Ca^{2+}-dependent isoforms of the PKC, which has been reported to be involved in mGluR-mediated LTD (Olieat *et al.*, 1997; Otani & Connor, 1998), might operate as coincidence detectors for pre and postsynaptic stimulation, onto which the activation of group I mGluR and VDCCs converge (Normann *et al.*, 2000). A similar mechanism might operate for the spike-timing-dependent de-potentiation and de-depression reported here at the LPP synapse, as their induction was inhibited by blocking the L-type VDCC or the mGluR5. As the induction of LTP and LTD at resting synapses and the induction of de-depression and de-potentiation at depressed and potentiated synapses in the present study were both produced by the same paired pre and post stimulation protocol, the glutamate concentration gradient in the extracellular space following presynaptic stimulation and the excitability of postsynaptic neurons following somatic stimulation would be very similar. This raises the fundamental question of what is the determining factor favoring NMDARs or group I mGluRs and VDCCs as the coincidence detector for plasticity at LPP synapses. The time-dependent nature of de-potentiation and de-depression suggests a possible candidate factor, namely the temporal change in molecular state before or after conditioning stimulation in the local dendritic spine area (see also Lee *et al.*, 2000). A recent study has provided evidence for regulation (phosphorylation) of the mGluR5 by NMDARs (Alagaisamy *et al.*, 2005). Activation of NMDARs at resting LPP synapses would therefore not only lead to expression of NMDAR-dependent LTP and LTD but also modify mGluR5 and VDCC function. The modified mGluR5s and VDCCs could then serve as detecting molecules for bidirectional plasticity at conditioned LPP synapses, and the turn-over of these modified mGluR5s and VDCCs would account for the time-dependent nature of de-potentiation and de-depression. However, the detailed signaling mechanism remains to be uncovered.

In conclusion, the results of the present study show that spike-timing-dependent bidirectional plasticity can occur at both resting and conditioned LPP synapses in the dentate gyrus. At resting synapses, the coincidence detector for STDP induction is the NMDAR, whereas at activated and depressed synapses, the coincidence detector is PKC isoforms, the activation of which requires the simultaneous activation of VDCCs and group I mGluRs.

Acknowledgements

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Abbreviations

aCSF, artificial cerebral spinal fluid; afSS, antidromic field somatic spike; AP5, D,L-2-amino-5-phosphonopentanoic acid; CaMKII, calcium–calmodulin kinase II; CHPG, (RS)-2-chloro-5-hydroxyphenylglycine; CNS, central nervous system; DHPG, (S)-3,5-dihydroxy-phenylglycine; ERK, extracellular signal-regulated kinase; fEPSP, field excitatory postsynaptic potential; HFS, high-frequency stimulation; LFS, low-frequency stimulation; LPP, lateral perforant pathway; LTD, long-term depression; LTP, long-term potentiation; MAPK, mitogen-activated protein kinase; mGluR, metabotropic glutamate receptor; MPEP, 2-methyl-6-(phenylethynyl) pyridine hydrochloride; NMDAR, *N*-methyl-D-aspartate receptor; PKC, protein kinase C; STDP, spike-timing-dependent plasticity; VDCC, voltage-dependent calcium channel.

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