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# Bidirectional Synaptic Plasticity Induced by Conditioned Stimulations With Different Number of Pulse at Hippocampal CA1 Synapses: Roles of *N*-Methyl-D-Aspartate and Metabotropic Glutamate Receptors

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*KEY WORDS* LTP; LTD; hippocampus; pulse number; NMDA; mGluR5

*ABSTRACT* In the mammlian brain, the hippocampus has been established as a principle structure for learning and memory processes, which involve synaptic plasticity. Althought a relationship between synaptic plasticity and stimulation frequency has been reported in numerous studies, little is known about the importance of pulse number on synaptic plasticity. Here we investigated whether the pulse number can modulate bidirectional plasticity in hippocampal CA1 areas. When a CA1 area was induced by a paired-pulse (PP) with a 10-ms interval, the strength of the synapse was altered to form a long-term depression (LTD), with a 68 ± 4% decrease in expression. The PP-induced LTD (PP-LTD) was blocked by the metabotropic glutamate receptors subtype 5 (mGluR5) antagonist MPEP, suggesting that the PP-LTD relied on the activation of GluR5. In addition, this modulation of LTD was protein kinase C (PKC)- and Group II mGluR-independent. However, when increasing the pulse number to 4 and 6, potentiated synaptic strength was observed, which was *N*-methyl-D-aspartate receptor (NMDAR)-dependent but mGluR5-independent. Surprisingly, when blocking mGluR, the synaptic efficacy induced by triple-pulse stimulation was altered to form a long-term potentiation (LTP) with a 142 ± 7% enhancement, and was further blocked by NMDA antagonist APV. Following treatment with APV and PKC blocker chelerythrine, the LTP expression induced by 4- and 6-pulse stimulation was switched to LTD. We suggest that CA1 synaptic plasticity is regulated by the result of competition between NMDA and mGluR5 receptors. We suggest that the pulse number can bidirectionally modulate synaptic plasticity through the activation of NMDA and mGluR5 in hippocampal CA1 areas. **Synapse 00:000–000, 2011.** 

## INTRODUCTION

A central hypothesis for learning and memory at the cellular level is required for the experience-dependent enhancement and weakening of synaptic efficacy, which includes the induction of long-term potentiation (LTP) and long-term depression (LTD). LTP and LTD are considered models of such bidirectionally-changeable plasticity and are generally required for



J.-C.H. and S.-J.C. contributed equally to this work.

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*N*-methyl-D-aspartate receptor (NMDAR) activation followed by postsynaptic $Ca^{2+}$ influx, instigating the triggering of $Ca^{2+}$-dependent signaling pathways (Bliss and Collingridge, 1993; Malenka and Bear, 2004; Malenka and Nicoll, 1999). According to the BCM theory (Beggs, 2001; Dudek and Bear, 1992), the direction of the change in synaptic strength is modulated by the activation of NMDA-dependent $Ca^{2+}$ signaling. A small to moderate amount of NMDA-dependent $Ca^{2+}$ signaling leads to LTD, whereas a larger activation causes LTP (Dudek and Bear, 1992; Mulkey and Malenka, 1992).

LTP can be induced with high-frequency stimulation (HFS) or low-frequency stimulation (LFS) paired with postsynaptic membrane depolarization (Lin et al., 2003, 2006). In contrast, LTD can be induced with (a) relatively LFS from 1 to 3 Hz; (b) presynaptic LFS with a small postsynaptic potentiation; (c) two independent pathways paired within a narrow period of time (Bear and Melanka., 1994; Lin et al., 2008). HFS causes a large influx of calcium into the postsynaptic membranes, which leads to LTP induction via activation of calcium-dependent signaling transduction, such as calcium/calmodulin-dependent protein kinase II, adenylyl cyclase, protein kinase, and mitogen-activated protein kinase (Lin et al., 2003, 2006). However, LFS depolarizes a relatively small number of NMDA receptors, leading to LTD via activation of phosphatases, including calcineurin (Mulkey et al., 1993, 1994).

Another induction of LTD expression at the hippocampal CA1 synapses was required for the paired-pulse at low frequency (referred as to PP-LTD), causing activation of metabotropic glutamate receptor (mGluR) but not NMDAR (Kin et al., 2003; Otani and Connor, 1998). mGluR-LTD can also be induced by applying mGluR5 agonist (R,S)-3,5-dihydroxyphenylglycine (DHPG) by pharmacology (Palmer et al., 1997). PP-LTD and DHPG share a common signaling pathway mechanism, because PP-LTD was blocked by mGluR antagonists (Kemp and Bashir, 1999). Interestingly, LFS-induced LTD may be regulated developmentally, as LTD can be induced in slices from young animals within 4 to 6 weeks (Yang et al., 2002). In contrast, recent studies have reported that paired-pulse LFS can reliably induce LTD in adult animals (Alarcon et al., 2004; Huang and Kandel, 2006). Therefore, the switching mechanism of NMDAR-dependent LTD to PP-induced mGluR-dependent LTD remains unclear.

It is well documented that a low frequency of 1 Hz and PP stimulation at a low frequency can robustly induce LTD, which is NMDAR- and mGluR5-dependent, in the hippocampus. PP stimulation for a short period can also induce age-related LTP. Our hypothesis states that the paired-pulse number for low-frequency stimulation can affect the bidirectional plasticity. To verify our hypothesis, pulse numbers from 1 to 6 were applied with a 10-ms interval for 10 min to hippocampal CA1 areas. Our results showed that different pulse numbers can bidirectionally modulate synaptic plasticity by activation of NMDAR and mGluR5 receptors. We believe that these results will provide a better understanding of pulse number responses and bidirectional plasticity as they relate to natural burst firing.

## MATERIALS AND METHODS

### Experimental animals

Male Sprague-Dawley rats weighing 150 to 200 g were used in this study. Rats were free access to food and water. The usage of these animals was approved by the Institute Animal Care and Use Committee of China Medical University and followed the Guide for the use of Laboratory Animals (National Academy Press).

### Electrophysiology

Adult male SD rats were anesthetized with halothane and decapitated. The brains were quickly removed and placed in ice-cold artificial CSF (ACSF) containing the following (mM): 119 NaCl, 2.5 KCl, 26.2 $NaHCO_3$, 1 $NaH_2PO_4$, 1.3 $MgSO_4$, 2.5 $CaCl_2$, and 11 glucose (the pH was adjusted to 7.4 by gassing with 5% $CO_2$-95% $O_2$). Transverse hippocampal slices (450 μm thick) were cut with a vibrating tissue slicer (Campden Instruments, Loughborough, UK) and transferred to an interface-type holding chamber at room temperature (25°C). The slices were recovered for at least 90 min and then were transferred to an immersion-type recording chamber, perfused at 2 ml/min with ACSF containing 100 μM picrotoxin at room temperature. The border between the CA1 and CA3 areas was cut to prevent epileptiform discharge of pyramidal neurons (Lin et al., 2003, 2008). For extracellular field potential recording, a glass pipette filled with 3M NaCl was positioned in the stratum radiatum of the CA1 area, and the field excitatory postsynaptic potential (fEPSP) was recorded. Bipolar stainless steel stimulating electrodes (Frederick Haer Company, Bowdoinham, ME) were placed in the striatum radiatum to stimulate Schaffer collateral branches. The fEPSP was elicited by adjusting the intensity of stimulation to about 40-50% of maximum response which population spikes after fEPSP began to appear. Stable baseline fEPSP activity was recorded by applying a short-duration voltage pulse (~1 ms) at the determined intensity every 30 s for at least 10 min. Different pulse stimulations were used to induce LTP and LTD expression from 1 to 6 pulses at 10 ms intervals. All signals were filtered at 2 kHz using the low-pass Bessel filter provided with the amplifier and digitized at 5 kHz using a CED micro 1401 interface running Signal software (Cambridge Electronic Design, Cambridge, UK). All

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drugs were purchased from Sigma (St. Louis, MO). The initial slopes of the fEPSP were measured for data analysis. Synaptic responses were normalized to the average of the baseline. The average size of the slope of the fEPSPs recorded from the last 10 min after different pulse stimulation was used for statistical comparisons. All data are presented as the mean ± standard error. Statistical significance was tested by paired *t*-test and Mann-Whitney *U* test. $P < 0.05$ was considered statistically significant.

## RESULTS

### Application of prolonged paired-pulse (PP) stimulation at low frequency induces LTD at CA1 synapses

First of all, we confirmed that the novel LTD expression in CA1 areas was induced by PP stimulation (Lin et al., 2003), which is two pulses with a 10-ms interpulse interval (IPI) at 0.167 Hz for 10 min. PP-induced LTD (PP-LTD) decreased to 68 ± 4% of the baseline ($n = 16$ slices, $P < 0.01$, paired *t*-test, Fig. 1A). However, the application of single-pulse stimulation at 0.167 Hz for 10 min did not result in a significant change in synaptic efficacy (Fig. 1B, 96 ± 4% of baseline, $n = 13$, $P = 0.12$, paired *t*-test). These results suggest that the induction of PP-LTD required a two-stimuli pattern. Furthermore, the induction of PP-LTD was not required for the activation of NMDA receptors, as demonstrated by adding NMDAR antagonist APV. Following the application of 50 μM APV in a bath, PP stimulation can reliably induce LTD (Fig. 1C, 79 ± 3% of baseline, $n = 10$ slices, $P < 0.01$, paired *t*-test).

### Induction of PP-LTD is required for the activation of group I mGluR, including mGluR5, but not NMDA receptors

It has been shown that LTD induced by PP stimulation with an IPI of 50 ms at 1 Hz for 15 min was not NMDAR-dependent but was Group I mGluR-dependent (Bear et al., 2001; Kemp and Bashir, 2001). We speculated that PP-LTD was also dependent on the activation of Group I mGluR, including mGluR5 receptors. To test this possibility, we first examined whether the chemical activation of Group I mGluR could induce LTD. The application of 40 μM of Group I mGluR agonist, DHPG, for 10 min resulted in significant LTD expression (65 ± 3% of baseline, $n = 6$, $P < 0.01$, paired *t*-test). The expression was measured 40 min after washing of DHPG. Second, we chose to examine whether DHPG saturated the Group I mGluR-dependent LTD. After repeated DHPG application (three times at 10-min intervals), saturated LTD expression resulted (45 ± 2% of baseline, $n = 7$ slices, $P < 0.01$, paired *t*-test). After the application of DHPG thrice, PP stimulation led to an altered plasticity of 90 ± 5% ($n = 7$ slices, $P = 0.13$, paired *t*-test, Fig. 2B) of the baseline; this recording was made 35 to 40 min after the final application of DHPG. DHPG-saturated LTD was occluded in the formation of PP-LTD, which suggests that the mechanism of PP-LTD is similar to DHPG-LTD. It has been reported that DHPG-induced LTD is independent of PKC activation (Schnabel et al., 1999, 2001). We

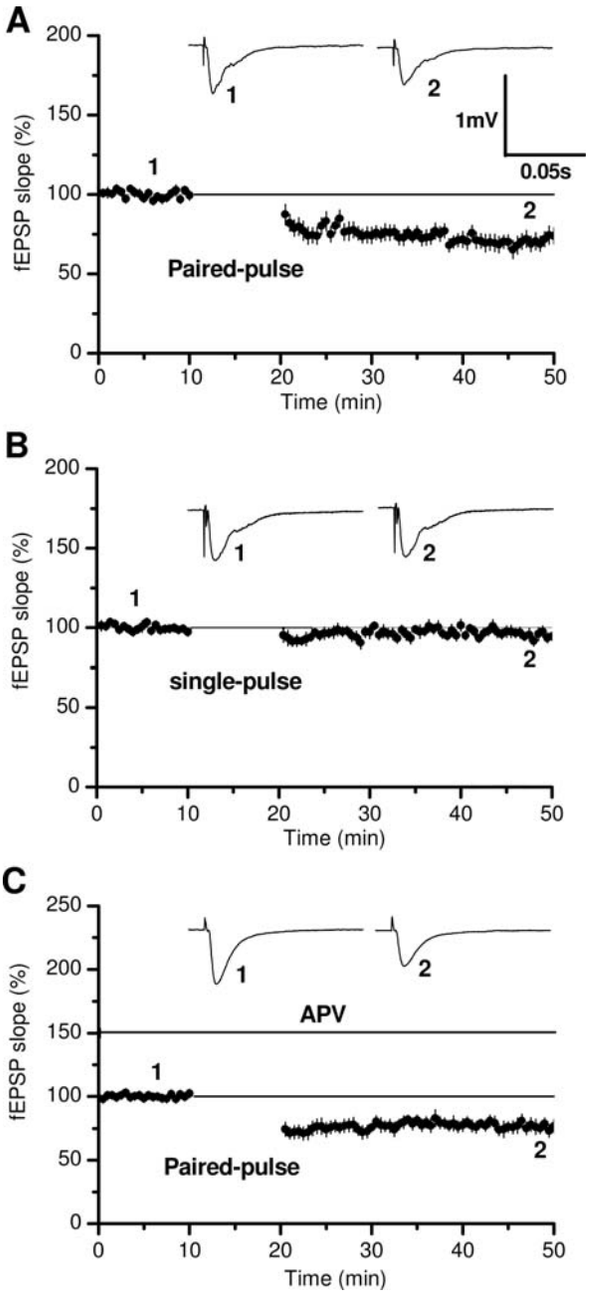


Fig. 1. Application of PP stimulation at low-frequency induced LTD and was NMDAR-independent. **A:** Application of PP stimulation with a 10 ms interval for 10 min resulted in LTD expression. **B:** Single-pulse stimulation did not significantly alter the synaptic efficacy. **C**: Induction of LTD by PP stimulation was NMDAR-independent.



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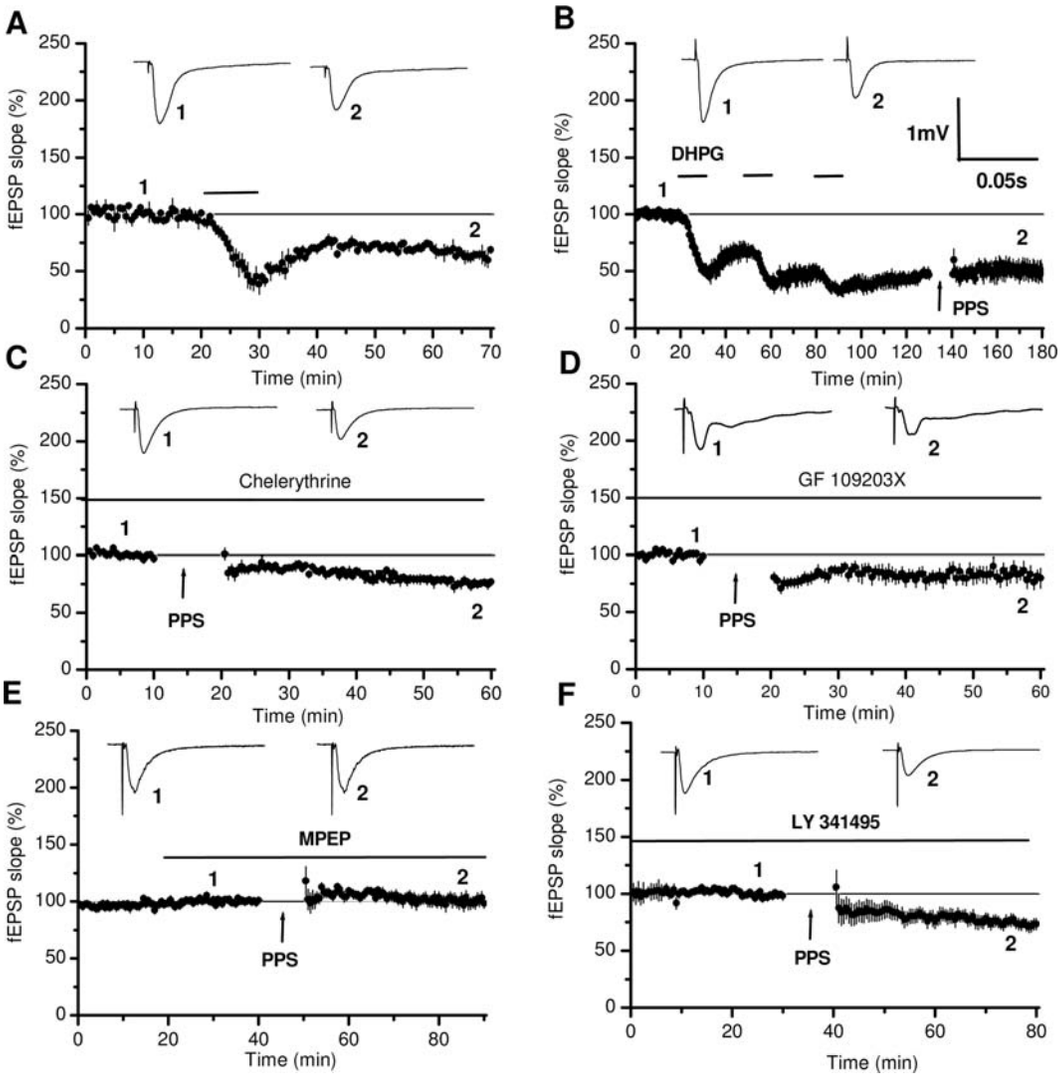


Fig. 2. Paired-pulse (PP) stimulation induced LTD and was NMDA receptor-independent but mGluR5-dependent. **A**: Application of DHPG induced a significant LTD. **B**: PP stimulation did not further induce LTD after DHPG saturation. **C**: DHPG-LTD was found to be PKC-independent by the addition of 3 μM chelerythrine, a PKC blocker. **D**: DHPG-LTD was found to be PKC-independent by the addition of 1 μM GF 109203X, a PKC blocker. **E**: DHPG-LTD was found to be mediated by the mGluR5 subtype of Group I mGluR by application of its blocker, MPEP. **F**: DHPG-LTD was Group II mGluR-independent.

further examined the role of PKC in PP-LTD. Following the addition of 3 μM chelerythrine, a PKC blocker, to the bath, PP-LTD was still inducible in CA1 areas (Fig. 2C, LTD = 78 ± 4% of baseline, $n$ = 6, $P < 0.01$, paired $t$-test). The phenomenon was also observed when using another PKC blocker, GF 109203X (Fig. 2D, LTD = 82 ± 5% of baseline, $n$ = 6, $P < 0.01$, paired $t$-test). Thus, PP-LTD was independent of PKC activation.

DHPG-induced LTD is mediated by the mGluR5 subtype of Group I mGluR (Faas et al., 2002; Mannaioni et al., 2001). Therefore, we examined the effect of the mGluR5 pathway on PP-LTD by applying 5 μM MPEP, a specific mGluR5 blocker. As shown in Figure 2E, under the conditions of an MPEP bath, PP-LTD was completely blocked in CA1 areas (101 ± 5% of baseline, $n$ = 12 slices, $P$ = 0.98, paired $t$-test). We also examined whether Group II mGluRs played any role in PP-LTD. Following the application of 250 nM LY341495, a specific Group II mGluRs blocker (Kingston et al., 1998), PP-LTD was not affected by blocking of the mGluR5 pathway in CA1 areas (LTD = 74 ± 5% of baseline, $n$ = 8 slices, $P < 0.01$, paired $t$-test, Fig. 2F). Taken together, in CA1 areas, LTD induced by the application of 10-min PP stimulation with a 10-ms IPI at 100 Hz was an analog of DHPG-LTD. PP-LTD was mediated by mGluR5 but not by Group II mGluRs.



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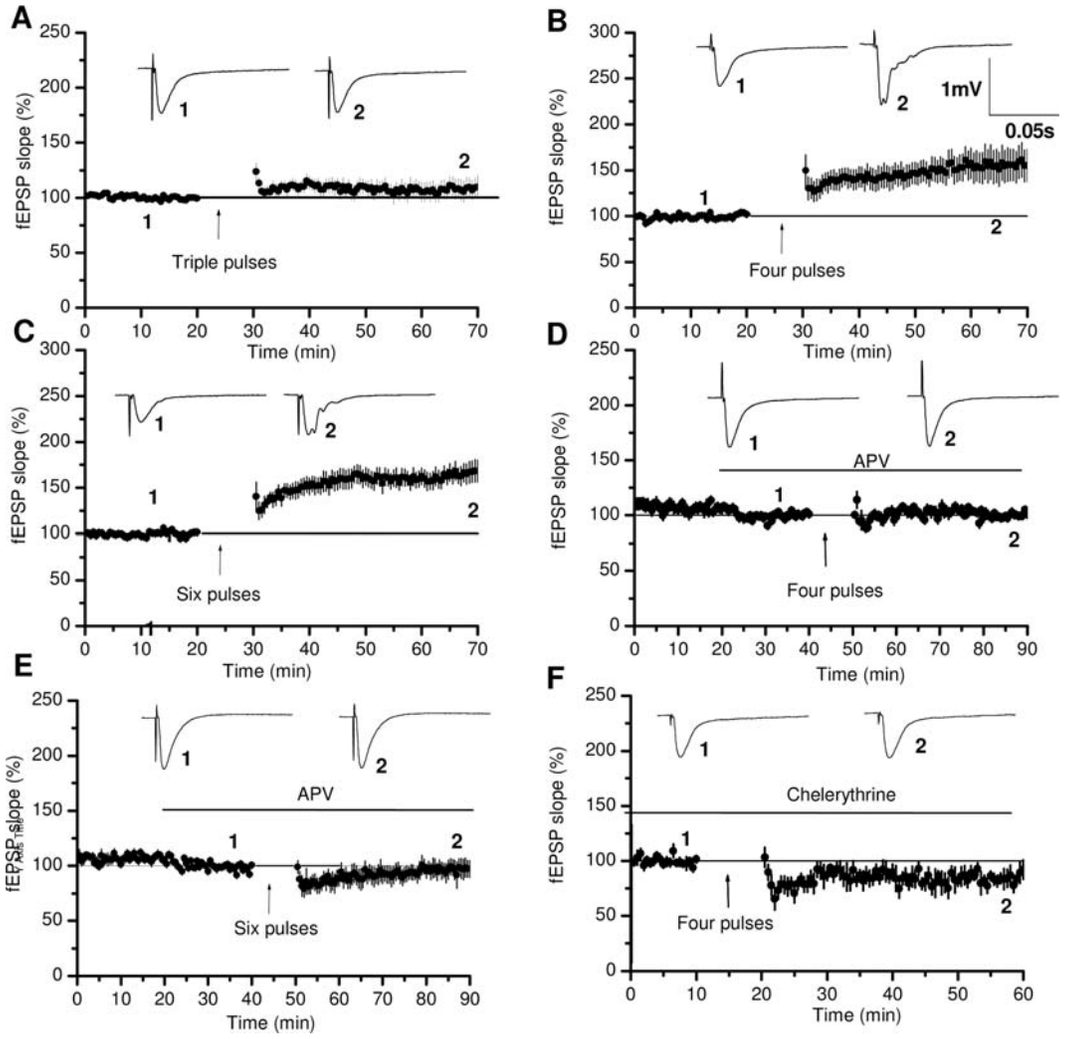


Fig. 3. Pulse stimulation can induce LTP expression by the mechanism of activation of NMDAR and PKC. **A**: Application of conditioned stimulation with a burst of three pulses did not alter synaptic efficacy. **B**: Application of four pulses induced LTP expression. **C:** Application of six pulses induced LTP expression. **D:** The 4-pulse stimulation-induced LTP was NMDA-dependent. **E**: The 6-pulse stimulation-induced LTP was NMDA-dependent. **F**: The 4-pulse stimulation-induced LTP was PKC-dependent.

### Pulse number stimulation bidirectionally regulates synaptic plasticity through activation of NMDA and mGluR in hippocampal CA1 areas

It is well known that the application of a theta burst, which consists of four stimulation pulses at 100 Hz, can induce LTP at CA1 synapses (Yang et al., 2002), whereas the application of two stimulation pulses at 100 Hz induces LTD, as shown here and by Lin et al. (2003). It would therefore be of interest to investigate the effect of application of conditioned stimulation with different pulse numbers on synaptic plasticity. The application of conditioned stimulation with a burst of three pulses at 100 Hz did not result in a significant change in synaptic efficacy (107 ± 9% of baseline, $n$ = 13 slices, $P$ = 0.41, Fig. 3A), while conditioned stimulation with bursts of four or six pulses at 100 Hz induced a significant LTP of 154 ± 16% and 163 ± 10%, respectively (Figs. 3B and 3C). In the conditioned stimulation, bursts of different numbers of pulses were given at 0.167 Hz for 10 min. The LTP induced by conditioned stimulation with bursts of four and six pulses was NMDAR-dependent (Figs. 3D and 3E, 100 ± 2% and 97 ± 8%, n = 8, $P$ = 0.89 and 0.68, respectively). LTP induction was blocked by the addition of 50 μM APV to the bath. These results showed a pulse-number-dependent bidirectional synaptic plasticity at CA1 synapses.

It is likely that LTP of a high magnitude was induced to counteract the expression of LTD mediated by mGluR5 when conditioned stimulation with a large number of pulses was used. Hence, we tested the effect of a specific blocker of mGluR5 on the application of conditioned stimulation with a moderate number of pulses (three pulses) in the burst. Notably, when

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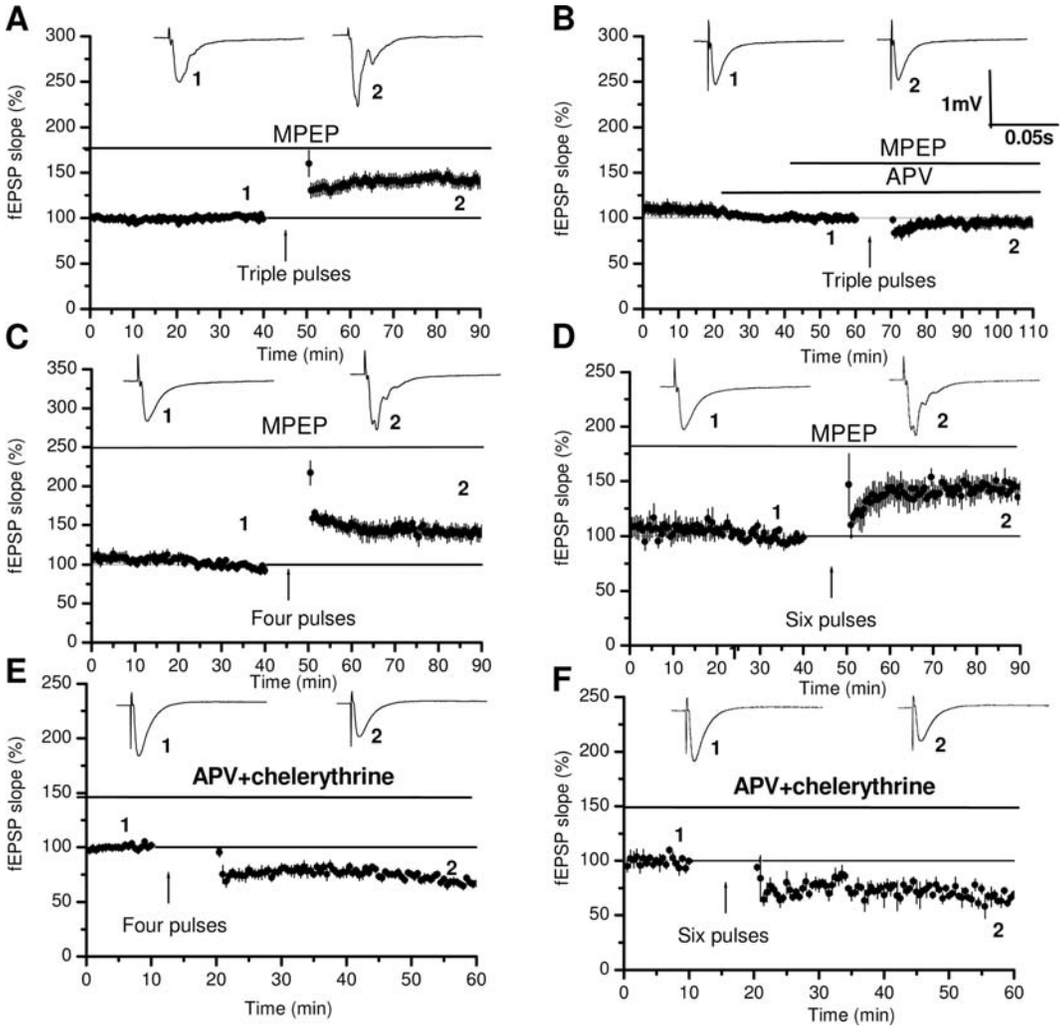


Fig. 4. Pulse number stimulation can alter the synaptic strength by activating NMDAR and mGluR5. **A**: LTP can be induced by the addition of MPEP with 3-pulse stimulation. **B**: LTP induced with MPEP under 3-pulse stimulation was NMDA-dependent. **C**: LTP induced by the application of four pulses was mGluR5-independent. **D**: LTP induced by the application of six pulses was mGluR5-independent. **E**: LTP can be switched to LTD expression by the application of NMDA and PKC blockers with 4-pulse stimulation. **F**: LTP can be switched to LTD expression by the application of NMDA and PKC blockers with 6-pulse stimulation.

blocking the possibility of LTD induction by adding MPEP to the bath, LTP induction by 3-pulse stimulation was observed (142 ± 7% of baseline, $n = 13$ slices, $P < 0.01$, Fig. 4A). This induction of LTP was NMDA-dependent, as demonstrated by applying 50 μM APV to the bath (95 ± 6% of baseline, $n = 11$, $P = 0.39$, Fig. 4B). Interestingly, bath addition of MPEP did not have a significant effect on the magnitude of LTP induced by conditioned stimulation with 4- or 6-pulse bursts (Figs. 4C and 4D, 140 ± 8 and 143 ± 8%, $n = 6$, $P < 0.01$, respectively). These observations were consistent with results that showed that blocking of NMDAR only eliminated LTP induction rather than LTD expression mediated by mGluR5. It has been shown that activation of PKC could cause desensitization of mGluR5, thereby inhibiting DHPG-LTD (Rush et al., 2002). It is possible that PKC is activated by conditioned stimulation with 4- or 6-pulse bursts. LTD was not able to counteract LTP expression, which explained the significant augmentation effect of MPEP application on the magnitude of LTP induced by conditioned stimulation with 4- or 6-pulse bursts. To test this possibility, chelerythrine and APV were both added to the bath to block PKC and NMDAR, respectively. Under these conditions, the application of conditioned stimulation with 4- or 6-pulse bursts resulted in a LTD of 70 ± 2% (Fig. 4E). Similar results were also observed with four-pulse stimulation and chelerythrine application (Fig. 4F, 83 ± 4%, $n = 6$, $P < 0.05$). These results suggested that synaptic efficacy can be bidirectionally regulated by different pulse number stimulation via the activation of NMDA and mGluR5 receptors.



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## DISCUSSION

It is known after long-term study that NMDAR and mGluR can bidirectionally regulate frequency-dependent synaptic plasticity, but it remains unclear whether they can influence pulse number-dependent synaptic plasticity. In this study, the bidirectional plasticity we demonstrated could be modulated through the pulse number, which activated NMDAR and mGluR. When single-pulse stimulation was delivered in hippocampal CA1 areas, no change in synaptic efficacy was observed. With an increase in pulse number to paired pulses with a 10-ms IPI, LTD was successfully induced and maintained for 30 min. PP-LTD was NMDAR-, PKC-, and Group II mGluR-independent but mGluR5-dependent. It was shown that MPEP blocked this induction and no further depression occurred after saturated DHPG-induced LTD. Furthermore, when we altered the pulse number from 3 to 6, the synaptic efficacy was massively altered. With three stimulation pulses, the synaptic strength was no longer depressed but was unchanged as compared with basal stimulation. When four or six stimulation pulses were delivered, the synaptic strength was potentiated, the mechanism of which was through activation of NMDAR and PKC. Furthermore, NMDAR-dependent LTP expression was also observed with 3-pulse stimulation when applying MPEP to unmask the effect of mGluR5 on synaptic plasticity.

It is well-known that field potential oscillations at a theta rhythmic frequency are important for the gleaning of new information about the hippocampus (Huh et al., 2010) and are implicated in novel spatial exploration. Modulation of synaptic efficacy during learning and memory occurs in a short period, with entorhinal-hippocampal network oscillations at a theta frequency playing a large role in this process. Theta burst stimulation, which consists of high-frequency theta oscillations, has been used to induce LTP in in vitro investigations (Yang et al., 2002). Hippocampal neurons are often either silent or discharge a single spike in normal physiological signaling during arousal (O'Keefe, 1976) but fire complex spike bursts in the learning process (Gothard et al., 2001). The high-frequency spike bursts significantly potentiate synaptic plasticity and lead to LTP, learning, and memory recall (Harris et al., 2001; Yang et al., 2002). Under real physiological conditions, locomotion-induced theta oscillations can successfully induce LTP in freely-behaving animals (Orr et al., 2001). This phenomenon is crucial and is usually observed in the hippocampus during exploration. Under pathological conditions, such as Alzheimer's and Parkinson's disease, hippocampal neurons can deliver theta rhythm stimulation but fail to induce LTP, leading to learning deficit (Yang et al., 2002). Here, we have used low-frequency stimulation with different pulse numbers to identify its effect on synaptic plasticity. Our findings suggested that four to six pulses can induce LTP, the mechanism of which is through activating NMDA receptors. In contrast, 2-pulse stimulation can induce LTD by activating mGluR5 receptors.

Recently, it has been reported that altered burst firing patterns in the hippocampus have altered efficacies depending on the distinguishable receptors activated during the stimulation periods - this is called burst plasticity. Alteration of this plasticity does not require synaptic depolarization, especially the activation of AMPA or NMDA receptors; in contrast, it depends on synergistic activation of mGluR1, mGluR5, and muscarinic receptors (Moore et al., 2009). Therefore, it is interesting to examine how the synaptic plasticity of EPSPs is potentiated with increasing numbers of burst firings delivered by a train of somatic current injections. This differs from synaptic plasticity due to the resistance to NMDA blockage (Moore et al., 2009). Accordingly, it is desirable to investigate the relationship between pulse number and synaptic plasticity at a relatively low-frequency stimulation. Our results show that with increasing pulse number, synaptic plasticity can be switched from LTD to basal level and even LTP expression. This important finding is distinguished from previous studies, which show that the synaptic strength can be altered by high-frequency burst stimulation. Our results reveal that synaptic efficacy can also be modulated by relatively low-frequency stimulation with different pulse numbers.

Neurons can undergo potential mechanisms by modifying the firing frequency and spike pattern, referred to as synaptic plasticity. The synaptic plasticity can be potentiated by delivering the pairing of physiologically relevant presynapse with coincident postsynaptic burst discharge of neurons. Previous studies have reported that burst pulses mimicking a natural pattern delivered to CA1 neurons can raise the excitatory recurrent circuit of the CA3 region. King et al. reported that CA1 pyramidal neurons were discharged by direct current injection or extracellular stimulation in rats (King et al., 1999). In the Schaffer collateral branches, pairing physiological activation with burst discharge can significantly increase the excitability and discharge probability. This synaptic plasticity was also observed in the amygdale (Rogan et al., 1997) and in the auditory cortex (Ahissar et al., 1992). Furthermore, it is also well-documented that theta bursts at a high-frequency can clearly induce LTP expression (Yang et al., 2002). Herein, we report that four and six pulses can simulate the natural firing property of the hippocampal Schaffer collateral branches. Stimulation patterns of a low- and high-frequency inducing pulse-number-dependent LTP share the common NMDAR-dependent mechanism

LTD is most commonly induced by prolonged (15 min) low-frequency stimulation, leading to an increase

in the relatively small but long-term influx of $Ca^{2+}$ through NMDAR. Interestingly, brief high-frequency stimulation with partial blockage of NMDAR can also produce LTD (Cummings et al., 1996). Moreover, PP stimulation at low frequency can also induce LTD, the mechanism of which is through activation of mGluR5 and ERK (Volk et al., 2006). The same phenomenon can be also induced by application of mGluR5 agonist DHPG (Huang and Hsu, 2006). These results support the hypothesis that PP stimulation can induce sufficient $Ca^{2+}$ influx, contributing to LTD expression. In contrast, single-pulse stimulation of synapses was not able to generate a postsynaptic spike to activate enough NMDAR to cause a large calcium influx leading to LTD.

Huang et al. reported that the synaptic plasticity induced by PP stimulation at 1 Hz is bidirectional (Huang et al., 2006). The direction of synaptic efficacy depends on the number of stimulation pulses and the timing of pulses. Classical stimulation of a prolonged single pulse at 1 Hz for 15 min can reliably induce LTD in young animals. However, brief PP stimulation for 10 min induces LTD in adult animals (Huang et al., 2006). These results demonstrated that a shorter induction period of 1 to 3 min dramatically induced LTP, whereas a longer period led to LTD; they also suggested that, in 1.5- to 2-month-old mice, PP stimulation can only induce early LTP that decays with time within 90 min. In contrast, the same manipulation induces the expression of late-LTP in 12- to 18-month-old mice (Huang et al., 2006). This stimulation protocol inducing late-LTP is dependent on NMDAR activation and voltage-dependent calcium channels. In comparison, we show that PP stimulation can successfully induce LTD in hippocampal CA1 areas. This result is consistent with previous studies showing that the number of stimulation pulses is important for synaptic strength.

In conclusion, we report that the pulse number can modulate bidirectional plasticity in hippocampal CA1 areas. When PP stimulation was delivered, the synaptic strength was decreased to 68 ± 4% as LTD induction. PP-LTD was found to be mGluR-dependent, because LTD was not further induced in completely DHPG-treated slices. Increasing the pulse number to 4 or 6 potentiated synaptic efficacy; this was NMDAR-dependent but mGluR5-independent, as single- and triple-pulse stimulation could not alter synaptic efficacy further. We suggest that this may due to the balance of activating NMDA and mGluR5 receptors. With the blockage of mGluR5, the strength of the synapse was potentiated to express LTP, and was further blocked by an NMDAR antagonist. Interestingly, following treatment with NMDAR antagonist APV and PKC blocker chelerythrine, the potentiated synaptic strength switched to LTD. We highly suggest that NMDAR and mGluR can bidirectionally modulate the expression of synaptic strength in hippocampal CA1 areas.

## REFERENCES


Ahissar E, Vaadia E, Ahissar M, Bergman H, Arieli A, Abeles M. 1992. Dependence of cortical plasticity on correlated activity of single neurons and on behavioral context. Science 257:1412–1415.

Alarcon JM, Hodgman R, Theis M, Huang YS, Kandel ER, Richter JD. 2004. Selective modulation of some forms of schaffer collateral-CA1 synaptic plasticity in mice with a disruption of the CPEB-1 gene. Learn Mem 11:318–327.

Bear MF, Malenka RC. 1994. Synaptic plasticity: LTP and LTD. Curr Opin Neurobiol 4:389–399.

Beggs JM. 2001. A statistical theory of long-term potentiation and depression. Neural Comput 13:87–111.

Bliss TV, Collingridge GL. 1993. A synaptic model of memory: Long-term potentiation in the hippocampus. Nature 361:31–39.

Cummings JA, Mulkey RM, Nicoll RA, Malenka RC. 1996. $Ca^{2+}$ signaling requirements for long-term depression in the hippocampus. Neuron 16:825–833.

Dudek SM, Bear MF. 1992. Homosynaptic long-term depression in area CA1 of hippocampus and effects of *N*-methyl-D-aspartate receptor blockade. Proc Natl Acad Sci USA 89:4363–4367.

Faas GC, Adwanikar H, Gereau RWt, Saggau P. 2002. Modulation of presynaptic calcium transients by metabotropic glutamate receptor activation: A differential role in acute depression of synaptic transmission and long-term depression. J Neurosci 22: 6885–6890.

Gothard KM, Hoffman KL, Battaglia FP, McNaughton BL. 2001. Dentate gyrus and ca1 ensemble activity during spatial reference frame shifts in the presence and absence of visual input. J Neurosci 21:7284–7292.

Harris KD, Hirase H, Leinekugel X, Henze DA, Buzsaki G. 2001. Temporal interaction between single spikes and complex spike bursts in hippocampal pyramidal cells. Neuron 32:141–149.

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

Huang YY, Kandel ER. 2006. Age-related enhancement of a protein synthesis-dependent late phase of LTP induced by low frequency paired-pulse stimulation in hippocampus. Learn Mem 13:298–306.

Huh CY, Goutagny R, Williams S. 2010. Glutamatergic neurons of the mouse medial septum and diagonal band of broca synaptically drive hippocampal pyramidal cells: Relevance for hippocampal theta rhythm. J Neurosci 30:15951–15961.

Kemp N, Bashir ZI. 1999. Induction of LTD in the adult hippocampus by the synaptic activation of AMPA/kainate and metabotropic glutamate receptors. Neuropharmacology 38:495–504.

Kemp N, Bashir ZI. 2001. Long-term depression: A cascade of induction and expression mechanisms. Prog Neurobiol 65:339–365.

King C, Henze DA, Leinekugel X, Buzsaki G. 1999. Hebbian modification of a hippocampal population pattern in the rat. J Physiol 521:159–167.

Lin YW, Min MY, Chiu TH, Yang HW. 2003. Enhancement of associative long-term potentiation by activation of beta-adrenergic receptors at CA1 synapses in rat hippocampal slices. J Neurosci 23:4173–4181.

Lin YW, Yang HW, Min MY, Chiu TH. 2008. Inhibition of associative long-term depression by activation of beta-adrenergic receptors in rat hippocampal CA1 synapses. J Biomed Sci 15:123–131.

Lin YW, Yang HW, Wang HJ, Gong CL, Chiu TH, Min MY. 2006. 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. Eur J Neurosci 23:2362–2374.

Malenka RC, Bear MF. 2004. LTP and LTD: An embarrassment of riches. Neuron 44:5–21.

Malenka RC, Nicoll RA. 1999. Long-term potentiation–A decade of progress? Science 285:1870–1874.

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

Moore SJ, Cooper DC, Spruston N. 2009. Plasticity of burst firing induced by synergistic activation of metabotropic glutamate and acetylcholine receptors. Neuron 61:287–300.

Mulkey RM, Endo S, Shenolikar S, Malenka RC. 1994. Involvement of a calcineurin/inhibitor-1 phosphatase cascade in hippocampal long-term depression. Nature 369:486–488.

Mulkey RM, Herron CE, Malenka RC. 1993. An essential role for protein phosphatases in hippocampal long-term depression. Science 261:1051–1055.

Mulkey RM, Malenka RC. 1992. Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. Neuron 9:967–975.

O'Keefe J. 1976. Place units in the hippocampus of the freely moving rat. Exp Neurol 51:78–109.

Orr G, Rao G, Houston FP, McNaughton BL, Barnes CA. 2001. Hippocampal synaptic plasticity is modulated by theta rhythm in the fascia dentata of adult and aged freely behaving rats. Hippocampus 11:647–654.

Otani S, Connor JA. 1998. Requirement of rapid $Ca^{2+}$ entry and synaptic activation of metabotropic glutamate receptors for the induction of long-term depression in adult rat hippocampus. J Physiol 511:761–770.

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

Rogan MT, Staubli UV, LeDoux JE. 1997. Fear conditioning induces associative long-term potentiation in the amygdala. Nature 390:604–607.

Rush AM, Wu J, Rowan MJ, Anwyl R. 2002. Group I metabotropic glutamate receptor (mGluR)-dependent long-term depression mediated via p38 mitogen-activated protein kinase is inhibited by previous high-frequency stimulation and activation of mGluRs and protein kinase C in the rat dentate gyrus in vitro. J Neurosci 22:6121–6128.

Schnabel R, Kilpatrick IC, Collingridge GL. 1999. An investigation into signal transduction mechanisms involved in DHPG-induced LTD in the CA1 region of the hippocampus. Neuropharmacology 38:1585–1596.

Schnabel R, Kilpatrick IC, Collingridge GL. 2001. Protein phosphatase inhibitors facilitate DHPG-induced LTD in the CA1 region of the hippocampus. Br J Pharmacol 132:1095–1101.

Schnabel R, Palmer MJ, Kilpatrick IC, Collingridge GL. 1999. A CaMKII inhibitor. KN-62, facilitates DHPG-induced LTD in the CA1 region of the hippocampus. Neuropharmacology 38:605–608.

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

Valli MJ, Schoepp DD, Wright RA, Johnson BG, Kingston AE, Tomlinson R, Monn JA. 1998. Synthesis and metabotropic glutamate receptor antagonist activity of N1-substituted analogs of 2R,4R-4-aminopyrrolidine-2,4-dicarboxylic acid. Bioorg Med Chem Lett 8:1985–1990.

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

Yang HW, Lin YW, Yen CD, Min MY. 2002. Change in bi-directional plasticity at CA1 synapses in hippocampal slices taken from 6-hydroxydopamine-treated rats: The role of endogenous norepinephrine. Eur J Neurosci 16:1117–1128.

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