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大鼠梨狀皮質之動作電位-時間差引導的突觸可塑特性研究

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Spike-timing-dependent plasticity (STDP) at resting and conditioned lateral perforant path synapses on granule cells in the dentate gyrus: different roles of NMDA and group I metabotropic glutamate receptors.

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摘要

本研究中，我們檢視在大鼠鋸齒腦迴之 lateral perforant pathway (LPP)神經路徑與顆粒細胞間突觸其『電位-時間差引導的突觸可塑特性 (Spike-timing-dependent plasticity; STDP)』機制. 我們使用海馬迴腦薄片為材料，並放置兩隻刺激電極於齒腦迴之分子層之外 1/3 處與顆粒細胞層下緣，以分別誘發 field EPSPs (fEPSPs) 與 antidromic field somatic spikes (afSSs). 藉由連續的 fEPSPs-afSSs 的配對刺激，我們可以成功的在 LPP 突觸引 ”發長期增益 (long-term potentiation; LTP)”. 倒轉 fEPSPs- afSSs 配對刺激的時間次序，則導致”長期抑制(long-term depression; LTD)”. 投與 NMDA 受器的抑制劑-AP5 能阻斷 LTP/ LTD 的引發，此外抑制 calcium-calmodulin kinase II, PKC，或 mitogen-activated / extracellular-signal regulated kinase，等酵素的活性，也能阻斷 LTP 的引發，顯示這些酵素訊息系統有參與 LTP 誘發的分子機制. 另一方面，抑制 PKC 與 protein phosphatase 2B，則能阻斷 LTP/ LTD 引發.

在經以高頻率刺激以誘發 LTP 或以低高頻率刺激以誘發 LTD 等經制約後的 LPP 突觸，配對 fEPSP-afSS 無法對經過”增益性制約”後的突觸有任何影響；而配對 afSS-fEPSP 刺激，則無法對經過”抑制性制約”後的突觸有任何影響. 然而在經過”抑制性制約”後的突觸施以配對 fEPSP-afSS 刺激，則能反轉”抑制性制約”；而同樣地，在經過”增益性制約後”的突觸以配對 afSS -fEPSP 刺激，則能反轉”增益性制約”. 此種”增益性制約”或”抑制性制約”的反轉與 NMDA 受器的活化無關，但能被 group I metabotropic glutamate receptor (mGluR)的抑制劑 MPEP 所阻斷，顯示此反轉效應與活化 group I mGluR 有關. 綜合本研究結果我們結論: STDP 可同時在休止狀態與制約後狀態下的 LPP 突觸引發，並且期引發過程中在休止狀態突觸須有 NMDA 受器的參與，而在制約後狀態下的 LPP 突觸則須 group I mGluR 受器的參與.

Abstract

We examined the mechanisms underlying STDP 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 the granule cells layer to evoke, respectively, field EPSPs (fEPSPs) and antidromic field somatic spikes (afSSs) in hippocampal slices. 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 LTD. Induction of LTP/LTD was blocked by AP5, showing that both effects are NMDA receptor (NMDA-R)-dependent. Induction of LTP was also blocked by inhibitors of calcium-calmodulin kinase II, PKC, or mitogen-activated / extracellular-signal regulated kinase, suggesting these are downstream effectors after NMDA-R activation, while induction of LTD was blocked by inhibitors of PKC and protein phosphatase 2B.

At LPP synapses previously potentiated or depressed by, respectively, high-frequency or low-frequency stimulation, paired fEPSP-afSS stimulation resulted in "de-depression" at depressed LPP synapses, but had no effect on potentiated synapses, while reversing 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 MPEP, a group I metabotropic glutamate receptor (mGluR) blocker, showing that the both are NMDA-R-independent, but group I mGR-dependent. In conclusion, our results show that STDP can occur at both resting and conditioned LPP synapses, its induction in the former case being NMDA-R-dependent and, in the latter, group I mGluR-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 and 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 in the CNS, including the hippocampus (Magee & Johnston, 1997; Markram et al, 1997; Lin et al., 2003), visual cortex (Froemke & Dan, 2002), and sensory cortex (Feldman, 2000), and in cultured neurons (Bi and Poo, 1998; Debanne et al,.1998); this phenomenon is referred to as spike-timing-dependent-plasticity (STDP, for review see, Bi & Poo; 2001; Dan and Poo, 2004). STDP is bi-directional, i.e. synaptic efficacy can be either potentiated or depressed by paired pre/postsynaptic spiking, depending both on the timing interval and the temporal order of the pre/postsynaptic spiking. In general, to induce STDP, the timing interval between paired pre/postsynaptic spiking has to be less than tens of millisecond, whereas a significant 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/post spiking, repeated paired pre/post spiking results in LTP if presynaptic stimulation precedes postsynaptic stimulation, but results in LTD if the temporal order of pre/post spiking is reversed. STDP provides a good explanation for synaptic modifications that are dependent on correlative activity between pre- and post-synaptic neurons, and are considered as the cellular mechanism underlying learning/memory and the activity-driven 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 STDP induction and expression are not fully understood. For example, although it has been reported that

the induction of both spike-timing-dependent LTP and LTD requires activation of the N-methyl-D-aspartate (NMDA) subtype of the glutamate receptor (NMDA-R) (Bi and Poo, 1998; Magee & Johnston, 1997; Markram et al, 1997; Lin et al., 2003; Feldman, 2000), some reports have suggested that, in LTD induction, voltage-dependent calcium channels (Bi & Poo, 1998) or internal calcium sources (Nishiyama et al., 2000) are involved in addition to the NMDA-R. Furthermore, it remains unclear whether signal effectors acting downstream of the NMDA-R, including calcium-calmodulin kinase II (CaMKII), protein kinase C (PKC), and mitogen-activated / extracellular-signal regulated kinase (MAP/ERK), which have been reported to be involved in induction of homosynaptic LTP/LTD (Roberson et al., 1996; Otmakhova et al., 2000; Impey et al., 1999; Yang et al., 2002), are also involved in spike-timing-dependent LTP/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 where temporal specificity of associative synaptic modification was first reported in vivo (Levey & Steward; 1983), the cellular mechanisms for induction of homosynaptic LTP/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 ^{0}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 ^{0}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 04008 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. 1A1). The first stimulating electrode was used to elicit field excitatory postsynaptic potentials (fEPSP); only activity that showed paired-pulsed (50 ms inter-pulse 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 (afSS) in granule cells. Stable baseline fEPSP activity of the LPP was elicited by stimulation every 30 seconds for at least 10 minutes. Bidirectional plasticity of the LPP was then induced by pairing fEPSP and afSS stimulation at 6 second intervals for 10 minutes. During the pairing protocol, the delivery of fEPSP stimulation preceded, or followed, that of afSS stimulation by several tens of milliseconds. Baseline fEPSP activity of the LPP was again elicited every 30 seconds for another 30 minutes. All signals were filtered at 2 kHz by a low pass Bessel filter provided by the amplifier (Axopatch-1D, Axon Instruments, Foster City, CA 94404 USA), and digitized at 5 kHz using CED micro 1401 interface running Signal software (Cambridge Electronic Design, Cambridge, UK). All

drugs were bath applied and were purchased from Sigma (St. Louis, MO 63178 USA), except for D,L-2-amino-5- phosphonopentanoic acid (AP5), chelerythrine, (S)-3,5-dihydroxyphenylglycine (DHPG), H-7, 2-methyl-6- (phenylethynyl) pyridine hydrochloride (MPEP), PD98059, and U0126, which were from Tocris-Cookson (Bristol, UK). 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 comparisons. All data are presented as the mean ± standard error, 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. 1A1, a synaptic response (Fig.1A2, upper left trace), confirmed by its sensitivity to bath application of 10 μM DNQX, was elicited by the stimulating electrode placed 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. 1A2, upper middle trace), and this was completely blocked by 1 μM TTX (Fig. 1A2, 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 placed 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. 1A2, lower traces), suggesting 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) /post (granule cell body) stimulation and record it using an extracellular field potential recording technique. Following recording of a stable fEPSP by stimulation every 30 sec for at least 10 minutes, LTP of fEPSP could be induced by paired fEPSP/afSS stimulation, repeated 100 times at 6 sec intervals with the evoking of fEPSP preceding that of afSS (see insets in Fig. 1B). The success rate for LTP induction depended on the timing interval between the evoking of the fEPSP and the afSS. Paired fEPSP-afSS stimulation with a timing interval (Δt) <30 ms resulted in LTP in all 9 slices tested. The average magnitude of the LTP 25-30 min later was 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 8 out of 9 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 (Figs. 1C, E); in the text hereafter, the Δt is shown with a negative sign for this pairing protocol. Again, the success rate for LTD induction

depended on the value of Δt. Significant synaptic depression was induced in 8 out of 9 slices tested by paired afSS-fEPSP stimulation with (Δt) $\leq$ $|-40|$ ms. The average magnitude of the LTD was 78±10% of baseline (Fig. 1C). However, paired afSS-fEPSP stimulation with (Δt) $>|-40|$ ms resulted in no significant change in synaptic strength in 6 out of 6 slices tested (LTD = 96±3% of baseline; Fig. 1E). Figure 1F and 1G show the relationship between the resultant 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):

$$F(\Delta t)=100\%\pm A\cdot e^{-\Delta t/\tau},$$

where $F(\Delta t)$ is the synaptic potentiation/depression induced by paired fEPSP-afSS/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), while 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 62% and 25.6 ms, respectively (Fig. 1F), the corresponding values for the depression induced by paired afSS-fEPSP stimulation being 50% and 36.3 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 7 out of 7 slices tested using this pairing protocol (LTP = 148±15% of baseline), but no induction of LTP was seen in 8 out of 8 slices tested in the presence of 50 μM AP5, an NMDA-R antagonist (LTP = 105±6% of baseline). Induction of LTP also failed in 6 out of 6 slices tested in the presence of 20 μM KN-93, a CaMKII inhibitor (LTP = 100±2% of baseline; Fig. 2B, white circles), but was seen in 6 out of 6 slices tested when 20 μM KN-92, an inactive analogue of KN-93, was used (LTP = 135±6% of baseline; Fig. 2B, black circles). LTP induction was also blocked in 6 out of 6 slices pretreated with

10 μM H-7, a protein kinase inhibitor, (LTP = 104±4% of baseline; Fig. 2C) and in 7 out of 7 perfused with 3 μM chelerythrine, a PKC inhibitor (LTP = 105±7% of baseline; Fig. 2D) Finally, LTP induction also failed in 6 out of 6 slices that were pretreated either with 50 μM PD98059, a MAP inhibitor, (LTP = 103±3% of baseline; Fig. 2E) or 20 μM U0126, an ERK inhibitor (LTP = 100±3% of baseline; Fig. 2F), suggesting that MAP/ERK kinase are downstream effectors acting after activation of PKC, which is activated following activation of NMDA-R / CaMKII during paired fEPSP-afSS stimulation.

To examine LTD induction, we used paired afSS-fEPSP stimulation with a Δt = -10 ms. As shown in Fig. 3A (black squares), this stimulation protocol resulted in LTD at LPP synapses in 7 out of 7 slices tested (LTD = 71±4% of baseline). In the presence of 50 μM AP5, LTD induction failed in 7 out of 7 slices (LTD = 98±2% of baseline; Fig. 3A, white squares). Inhibition of PKC activity by perfusion of slices with chelerythrine blocked LTD induction in 6 out of 6 slices tested. In addition, perfusion of slices with aCSF containing 10 μM cypermethrin, an inhibitor of protein phosphatase 2B (PP2B), also blocked LTD induction in 7 out of 7 slices. Taken together, the above results show that LTD induction using the pairing protocol in this study is NMDA R-dependent and involves the PKC-PP2B signaling pathway.

Since it is well known that the CaMKII/PKC/MAP/ERK pathway and PKC/PP2B pathway are involved, respectively, in induction of homosynaptic LTP and LTD (Kemp & Bashir, 2001; Roberson et al., 1996; Otmakhova et al., 2000; Impey et al., 1999; Yang et al., 2002), our results suggested that the LTP/LTD induced by paired pre/post stimulation might share some cellular mechanisms with homosynaptic LTP/LTD induced by HFS/LFS. To determine if this were the case, we tested whether induction of LTP/LTD by HFS/LFS could occlude the induction of LTP/LTD using paired pre/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 3 trains of HFS repeated

3 times at 5 minutes interval 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 could be induced by paired fEPSP-afSS stimulation with Δt = 10 ms (Fig. 4C; LTP = 135 ±6% of baseline). Similarly, following depression of LPP by LFS, no further synaptic depression could be induced by paired afSS-fEPSP stimulation with Δt = -10 ms (Fig. 4D; LTD = 67±5% of baseline). These results are therefore consistent with our argument that the LTP/LTD induced by paired pre/post stimulation shares common cellular mechanisms with the homosynaptic LTP/LTD induced by HFS/LFS.

In addition to inducing LTP and LTD at resting synapses, paired pre/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 Δt = -10 ms reversed the homosynaptic LTP induced by HFS to almost the baseline level in 6 out of 6 slices tested (de-potentiation = 106 ±8% of baseline). In addition, paired fEPSP-afSS stimulation with Δt = 10 ms reversed the LTD induced by prolonged LFS to the baseline level in 6 out of 6 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 7 out 7 slices tested (de-potentiation = 130 ±6% of baseline; Fig. 5C), and paired fEPSP-afSS stimulation with Δt >30 ms resulted in no de-depression of homosynaptic LTD in 6 out of 6 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 & Co Collingridge, 1992; O'Dell & Kandel, 1994; Staubli & Chun, 1996; Staubli & Scafidi, 1999; Huang et al., 1999; Chen et al., 2001). We therefore tested whether the same property was seen in our system. As shown in Fig. 5E, paired afSS-fEPSP stimulation with Δt = -10 ms applied 1 hour after induction of homosynaptic LTP by HFS did not result in de-potentiation (142 ±10% of

baseline) and similar results were obtained for de-depression (Fig. 5F; de-depression = 66 ± 10 % of baseline). These results show that there is also 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 Figure 6A, de-potentiation was induced in 6 out of 6 slices tested in the presence of 50 μM AP5 (de-potentiation = 116 ± 4 % of baseline), as was de-depression (6 out of 6 slices; de-depression = 106 ±2% of baseline), showing that both processes were NMDA-R-independent. Interestedly, induction of both de-depression and de-potentiation was blocked by bath application of MPEP, a group I mGluR antagonist (Fig. 6 C, D). The above results therefore 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. At CA1 synapses, an NMDA-R 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 condition. 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 minutes washout of DHPG, resulting in LTD of 56±7% of baseline (Fig. 7A, black circles). 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 minutes washout of DHPG in 6 out of 7 slices tested (Fig. 7A, white squares). 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. At CA1 synapses, the DHPG-induced LTD was independent of the NMDA-R-dependent LTD induced by LFS in 6 out of 6 slices tested (Fig. 7B). However, bath application of DHPG resulted in

de-depression at LPP synapses previously depressed by LFS (Fig. 7C) or by paired afSS-fEPSP stimulation with Δt = 10 ms (Fig. 7D). These results are therefore consistent with the results that de-depression of LPP synapses is dependent on activation of mGluR5.

Discussion

In the present study, we found that spike-timing-dependent plasticity could be induced at the LPP synapse; the estimated time constant for the timing interval between pre/post stimulation for induction of LTP was 25.6 ms and that for induction of LTD 36.3 ms, both compatible with data for other synapses in the CNS (for review, see Bi and Poo, 2001). We also demonstrated that, at LPP synapses, induction of bidirectional STDP could be occluded when the synaptic strength was previously potentiated/depressed by FFS/LFS, suggesting that, at LPP synapses, induction of STDP and homosynaptic plasticity share some molecular mechanisms (Kemp & Bashir, 2001; Roberson et al., 1996; Otmakhova et al., 2000; Impey et al., 1999; Yang et al., 2002). This argument was further supported by the finding that inhibitors of NMDA-R, 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/LTD at resting LPP synapses, paired fEPSP-afSS stimulation induced potentiation at synapses previously depressed by LFS, and paired afSS-fEPSP stimulation induced depression at synapses previously potentiated by HFS. The reversal of LTP by prolonged LFS is commonly referred to as de-potentiation, and has been found in many CNS synapses (O'Dell and Kandel, 1994; Wagner & Alger, 1996; Huang et al., 199; 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. Since we have demonstrated that induction of homosynaptic plasticity and STDP may share the same cellular mechanisms, our results also suggest that new potentiation/depression of synapses can be erased if the original temporal order of pre/post stimulation is reversed within, or is not

maintained for, a short period of ~10 minutes 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/post stimulation was applied 1 hr after induction of LTP/LTD (Fig. 5E and F), suggesting 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 & Co Collingridge, 1992; O'Dell & Kandel, 1994; Staubli & Chun, 1996; Staubli & Scafidi, 1999; Huang et al., 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 minutes 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 NMDA-R-dependent (see Fig. 2A and B), spike-timing-dependent de-potentiation and de-depression appeared to be NMDA-R-independent, but dependent on group I mGluR activation (see Fig. 6; also O'Mara et al., 1995). Like LTD induced by LFS at resting synapses, de-potentiation at CA1 synapses induced by prolonged LFS is also reported to be NMDA-R-dependent (Fujii et al., 1991; Holland & Wagner, 1998; Huang, 2001; but see Bashir & Collingridge, 1994; Staubli & Chun, 1996). However, a growing number of 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., 201), 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 naïve) CA1 synapses, activation of group I mGluRs by bath application of DHPG results in a persistent decrease in synaptic transmission, i.e. DHPG-LTD (Bolshakov & Siegelbaum, 1994; Oliet 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, and this effect can be quickly washed out, suggesting it is completely drug-dependent; this component has been shown to be mediated by the mGluR 1 subunit (Faas et al., 2001). The second component, which is mediated by the mGluR 5 subunit, since 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 NMDA-R-dependent LTD induced by prolonged LFS, as application of DHPG can cause a further depression of synaptic strength after saturation of NMDA-R-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 (see Fig.7 A&B), and found some distinct features of LPP synapses regarding the role of group I mGluR 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 mGluR 1 receptor mediating the first component of the DHPG-induced decrease in synaptic transmission at CA1 synapses was not be seen at LPP synapses. However, a small (compared to 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 mGluR 1 and the presence of mGluR 5 receptors in this area (see also Rush et al., 2002; Romano et al., 1995; Shigemoto et al., 1997). 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. 7 C and D). Interestedly, 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 is required at both LPP and CA1 synapses. Taken together, the above results suggest that activation of group I mGluRs plays different roles in modulating synaptic plasticity at LPP and CA1 synapses, though a previous study suggested that LPP synapses are more similar to CA1 synapses than medial perforant synapses are 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 intercellular calcium concentration (Mannaioni et al., 2001), which then activates the Ca^{++}-dependent signaling pathway, leading to de-potentiation/ 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/de-depression in potentiated/depressed conditions, similar to the role of the NMDA-R in plasticity in the resting condition (Bear, 1996; Bear & Kirkwood, 1996). Since induction of LTP/LTD at resting synapses and induction of de-depression/de-potentiation at depressed/potentiated synapses in the present study were both produced by the same paired pre/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 NMDA-R or group I metabotropic receptors as the detecting molecule 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/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 mGluR 5 subunit by NMDA-Rs (Alagaisamy et al., 2005). Activation of NMDA-Rs at resting LPP synapses would therefore not only lead to expression of NMDA-R-dependent LTP/LTD, but also modify mGluR 5

receptor function. The modified mGluR receptors could then serve as detecting molecules for bidirectional plasticity at conditioned LPP synapses, and the turn-over of these modified mGluR 5 receptors 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 and that the detecting molecule for STDP induction at resting synapses is the NMDA-R and that at activated/depressed synapses the group I mGluR.

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Figure legends

Figure 1. LTP/LTD induced by paired fEPSP-afSS/afSS-fEPSP stimulation at LPP synapses. *A.* Arrangement of the recording (***Rec***.) and stimulating (***Sti. 1*** & ***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 Δt = 15 ms. The lower plot shows the summarized results for 9 experiments, in which the Δt for the paired fEPSP-afSS stimulation was < 30 ms. (LTP=144±4%; p=0.007, 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 the Δt = -25 ms (see middle inset). The lower plot shows the summarized results for 8 experiments, in which the Δt for the paired fEPSP-afSS stimulation was < $\left|-40\right|$ ms. (LTD=78 ± 10 %; p = 0.009, paired t test). *D* & *E*. No significant LTP or LTD was induced by paired fEPSP-afSS/afSS-fEPSP stimulation when the Δt >30ms or > $\left|-40\right|$ ms, respectively. (LTP=98 ± 9 %, n = 9; p = 0.34, paired t test; LTD = 96 ± 3 %, n = 7; p = 0.84, paired t test). *F* & *G*. 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.

Figure 2. Cellular mechanisms underlying LTP induction. *A.* Plot showing the LTP induced using paired fEPSP-afSS stimulation with the Δt = 10 ms under control conditions (black circles) (LTP= 148 ±5 %, n= 7; p = 0.006, paired t test) or after bath application of 50 μM AP5 (white circles) (LTP= 105 ± 6 %, n= 8; p = 0.52, paired t test). The insets show averaged fEPSP activity at baseline (10 sweeps) or at 30 minutes after the end of the pairing protocol (10 sweeps). *B.* Induction of LTP is blocked by the CaMKII inhibitor, KN-93, (white circles) (LTP= 100 ± 2 %, n= 7; p= 0.87, paired t test), but not by KN-92, the inactive analogue of KN-93O (black circles) (LTP= 135 ± 6 %, n = 6; p = 0.0086, paired t test). The insets show averaged fEPSP activity at baseline (10 sweeps) and at 30

minutes after the end of the pairing protocol (10 sweeps). *C*. Induction of LTP is blocked by the protein kinase inhibitor, H7, (LTP= 104 ± 4 %, n= 6; p = 0.34, paired t test). *D*. Induction of LTP is blocked by the PKC inhibitor, chelerythrine (LTP= 105 ± 7 %, n = 7; p = 0.5, paired t test). *E*. Induction of LTP is blocked by the MAP inhibitor, PD98059 (LTP= 103 ± 3 %, n = 6; p = 0.41, paired t test). *F*. Induction of LTP is blocked by the ERK 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 minutes after the end of the pairing protocol (10 sweeps).

Figure 3. Cellular mechanisms underlying induction of LTD. *A.* Induction of LTD by paired afSS-fEPSP stimulation with Δt = -10 ms (black squares; LTD= 71 ± 4 %, n = 7; p = 0.004, paired t test) and its blockade by AP5 (white squares; LTP= 98 ± 2 %, n = 7; p = 0.37, paired t test). *B*. Induction of LTD is blocked by chelerythrine (LTD = 97 ± 3 %, n = 6; p = 0.29, 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 minutes after the end of the pairing protocol (10 sweeps).

Figure 4. Occlusion of STDP by saturated homosynaptic LTP/LTD. *A.* LTP induced by 3 trains of 100 pulses at 100 Hz (inter-train interval = 30 s), repeated 3 times at intervals of 5 minutes (see arrows) (LTP= 130 ± 5 %, n = 7; p = 0.001, paired t test). *B*. LTD induced by stimulation with 900 pulses at 1 Hz (LTD= 68 ± 8 %, n= 7; p = 0.002, paired t test). *C.* LTP induction by fEPSP-afSS stimulation with Δt = 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 Δt = -10 ms is occluded by homosynaptic LTD (67 ± 5 %, n = 7; p < 0.01, 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.

Figure 5. Induction of de-potentiation and de-depression by paired pre/post stimulation and its

time-dependent nature. A. The homosynaptic LTP of 152 ± 6 % ($p < 0.005$, paired t test) induced by HFS and measured 10 minutes after application of the last train of HFS is reversed to 106 ± 8 % of baseline 40 minutes after application of paired afSS-fEPSP stimulation with Δt = -10 ms. The data are averaged from 7 slices. *B*. The homosynaptic LTD of 74 ± 2 % ($p < 0.005$, paired t test) induced by LFS and measured 20 minutes after application of LFS is reversed to 106 ± 8 % of baseline 40 minutes after application of paired fEPSP-afSS stimulation with Δt = 10 ms. The data are averaged from 7 slices. *C*. Reversal of homosynaptic LTP cannot be induced by paired afSS-fEPSP stimulation with $\Delta t > |-40|$ ms. The LTP before and after application of paired afSS-fEPSP stimulation was 138 ± 6 % and 130 ± 8 %, respectively ($p > 0.3$; paired t test). The data are averaged from 6 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 ($p > 0.4$ paired-t test). E. Reversal of homosynaptic LTP also failed when paired afSS-fEPSP stimulation with Δt = -10 ms was applied 1 hour after LTP induction. The LTP before and after application of paired afSS-fEPSP stimulation was 143 ± 11 % and 133 ± 9 %, respectively ($p > 0.1$; paired t test). The data are averaged from 6 slices. F. Reversal of homosynaptic LTD fails when paired fEPSP-afSS stimulation with Δt = 10 ms is applied 1 hour after LTD induction. The LTD before and after application of paired afSS-fEPSP stimulation was 66 ± 10 % and 61 ± 9 %, respectively ($p > 0.1$; 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.

Figure 6. De-potentiation/de-depression of LTP/LTD by paired pre/post stimulation is NMDA-R-independent, but group I mGluR-dependent. *A/B*. Reversal of LTP/LTD by paired afSS-fEPSP/fEPSP-afSS stimulation with Δt = -10/10 ms is unaffected by application of AP5 (white circles/squares). The data are averaged from 6 slices. For comparison, the data from Fig. 5A/B are superimposed as the control (black circles/squares). No significant difference was found

between the two groups of data (p = 0.27 / 0.52, one way ANOVA). *C/D*. Reversal of LTP/LTD by paired afSS-fEPSP/fEPSP-afSS stimulation with Δt = -10/10 ms is blocked by application of MPEP (white circles/squares). The data are averaged from 6 slices. For comparison, the data from A/B. are superimposed as controls (black circles/squares). A significant difference was found between the two groups of data (p <0.008 / 0.007, one way ANOVA). The insets in *A-D* are raw synaptic responses averaged at the points shown by the numbers on the recording.

Figure 7. Comparison of the responses at LPP and CA1 synapses to short exposure to DHPGOK??. *A*. The LTD induced by bath application of 50 μM DHPG at LPP synapses (white squares) and CA1 synapses (black circles) is 91 ± 2 % (p < 0.01, paired t test) and 57 ± 6 % (p < 0.003; paired t test) of baseline, respectively. The data are averaged for 7 slices for LPP synapses and for 6 slices for CA1 synapses. 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 even 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). *C/D*. Application of DHPG reverses the LTD (i.e. produces de-depression) induced by LFS (C, LTD = 66 ± 4 % of baseline, n = 6, p < 0.0005, paired t test) or paired fEPSP-afSS stimulation with Δt = -10 ms (D, LTD = 76 ± 3 %of baseline, n = 6, p < 0.0005, paired t test) to 87 ± 3 % and 93 ± 3 % of baseline, respectively at LPP synapses. The reversal was significant in both cases (p < 0.005 and < 0.005, respectively). The insets in *A-D* are raw synaptic responses averaged at the time epochs shown by the numbers on the recording.

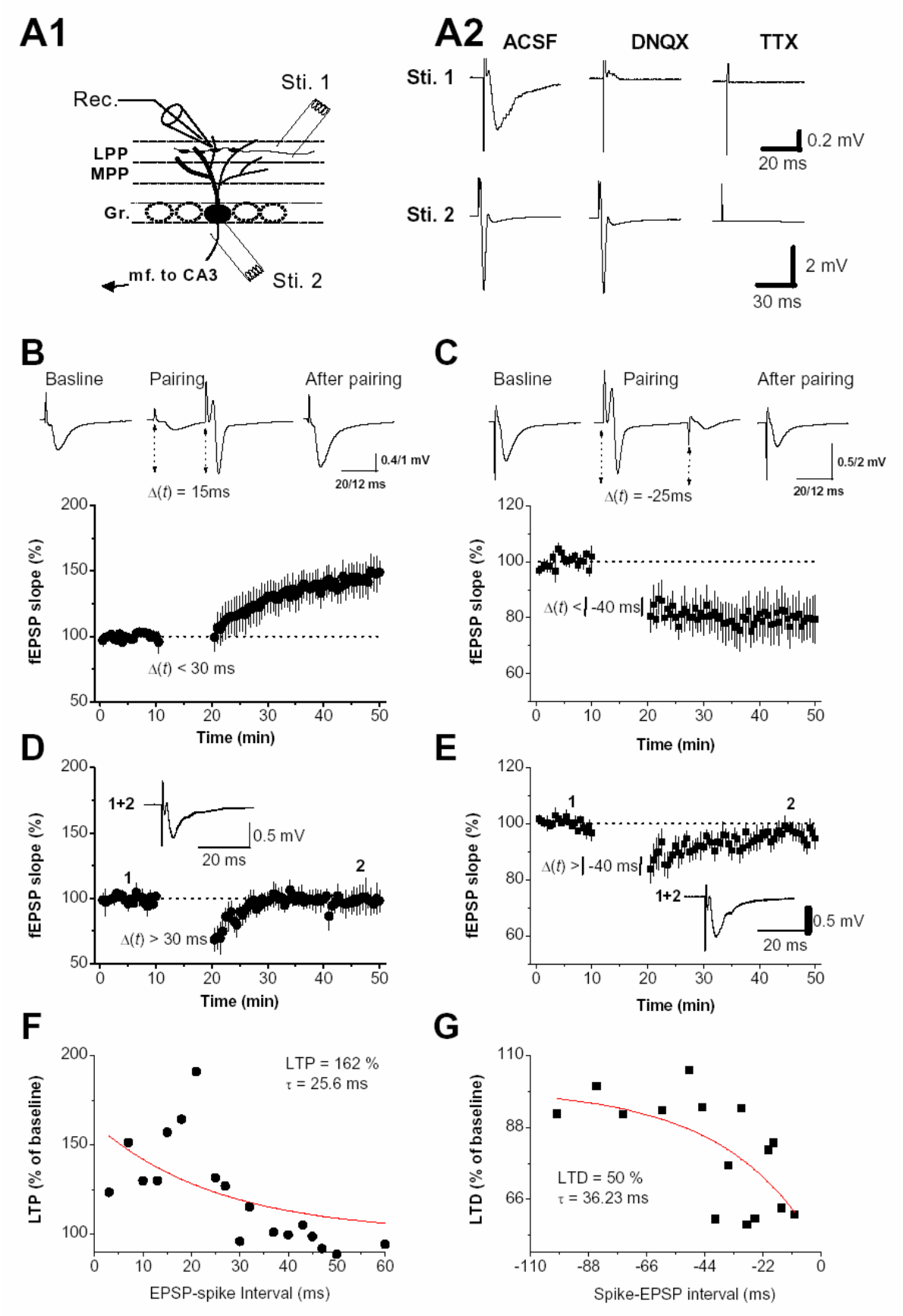
A1
Rec.
Sti. 1
LPP
MPP
Gr.
mf. to CA3
Sti. 2
A2
ACSF
DNQX
TTX
Sti. 1
0.2 mV
20 ms
Sti. 2
2 mV
30 ms
B
Basline
Pairing
After pairing
0.4/1 mV
20/12 ms
Δ(t) = 15ms
fEPSP slope (%)
Δ(t) < 30 ms
Time (min)
C
Basline
Pairing
After pairing
0.5/2 mV
20/12 ms
Δ(t) = -25ms
fEPSP slope (%)
Δ(t) < -40 ms
Time (min)
D
1+2
0.5 mV
20 ms
1
2
fEPSP slope (%)
Δ(t) > 30 ms
Time (min)
E
1
2
fEPSP slope (%)
Δ(t) > -40 ms
1+2
0.5 mV
20 ms
Time (min)
F
LTP (% of baseline)
LTP = 162 %
τ = 25.6 ms
EPSP-spike Interval (ms)
G
LTD (% of baseline)
LTD = 50 %
τ = 36.23 ms
Spike-EPSP interval (ms)

Fig. 1

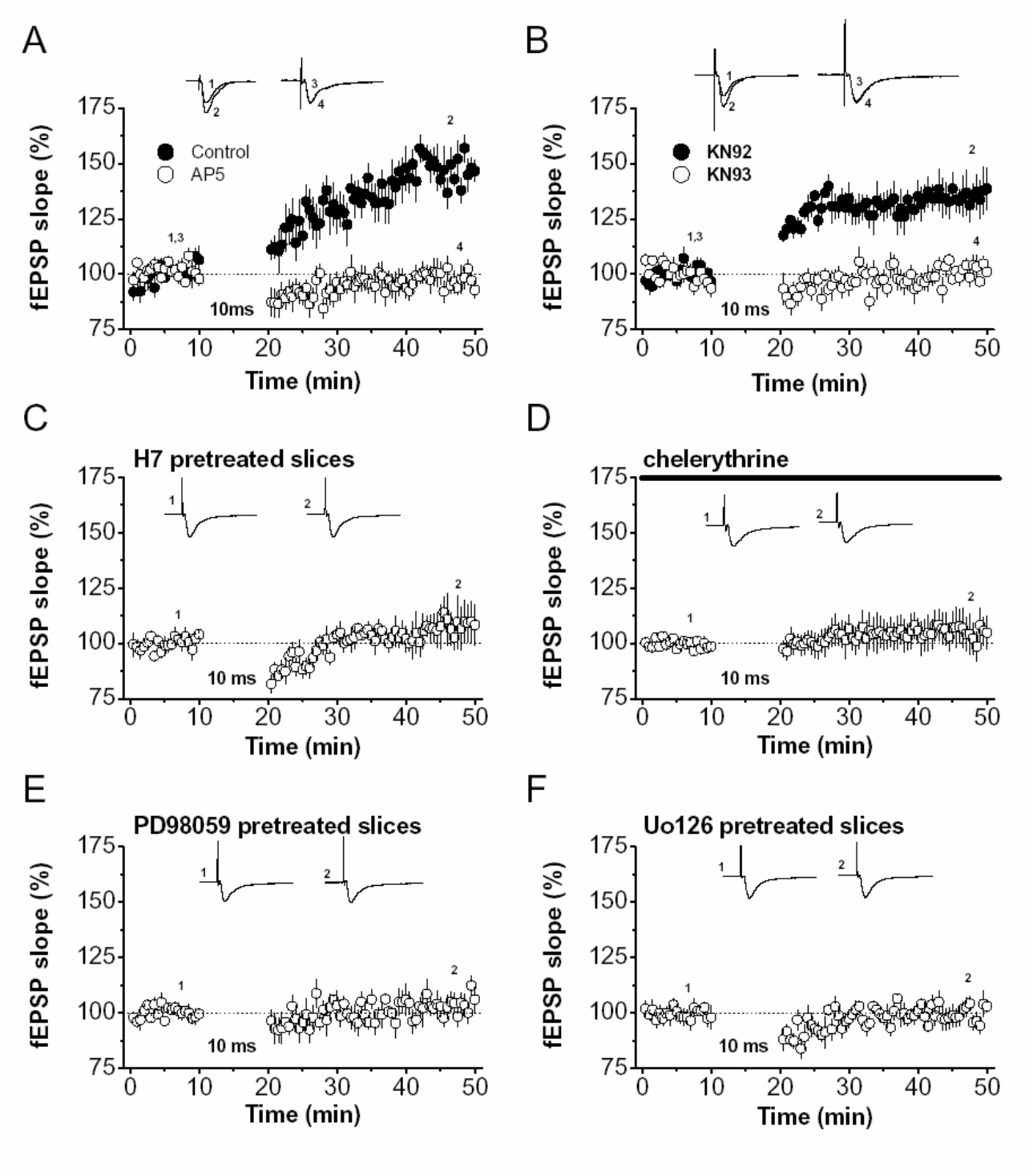
A
fEPSP slope (%)
Control
AP5
10ms
Time (min)
B
KN92
KN93
10 ms
C
H7 pretreated slices
D
chelerythrine
E
PD98059 pretreated slices
F
Uo126 pretreated slices

Fig. 2

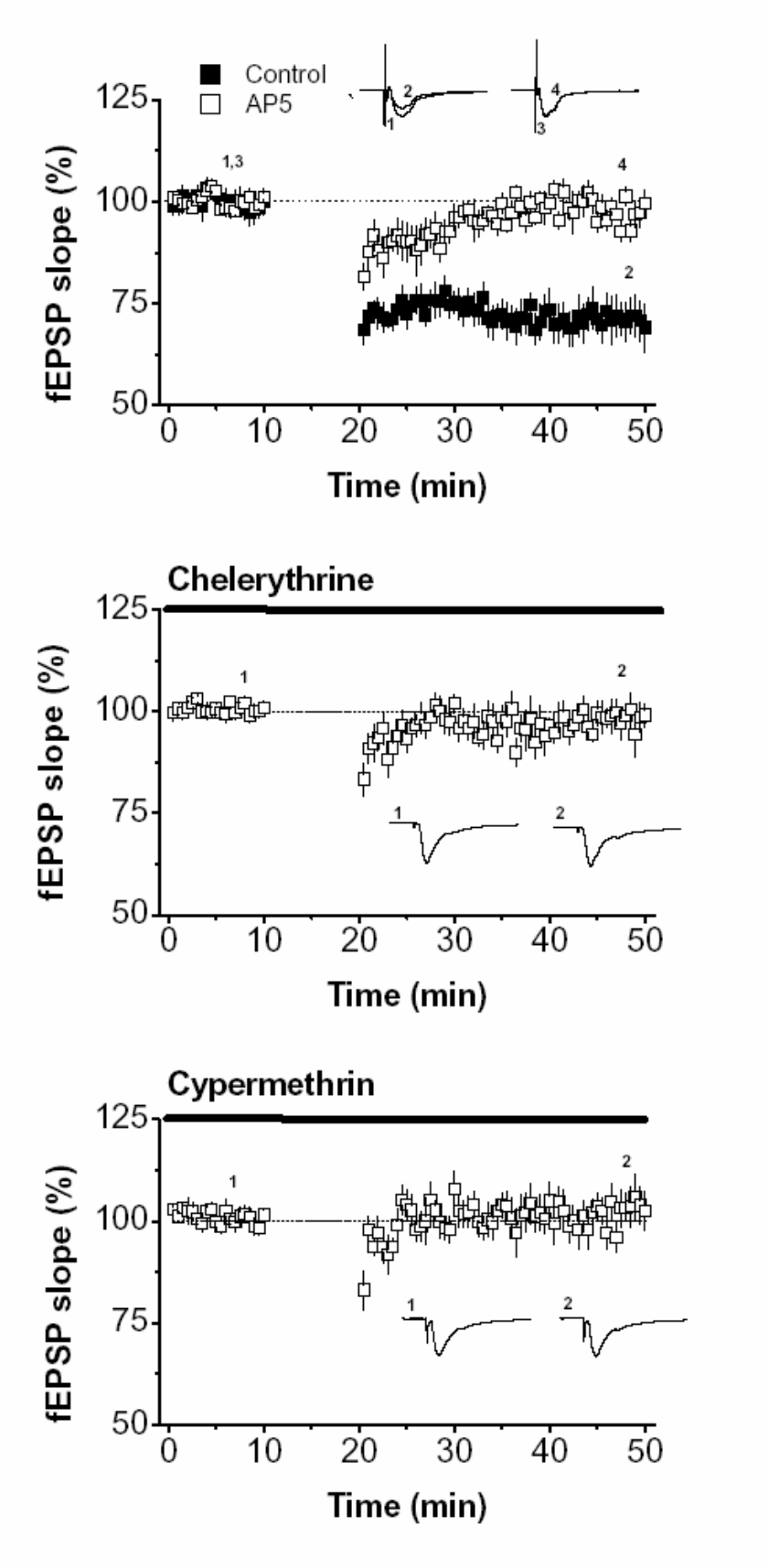
Control
AP5
fEPSP slope (%)
Time (min)
Chelerythrine
Cypermethrin

Fig. 3

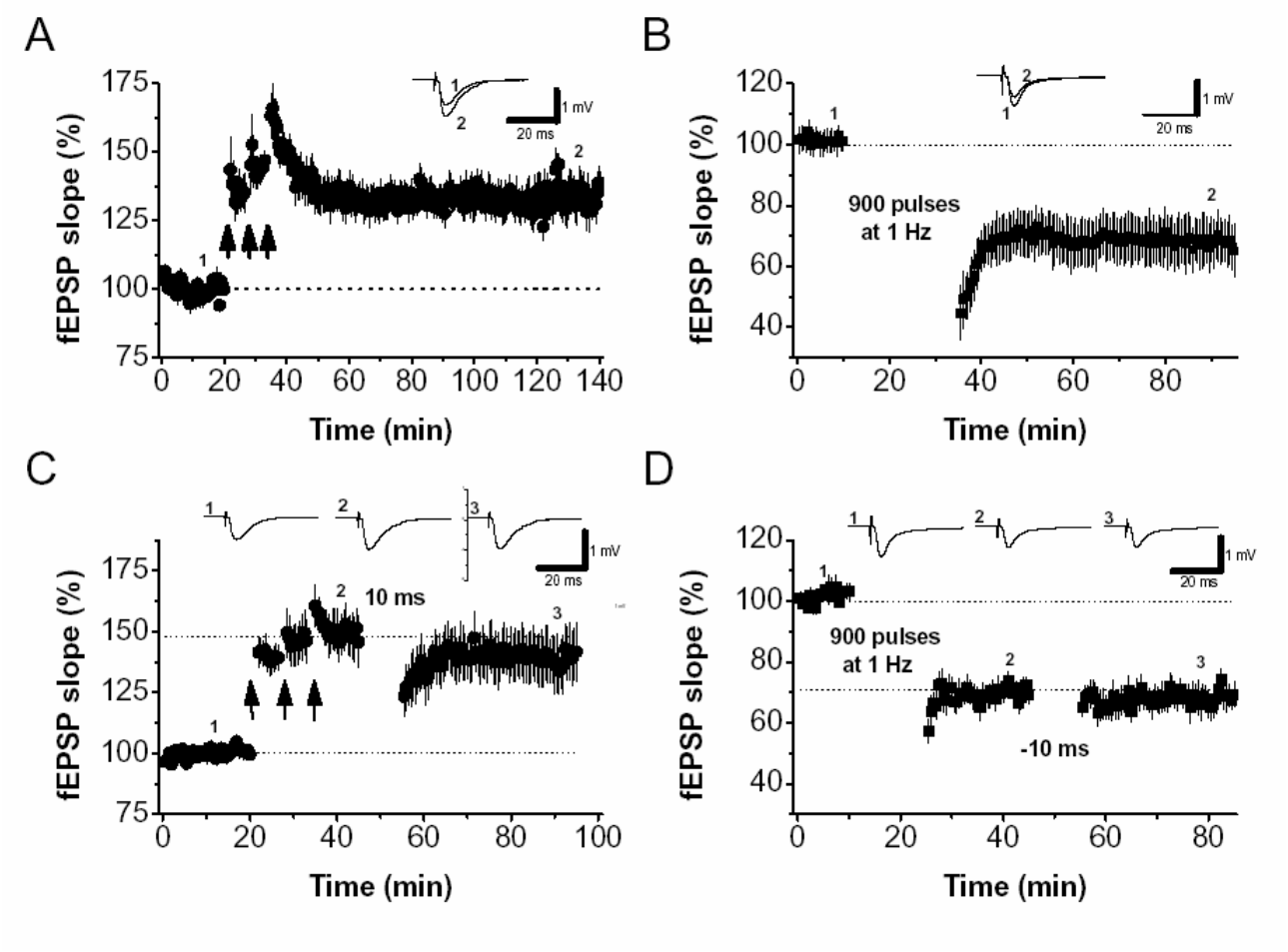
A
fEPSP slope (%)
Time (min)
1 mV
20 ms
B
900 pulses
at 1 Hz
C
10 ms
D
-10 ms

Fig.4

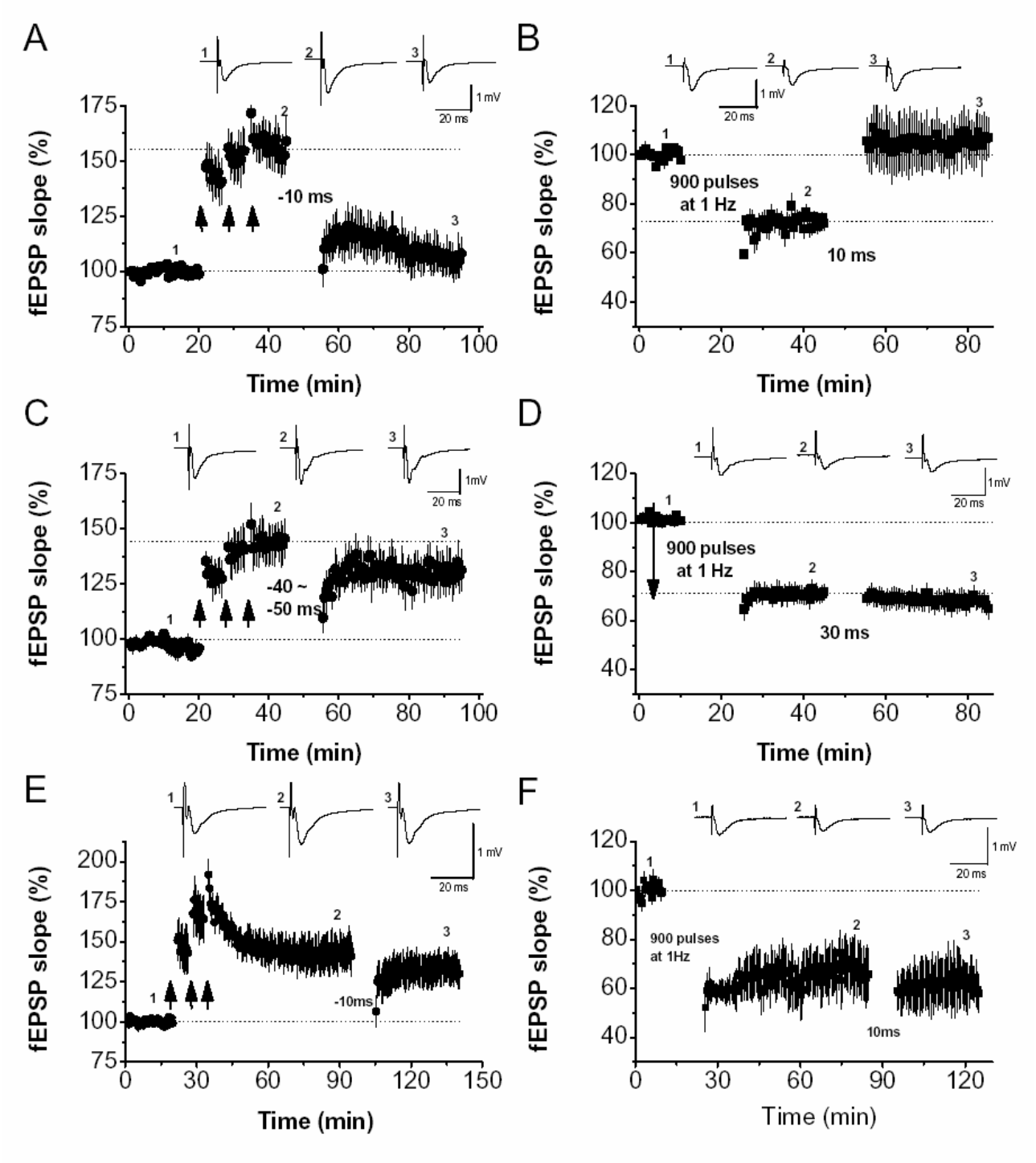
A
fEPSP slope (%)
Time (min)
-10 ms
1 mV
20 ms
B
900 pulses
at 1 Hz
10 ms
C
-40 ~
-50 ms
D
30 ms
E
-10ms
F
900 pulses
at 1Hz
10ms

Fig.5

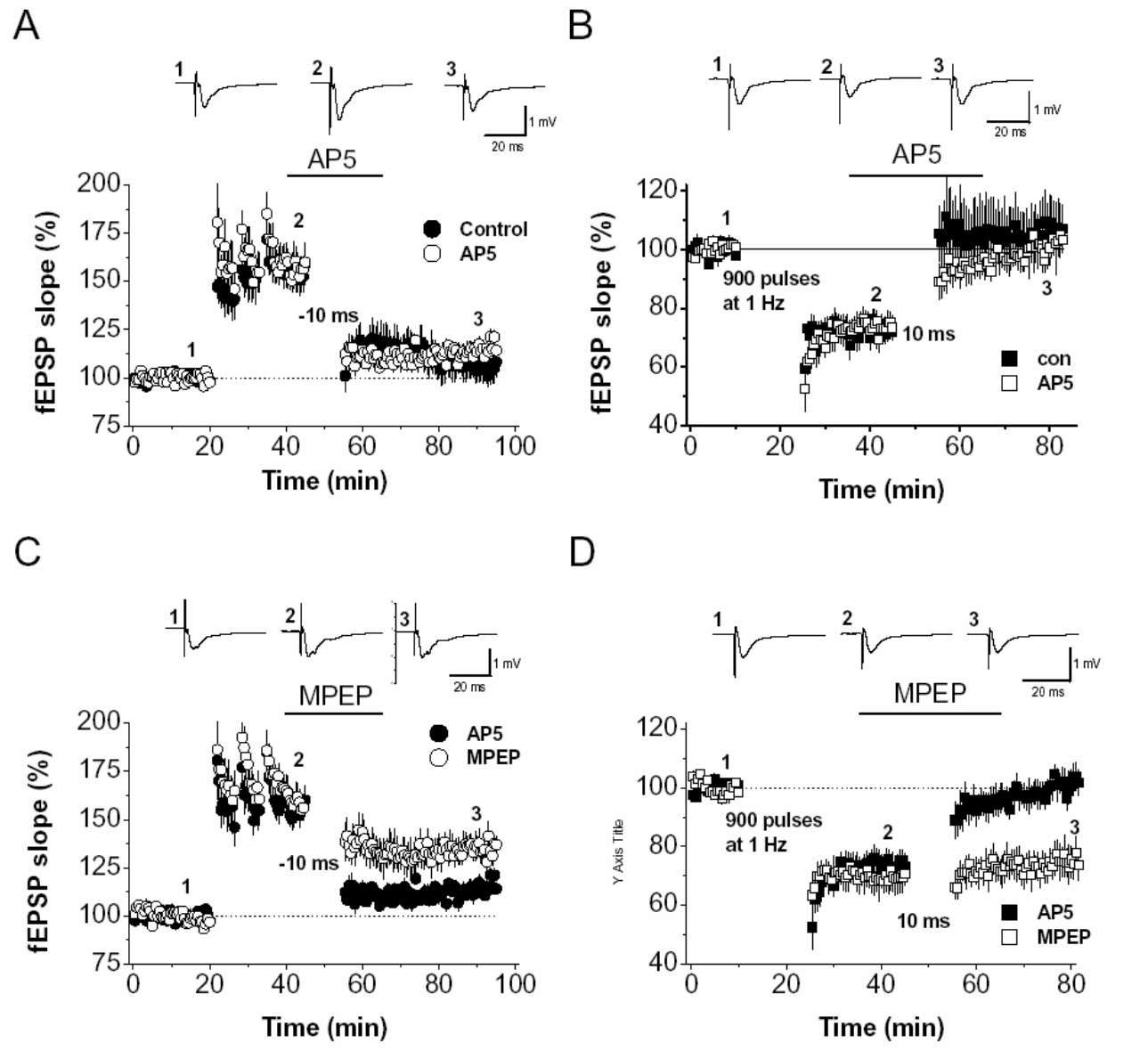
A
1
2
3
1 mV
20 ms
AP5
fEPSP slope (%)
Control
AP5
-10 ms
Time (min)
B
AP5
900 pulses
at 1 Hz
10 ms
con
AP5
C
MPEP
AP5
MPEP
D
MPEP
Y Axis Title

Fig. 6

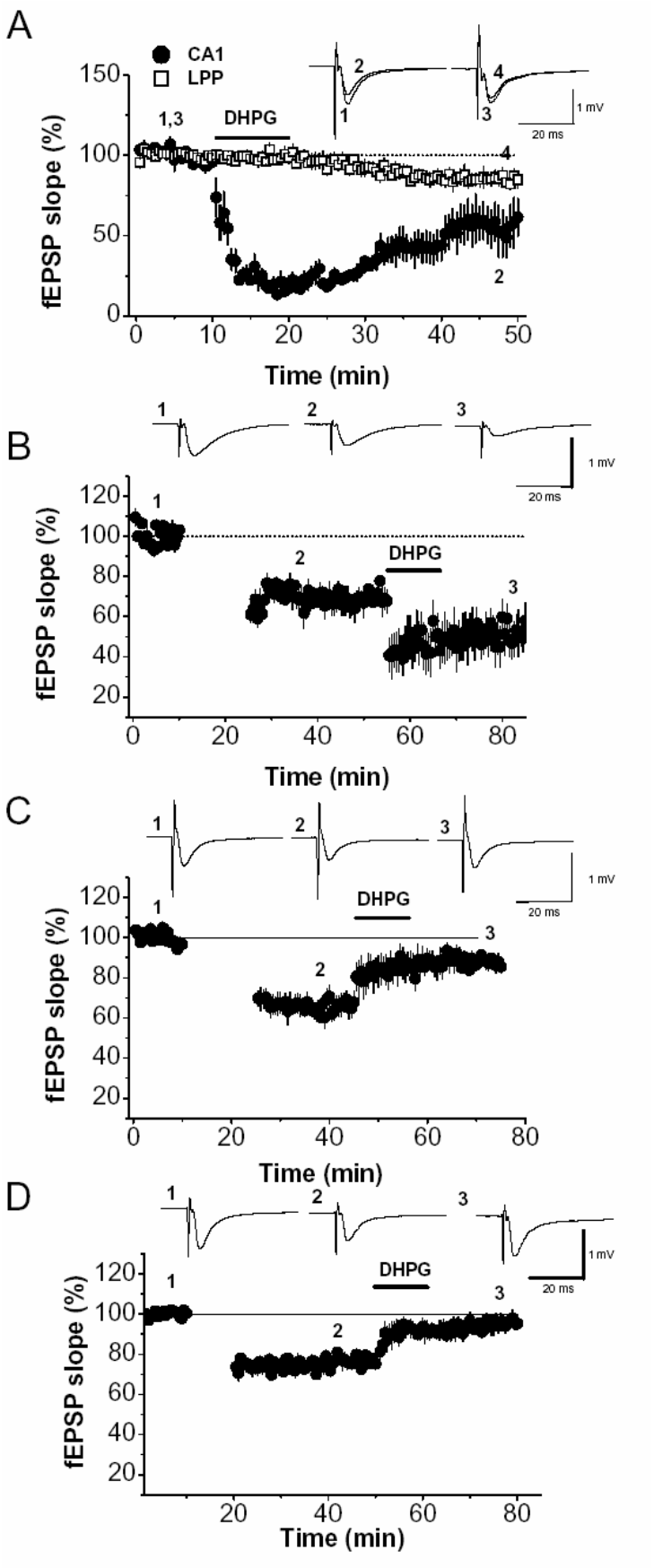

Fig. 7