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Original Article

Critical Differences in Magnitude and Duration of N-methyl-D-aspartate (NMDA) Receptor Activation between Long-term Potentiation (LTP) and Long-term Depression (LTD) Induction

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The induction of both long-term potentiation (LTP) and long-term depression (LTD) in the hippocampal CA1 region is triggered by the activation of N-methyl-D-aspartate (NMDA) receptors and the subsequent postsynaptic intracellular Ca^{2+} increase. However, how NMDA receptor activation differs between LTP and LTD induction is unclear. In the present study, we examined the effects of the magnitude and duration of NMDA receptor activation on the induction of LTP and LTD. Partial blockage of NMDA receptors by a low concentration of aminophosphonovaleric acid (APV) (2 μM) prevented the induction of LTP, but not LTD. In contrast, a high concentration of APV (25 μM) blocked both LTP and LTD. Tetanus stimulation-induced LTP was impaired when hippocampal slices were given the tetanus stimulation for more than 5 min. Under partial blockage of NMDA receptors, the prolonged-tetanus stimulation induced LTD but not LTP. This phenomenon was mimicked by the application of glutamate to the slices. Finally, LTD induced by prolonged activation of NMDA receptors was not affected by inhibition of the desensitization of α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors. These results suggest that critical differences exist between the induction of LTP and that of LTD in terms of both the magnitude and the duration of NMDA receptor activation. The duration of the increase in intracellular Ca^{2+} concentration may be critical for determining whether LTP or LTD induction occurs.

Key words: LTP, LTD, NMDA receptor, learning and memory, hippocampus

Long-term potentiation (LTP) and long-term depression (LTD) in the hippocampus have been widely accepted as providing the basis of synaptic models of learning and memory [1, 2]. Excitatory synaptic transmission in the CA1 region of the hippocampus is mediated by glutamate. The induction of both LTP and LTD in the CA1 region requires a conditioning stimulation of glutamate receptors [1, 2]. The conditioning stimulation activates N-methyl-D-aspartate (NMDA) receptors and the subsequent postsynaptic intracellular Ca^{2+} increase [2, 3]. A high level of Ca^{2+} increase activates various protein kinases, such as protein kinase C, Ca^{2+}/calmodulin-dependent protein kinase II and mitogen-activated protein kinase (MAPK). The kinases phosphorylate α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor, resulting in an increase in the ionic conductance of the channel and the insertion of

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entirely new AMPA receptors into the postsynaptic membrane [4]. Consequently, LTP is induced [4]. In contrast, a lower Ca^{2+} increase results in the activation of protein phosphatases such as calcineurin and the subsequent induction of LTD by dephosphorylation of AMPA receptors [5]; thus, the difference of the magnitude of Ca^{2+} influx from NMDA receptors is thought to determine whether LTP or LTD is induced [6].

Reviewing most experimental protocols for the induction of LTP and LTD, one finds that there are 2 basic factors involved in the conditioning stimulation protocol, that may determine whether LTP or LTD is induced. One factor is stimulation strength, which is mainly determined by stimulus frequency, although stimulus intensity is also used to adjust the stimulus strength in some cases. High-frequency stimulation, such as tetanic stimulation or theta-burst stimulation (TBS), has been used to induce LTP [10] whereas low-frequency stimulation, such as 1 Hz stimulation, has been used to induce LTD [11]. The stimulus frequency is thought to affect the transmitter release and to control the degree of depolarization of the postsynaptic cell and thereby to affect the degree of NMDA receptor activation, by which the magnitude of the Ca^{2+} influx is subsequently regulated. The other factor is the duration of the stimulation. A short duration, such as 1 sec to several seconds, is generally used for LTP induction [10], whereas a much longer duration, such as 15 min, is generally used to induce LTD [11]. However, it is not clear whether the duration of the stimulation is crucial in determining whether LTP or LTD is induced.

In the present study, we examined the effect of a partial blockade of NMDA receptor activation by APV on the induction of LTP and LTD. In addition, we also examined the characteristics of the duration of stimulation required to induce LTP and LTD.

Materials and Methods

Electrophysiological study. All experiments were performed using male Wistar rats (5–10 weeks old) or young rats (2–4 weeks old). Hippocampal slices were prepared as previously described [10, 12]. Briefly, the animals were anesthetized with ether and were then decapitated. The brains were quickly removed and immersed in ice-cold artificial cerebrospinal fluid (ACSF). The hippocampi were dissected and transversal hippocampal slices (400 μm thick) were prepared. The CA3 area was surgically removed for some experiments. The slices were incubated in ACSF saturated with 95% O_2 and 5% CO_2 at room temperature for at least 1 h before being transferred to a recording chamber. The standard ACSF was composed of (in mM) NaCl, 124; KCl, 3; KH_2PO_4, 1.25; $CaCl_2$, 3; $MgCl_2$, 1; $NaHCO_3$, 26 and glucose, 10.

A single slice was transferred to a submersion-type recording chamber and continually perfused with oxygenated ACSF at 34 °C at a flow rate of 1.5–2 ml/min. A glass electrode filled with ACSF (resistance 1–5 MΩ) was used for recording. A bipolar stainless steel electrode (100 μm in diameter) was used to deliver stimulation. The recording electrode was positioned in the dendritic layer of field CA1 to record the field excitatory postsynaptic potentials (EPSPs). EPSP responses were evoked by 0.1-ms duration impulses (intensity 5–8 V) at a frequency of 0.033 Hz on the Schaffer collateral/commissural pathway. The stimulus intensity that elicited a half-maximal response was always used for test stimulation and conditioning stimulation.

LTP was induced by TBS, which consisted of 10 stimulus trains delivered at 5 Hz. Each train consisted of 4 pulses at 100 Hz. Prolonged conditioning stimulation consisted of repeated TBS for as long as 30 sec to 15 min, as indicated. LTD was induced by delivering low-frequency stimulation at 1 Hz for 15 min (LFS). LTD was performed with slices prepared from young rats and induced in naive slices without previous LTP-inducing stimulation.

In order to record the NMDA receptor-mediated potentials, we applied the following procedures modified from those previously reported [13]. The CA3 area of a hippocampal slice was cut and discarded. The perfusing medium was modified by reducing the concentration of $MgCl_2$ to 50 μM and adding 10 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX) to the standard ACSF. Furthermore, priming stimulation was delivered as 2 stimuli 200 ms apart in order to reduce the GABAergic inhibition and increase the NMDA responses.

Aminophosphonovaleric acid (APV), DNQX, and cyclothiazide (CYZ) were purchased from Sigma. In most cases, drugs were dissolved in the ACSF to

make a stock solution. DNQX was initially dissolved in dimethylsulfoxide (DMSO) at a concentration of 10 mM, so that the final concentration of DMSO during perfusion did not exceed 0.1%.

LTP was evaluated 60 min after TBS. LTD was evaluated 30 min after conditioning stimulation (LFS or prolonged TBS). The initial slope of the EPSPs was measured to evaluate the synaptic response.

Statistics. Data are shown as the percentage of the average 10-min baseline before conditioning stimulation. Values are reported as the mean ± s.e.m. Data were analyzed using Student's t test to compare 2 conditions and one-way ANOVA was followed by Scheffe's post-hoc analysis to compare multiple conditions. $P < 0.05$ was considered to be significant.

Results

Partial blockade of NMDA receptors abolishes LTP, but does not prevent LTD. To elucidate the characteristics of the NMDA receptor dependency of the induction of LTP and LTD, we investigated the effects of NMDA receptor blockade on the induction. As a control experiment, we first examined the dose-response relationships of APV, a specific antagonist for NMDA receptors, on NMDA receptor-mediated responses. When the AMPA receptor-mediated responses were blocked by DNQX (10 μM) and the Mg^{2+} concentration in the perfusant was reduced to 50 μM, NMDA receptor-mediated responses could be isolated in the synapses between the Schaffer collateral fibers and CA1 pyramidal cells in the hippocampal slices. APV at concentrations ranging from 0.3–10 μM showed a dose-dependent suppression of the NMDA-receptor-mediated potentials (Fig. 1A). APV at 2 or 5 μM produced 40–60% inhibition compared with the control (0 μM APV) (Fig. 1A and B).

The effect of NMDA receptor blockade on LTP induced by TBS for 2 sec was next examined (Fig. 1C). Under the control condition in the absence of APV, TBS for 2 sec induced an 88% increase in the slope of the EPSPs (188 ± 17% 60 min after TBS, n = 8). Incubation with 2 μM APV, which produced a 40% inhibition of the NMDA receptor-mediated responses as shown in Fig. 1B, suppressed the TBS-induced increase of EPSPs to 38% (138 ± 8%, n = 7, Fig. 1C). A higher concentration of APV (10 μM) completely abolished TBS-induced LTP (98 ± 2%, n = 5, Fig. 1C). A lower concentration of APV (0.3 μM) produced no distinguishable effect on LTP compared with the control (data not shown). Consistent with its effects on NMDA potentials, APV produced a dose-dependent inhibition of TBS-induced LTP. These results suggest that more than 60% activation of NMDA receptors seems to be essential to induce LTP under the present experimental conditions.

The induction of LTD requires a relatively moderate increase of the cytoplasmic Ca^{2+} level [1], suggesting that it requires lower activation of NMDA receptors than does the induction of LTP. We examined the effect of a low concentration of APV on the induction of LTD. In the presence of 2 μM APV, which inhibited LTP, as shown in Fig. 1C, low-frequency stimulation (LFS) induced LTD (69 ± 3% 40 min after LFS, n = 7; Fig. 1D). The decrease in the slope of the EPSPs slope was not significantly different from that of the control in the absence of APV (70 ± 3%, n = 6). However, when a higher concentration of APV (10 μM) was applied, LTD was not induced (99 ± 4%, n = 5). Therefore, APV at a low concentration, which was sufficient to inhibit LTP, did not impair the induction of LTD. These results suggest that the induction of LTD requires less activation of NMDA receptors than the induction of LTP, although both the induction of LTP and that of LTD are NMDA-dependent.

Prolonged tetanus stimulation abolishes LTP and induces LTD under partial blockade of NMDA receptors. Under a partial blockade of NMDA receptors with 5 μM APV, TBS-induced LTP was blocked by a mechanism involving a reduced level of the intracellular Ca^{2+} increase. However, this treatment never converted the direction of synaptic plasticity to LTD, suggesting that the difference in the magnitude of the cytoplasmic Ca^{2+} level is not the only factor that determines whether LTP or LTD is induced. LTP is generally induced by a very brief stimulation, such as a 1 sec stimulation with tetanus, whereas the induction of LTD requires a relatively much longer stimulation, such as 15 min of low-frequency stimulation [4]. We therefore investigated the effect of the duration of TBS on the induction of LTP and LTD. The effect of prolonged high-frequency stimulation on LTP induction was then examined. A brief TBS (2 sec duration) induced robust LTP (199

±18%, n=8; Fig. 2A). Surprisingly, when the same stimulation was given for 5 min and 15 min in the hippocampal slices, no significant increase or decrease of EPSP slope was observed (TBS for 5 min, 109±7%, n=6; TBS for 15 min, 98±8%, n=7; Fig. 2A). TBS for 5 and 15 min produced an immediate inhibition of EPSP slope (Fig. 2A), probably due to glutamate exhaustion in the presynaptic terminals. The reduced EPSP slope recovered to baseline within 5–10 min, and the recovered EPSP slope was main-

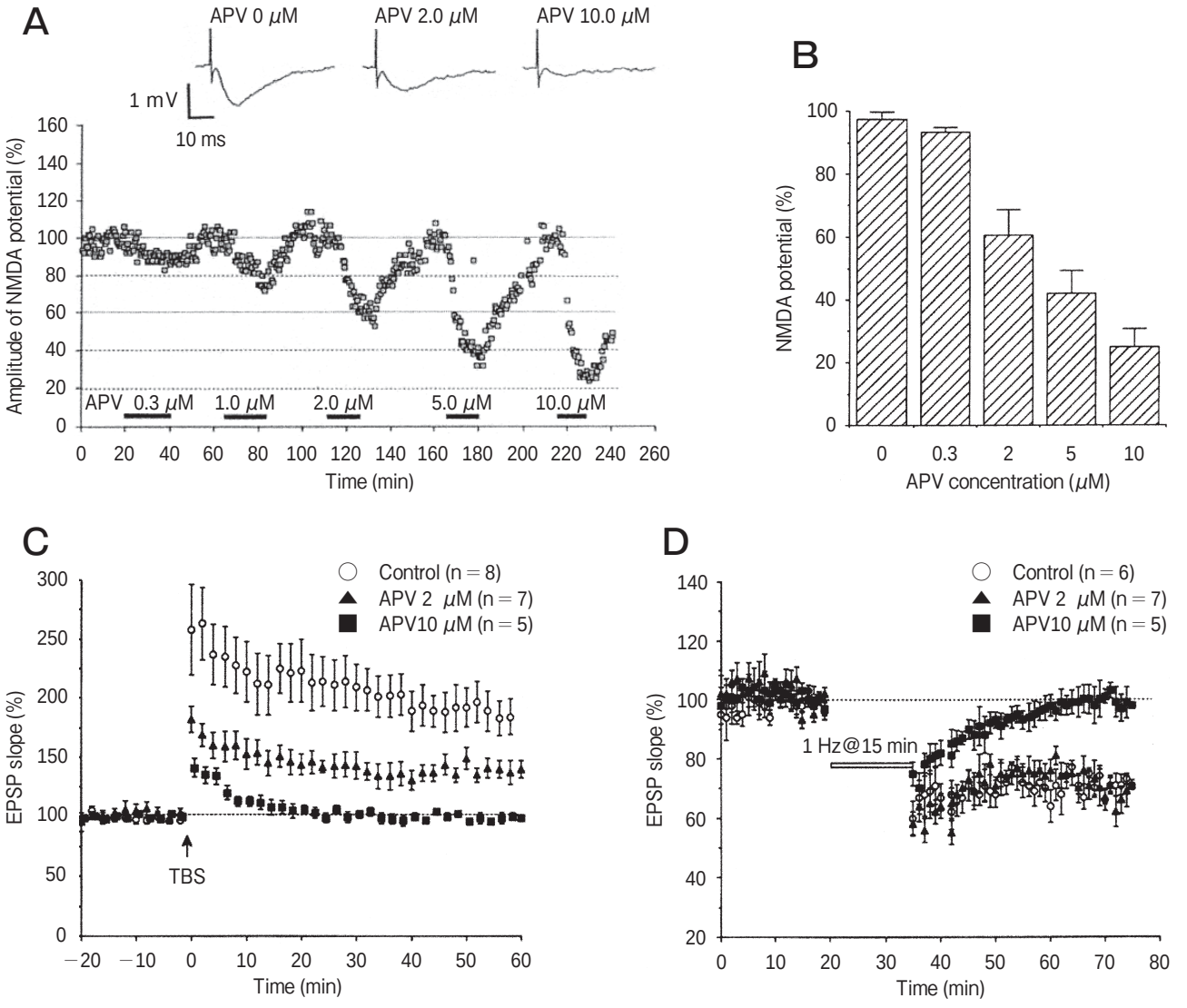

Fig. 1 Effects of partial blockade of NMDA receptors on the induction of LTP and LTD.
A, A representative example showing dose-dependent inhibition of NMDA receptor-mediated potential by APV. APV at concentrations of 0.3, 1.0, 2.0, 5.0, and 10 μM produced dose-dependent and reversible inhibition of the NMDA receptor-mediated response. Insets show the NMDA potential traces at APV 0 μM (control), 2.0 μM and 10 μM; **B**, Summary of the inhibitory effects of APV at various concentrations on NMDA receptor-mediated potential. Data show mean ± s.e.m. of inhibition from 5 experiments; **C**, Partial blockade of NMDA receptor impaired LTP. Theta-burst stimulation (TBS) for a 2 sec duration was used to induce LTP. APV at the indicated concentration was applied 20 min before and 15 min after TBS; **D**, A low concentration of APV did not block LTD. Low-frequency stimulation (LFS) at 1 Hz for 15 min was used to induce LTD. APV at the indicated concentration was applied 20 min before and 15 min after LFS.

tained for 40 min after the stimulation. Thus, prolonged TBS did not induce LTP.

We next examined the effect of prolonged TBS under partial blockade of NMDA receptors on the EPSP slope. When 5 μM APV was added in the perfusant, which produced 60% inhibition of NMDA receptors (Fig. 1B), TBS for 15 min produced a 20% decrease of the EPSP slope 60 min after the stimulation ($80 \pm 4\%$, $n = 5$; Fig. 2B), suggesting that LTD induction occurred. However, TBS for 5 min did not affect the EPSP slope 60 min after the stimulation ($103 \pm 4\%$, $n = 5$; Fig. 2C).

Prolonged TBS-induced LTD is not due to the desensitization of AMPA receptors. Since the response of AMPA receptors might be suppressed due to desensitization caused by prolonged stimulation, one concern is whether the decrease of the EPSP induced by prolonged high frequency stimulation shown in Fig. 2B was simply a result of the desensitization of the AMPA receptors. To exclude this possibility, we examined the effect of cyclothiazide (CYZ), an inhibitor of AMPA receptor desensitization, on prolonged TBS-induced LTD. CYZ (30 μM) did not produce any significant changes in TBS (15 min)-induced LTD under a partial blockade of NMDA receptors with 5 μM APV ($85 \pm 8\%$, 60 min after TBS, $n = 4$; Fig. 3) compared with that induced in the absence of CYZ ($80 \pm 4\%$; Fig. 2B). These results suggest that desensitization of AMPA receptors is not involved in the induction of prolonged high-frequency stimulation-induced LTD.

Pronged application of glutamate induces LTD. Previous studies have shown that the application of glutamate or NMDA induces LTD in hippocampal slices [14, 15]. Finally, we examined whether prolonged application of glutamate induced LTD. Glutamate (1 mM) was bath-applied to hippocampal slices since the glutamate concentration in the synaptic cleft during normally evoked transmitter

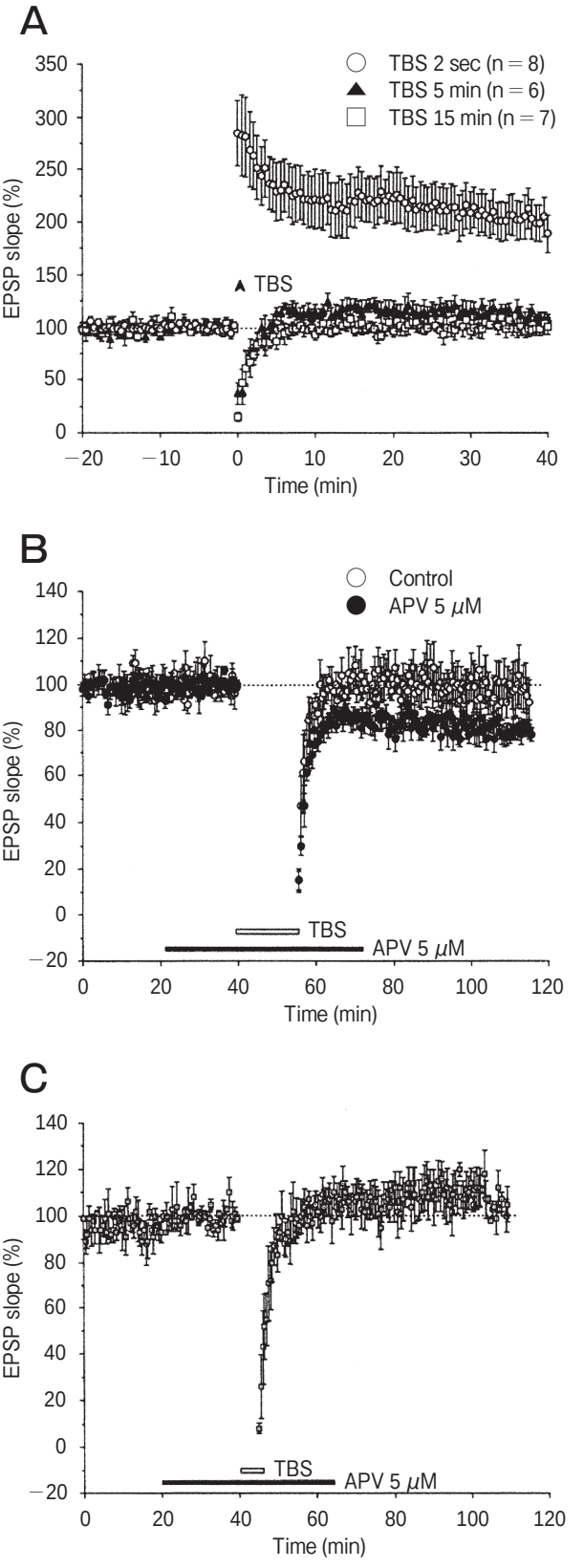

Fig. 2 Prolonged high-frequency stimulation abolished LTP and induced LTD under a partial blockade of NMDA receptors.
A, A brief TBS (2 sec) induced LTP, while prolonged TBS for 5 min or 15 min abolished LTP; **B**, In the presence of a low concentration of APV (5 μM), a prolonged TBS (15 min) induced LTD; **C**, In the presence of 5 μM APV, 5 min of TBS produced neither LTP nor LTD.

release was reported to be 1.1 mM at cultured hippocampal synapses [16]. Perfusion of glutamate for 5 min temporarily abolished the EPSP slope, but the EPSP slope recovered to the baseline 40 min after the glutamate was washed out ($100 \pm 4\%$, $n = 4$; Fig. 4A), suggesting that glutamate application for 5 min did not induce LTD. Hippocampal slices were next incubated with glutamate for 15 min. The application of 1 mM glutamate for 15 min also abolished the EPSPs slope. After the glutamate was washed out with normal ACSF, the evoked EPSPs gradually recovered, but not back to the same level as before the application of glutamate ($85 \pm 4\%$ of baseline, 40 min after glutamate application, $n = 4$; Fig. 4B). Thus, LTD was produced by prolonged perfusion of glutamate for 15 min. Similar to the LTD induced by LFS in these synapses, glutamate-induced LTD was blocked by a blockade of NMDA receptors (Fig. 4B), although APV did not affect the glutamate-induced early abolition of EPSPs. These results are similar to those induced by 5-min and 15-min TBS, and they support the idea that the duration of conditioning stimulation is critical for synaptic plasticity, and that prolonged stimulation is necessary for the induction of LTD.

Discussion

This study reports the new finding of a differential threshold of NMDA receptor activation for the induction of LTP and LTD, and also finds that the duration of NMDA receptor activation is another critical factor that controls the direction of synaptic plasticity. Thus, both the level of the intracellular Ca^{2+} increase

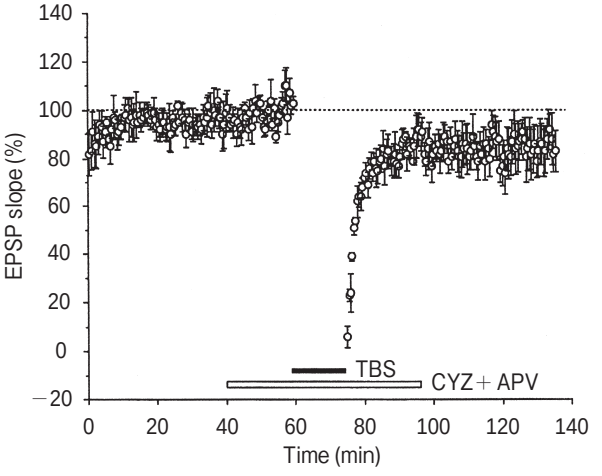

Fig. 3 Prolonged TBS-induced LTD was not due to desensitization of AMPA receptors.
CYZ did not block prolonged TBS-induced LTD. LTD was induced by 15 min of TBS in the presence of a partial blockade of NMDA receptors by 5 μM APV. CYZ (30 μM) was applied 20 min before and after TBS.

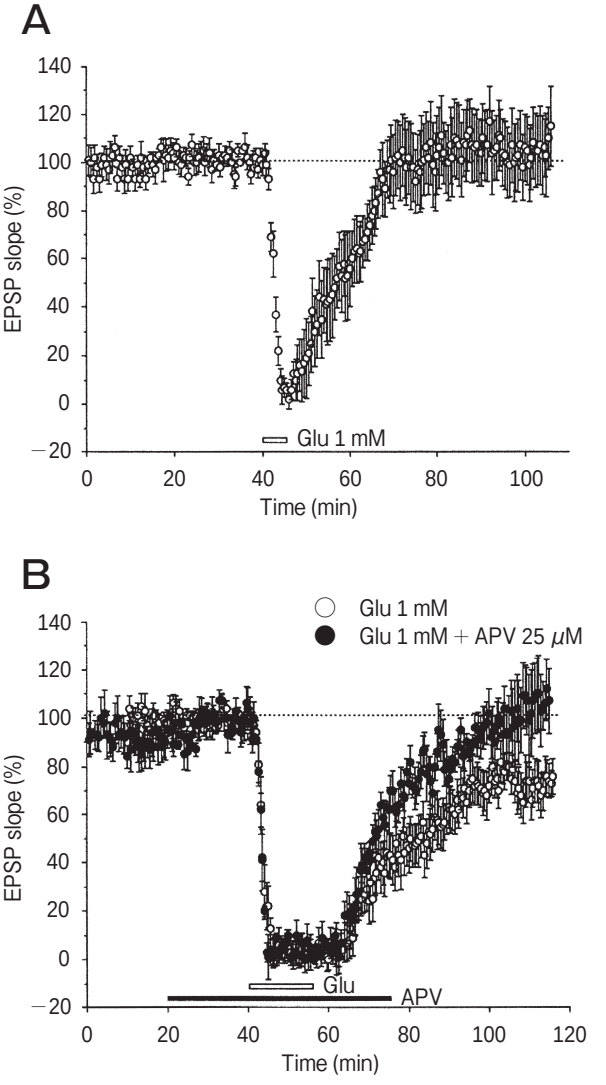

Fig. 4 Prolonged application of glutamate induced LTD.
A, The application of glutamate (1 mM) for 5 min induced neither LTP nor LTD; B, The application of glutamate (1 mM) for 15 min induced LTD. Hippocampal slices were treated with APV (25 μM) 20 min before starting the stimulation and were incubated for 55 min.

and the duration for which such a Ca^{2+} level is sustained may affect whether LTP or LTD is induced.

In the present study, we examined the effect of the partial inhibition of NMDA receptors on the induction of LTP and LTD. Partial blockade of NMDA receptors abolished LTP, but did not inhibit LTD, although a higher level of blockade of NMDA receptors prevented the induction of LTD (Fig. 1). These results suggest that LTP has a higher threshold than LTD for NMDA receptor activation and subsequent Ca^{2+} influx. The amount of Ca^{2+} influx from NMDA receptors is one of the critical factors determining whether LTP or LTD is induced.

While the magnitude of the cytoplasmic Ca^{2+} increase is a key factor in the induction of LTP and LTD, this factor is not sufficient to determine the direction of changes in synaptic strength. In the present study, we showed that the length of time for which the enhanced Ca^{2+} level is sustained is another critical factor. Reducing the intracellular Ca^{2+} level without changing the duration at that Ca^{2+} level resulted in blockage of LTP, but did not cause conversion to LTD (Fig. 1C). Moreover, prolonging the duration of the conditioning stimulation alone, but not changing the stimulation frequency, prevented the induction of LTP (Fig. 2). When TBS was prolonged to 5–15 min, neither LTP nor LTD was induced (Fig. 2A). When NMDA receptors were partially blocked and the tetanization-evoked Ca^{2+} increase was reduced, the prolonged TBS induced LTD but not LTP (Fig. 2B). These results suggest that a brief duration of high-level cytoplasmic Ca^{2+} may be critical for LTP induction, and that a sustained duration of a moderate elevation of the Ca^{2+} level may be critical for LTD induction.

It is thought that following the increase of the intracellular Ca^{2+} level, the activation of Ca^{2+}/calmodulin-dependent protein kinases and/or phosphatase is a key step leading to long-lasting changes in synaptic transmission [4]. The present results suggest that, in addition to the magnitude of the intracellular Ca^{2+} increase, the duration of the intracellular Ca^{2+} increase may be critical in determining which enzyme is activated, kinase or phosphatase. Investigating the activation of CaMKII and calcineurin during LTP and LTD induction in future studies is important to help address this question.

In summary, the present study provided strong evidence of the critical differential dependence on NMDA receptors between the induction of LTP and LTD. The magnitude of the intracellular Ca^{2+} increase is one key factor determing whether LTP or LTD is induced. The duration of the Ca^{2+} increase is also critical for determining whether the induction of LTP or LTD occurs.

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