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Postsynaptic induction of mossy fibre long term depression in developing rat hippocampus

János Györi, Marco Atzori and Enrico Cherubini[CA]

Biophysics Sector, International School for Advanced Studies (SISSA), Via Beirut 2-4, 34014 Trieste, Italy

[CA]Corresponding Author

THE whole cell configuration of the patch clamp technique was used to study the mechanisms of induction of long term depression (LTD) occurring at the mossy fibre–CA3 synapse between postnatal (P) day 6 and P13. In control conditions, when two pulses were delivered to the mossy fibres with an interval of 50 ms a potentiation of the EPSC evoked by the second pulse associated with a reduction in the number of failures was observed. Tetanization of the mossy fibres induced LTD of the responses to the first and second stimulus without affecting the paired pulse facilitation. Loading the postsynaptic cell with BAPTA prevented the induction of LTD but did not modify the paired pulse facilitation, suggesting that LTD induction occurs at the postsynaptic site.

Key Words: LTD; Development; Hippocampus; CA3 region; BAPTA; Excitatory postsynaptic currents; Failures; Patch clamp

Introduction

Activity-dependent processes such as those occurring during long term potentiation (LTP) or long term depression (LTD) play a crucial role during development, strengthening synaptic connections and contributing to the final tuning of the neuronal circuitry.[1] In the hippocampus, the expression of LTP or LTD changes markedly during development: LTD, which consists of a long lasting use-dependent decrease in synaptic efficacy, is more prominent in early postnatal life and precedes the developmental onset of LTP.[2,3] In the CA1 hippocampal region, both a non-NMDA and a NMDA-dependent type of LTD have been described.[2,4] The former is usually present during the first postnatal week and is induced by repetitive stimulation (5 Hz for 3 min) of the Schaffer collaterals whereas the latter occurs during the second postnatal week and is produced by a low frequency stimulation of the afferent pathway maintained for a prolonged period of time (1 Hz for 5–10 min). In the CA3 area of the hippocampus, during a critical period of postnatal development, between postnatal (P) day 6 and P14, we have recently found that a high frequency stimulation (HFS) train to the mossy fibres (100 Hz for 1 s) induces LTD of the field EPSPs which is NMDA-independent. After the second postnatal week, the same high frequency stimulation train induces LTP, as in adult neurones.[3] In this study we have used the patch clamp technique (whole cell configuration) to examine whether induction of this novel form of LTD involves pre- or postsynaptic mechanisms.

Material and Methods

Hippocampal slices (250–300 μm) were prepared from P6–P13 Wistar rats and superfused with ACSF containing (in mM): NaCl 126, KCl 3.5, $NaH_2PO_4.H_2O$ 1.2, $MgCl_2.6H_2O$ 1.3, $CaCl_2$ 2, $NaHCO_3$ 25, glucose 11, equilibrated with 95% O_2 and 5% CO_2 (pH 7.3) at 21–23°C. Whole cell recordings[5] were obtained from CA3 pyramidal neurones. Patch electrodes (3–5 MΩ) contained (in mM): $KMeSO_4$ 124, KCl 3, K_2ATP 1.5, $MgCl_2.6H_2O$ 3, HEPES 10; the pH was adjusted to 7.3 with KOH. In four experiments, to buffer $[Ca^{2+}]$ near its normal resting concentration, 1,2-bis(*o*-aminophenoxy)-ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA; 20 mM) was included in the pipette solution. In this case $CaCl_2$ 1 mM was also present. Membrane currents were recorded with a standard patch clamp amplifier (EPC-7, List Medical, Darmstadt, Germany) after optimizing capacitance and series resistance compensation. Typical values for series resistance compensation ranged between 10 and 15 MΩ; they were checked for stability throughout the experiment. Afferent fibres were stimulated at 0.06 Hz with a patch pipette (3–6 MΩ), filled with ACSF, positioned on the mossy fibre tract, close (~100 μm) to the recording pipette. To minimize the contribution of GABA-mediated synaptic currents, neurones were held at −70 mV, which is the calculated reversal potential value for Cl^-. Paired pulses (50 ms interval, 40–70 μs duration) were usually applied. The stimulation intensity was adjusted above threshold for eliciting EPSCs with a failure rate of 10–30% and was

kept constant throughout the exp
number of failures was obtained by
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tical to that of the background noise
To elicit LTD, one or two brief (1 s) h
(100 Hz) stimulation trains were applie
in current clamp conditions. To avoid
with NMDA-dependent forms of syna
the NMDA receptor antagonist (+)-3-(
azin-4-yl)-propyl-1-phosphonic acid
gift of Dr P.L. Herrling, Sandoz, Ba
applied for 10 min before and during
quency stimulation train. Acquisiti
analysis were performed with a pClam
Instruments, Foster City, CA, USA) s
a Digidata 1200 AD/DA board. Signals
at 5 kHz, filtered at 2 kHz and stored
recorder. EPSCs were analysed off-lir
ms window at the peak of the event,
baseline taken immediately before the
fact. The decay time constant of syn
was calculated by least square fitting o
records with a single exponential fu
recordings lasting > 1 h were obtainec
in 13 slices from nine rats. Data are exp
± s.e.m.

Results

Excitatory postsynaptic currents
recorded at −70 mV from CA3 pyran
in slices from P6–P13 rats, following
the mossy fibre tract. Under these co
difficult to elicit only a single fibre EP
with low stimulus intensity it was p
vate only a small number of synapse
tuated in amplitude from trial to trial
pA) and were associated with a 10–30
EPSCs had a 20–80% rise time of
latency of 1.8 and 2.2 ms and a decay p
be fitted by a single exponential with a
constant of 20.1 ± 4.5 ms. These value
those of the composite EPSC evoked
midal cells by extracellular stimulatio
fibre tract in which a major fast AMP
a minor slow NMDA-receptor-mediat
can be detected.[7] In the absence of
protocol, no run down of synaptic
observed for at least 60 min (Fig. 1A
experiments, two pulses, 50 ms apart,
to the mossy fibres in order to stud
facilitation, which is a widely acce
presynaptic function.[8] As shown in Fig
facilitation of the average response to
ulus was obtained (156 ± 13%). One

kept constant throughout the experiment. The number of failures was obtained by counting the number of responses that fell into the 0 pA bin in the amplitude distribution histogram with a s.d. identical to that of the background noise (1.9 ± 0.3 pA). To elicit LTD, one or two brief (1 s) high frequency (100 Hz) stimulation trains were applied (1 min apart) in current clamp conditions. To avoid contamination with NMDA-dependent forms of synaptic plasticity[6] the NMDA receptor antagonist (+)-3-(carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP, 20 μM; gift of Dr P.L. Herrling, Sandoz, Basel) was bath-applied for 10 min before and during the high frequency stimulation train. Acquisition and data analysis were performed with a pClamp 6.0.2 (Axon Instruments, Foster City, CA, USA) software, using a Digidata 1200 AD/DA board. Signals were sampled at 5 kHz, filtered at 2 kHz and stored on a video tape recorder. EPSCs were analysed off-line, using a 1–2 ms window at the peak of the event, relative to the baseline taken immediately before the stimulus artifact. The decay time constant of synaptic currents was calculated by least square fitting of experimental records with a single exponential function. Stable recordings lasting > 1 h were obtained from 13 cells in 13 slices from nine rats. Data are expressed as mean ± s.e.m.

Results

Excitatory postsynaptic currents (EPSCs) were recorded at −70 mV from CA3 pyramidal neurones in slices from P6–P13 rats, following stimulation of the mossy fibre tract. Under these conditions it was difficult to elicit only a single fibre EPSC.[7] However, with low stimulus intensity it was possible to activate only a small number of synapses. EPSCs fluctuated in amplitude from trial to trial (range 15–120 pA) and were associated with a 10–30% failure rate. EPSCs had a 20–80% rise time of 1.7 ± 0.3 ms, a latency of 1.8 and 2.2 ms and a decay phase that could be fitted by a single exponential with an average time constant of 20.1 ± 4.5 ms. These values are similar to those of the composite EPSC evoked in CA3 pyramidal cells by extracellular stimulation of the mossy fibre tract in which a major fast AMPA/kainate- and a minor slow NMDA-receptor-mediated component can be detected.[7] In the absence of a conditioning protocol, no run down of synaptic currents was observed for at least 60 min (Fig. 1A). In 10 of 13 experiments, two pulses, 50 ms apart, were delivered to the mossy fibres in order to study paired-pulse facilitation, which is a widely accepted index of presynaptic function.[8] As shown in Figure 1B, a clear facilitation of the average response to second stimulus was obtained (156 ± 13%). One or two HFS

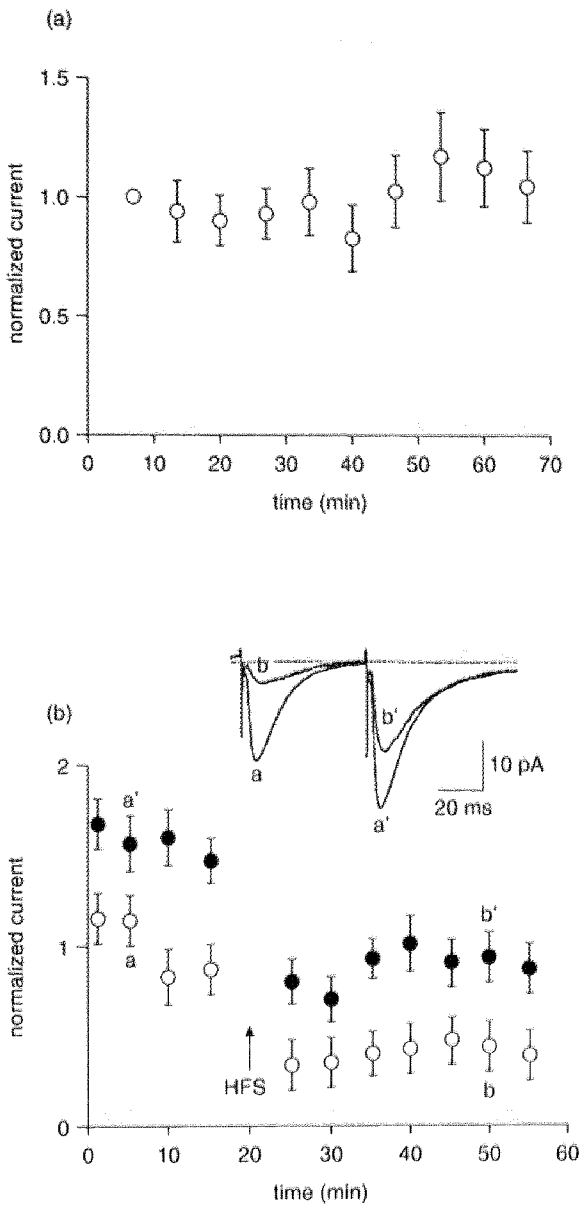

FIG. 1 (**A**). In the absence of conditioning protocol, the amplitude of EPSCs evoked by stimulation of the mossy fibres every 20 s (for a period of 70 min) was unchanged. EPSCs recorded at −70 mV from a CA3 pyramidal neurone at P7. Each point is the average of 20 responses. Bars represent s.d. Amplitudes are expressed as percentage of values averaged over the first 10 min. (**B**). Time course of the amplitude changes of EPSCs recorded at −70 mV from a CA3 pyramidal neurone (P8), evoked by a pair of stimuli delivered at 50 ms intervals (0.06 Hz) to the mossy fibres, before and after tetanization of the afferent pathway (arrow). First EPSC: open circle; second EPSC: closed circle. Amplitudes are expressed as percentage of values averaged over 15 min before the HFS. Each point is the average of 15 responses. Bars represent s.d. Inset shows the average of 10 responses taken at the time indicated by the letters above the graph.

trains to the mossy fibres (in the presence of the selective NMDA antagonist CPP, 20 μM) induced in 6 of 10 neurones a depression in the amplitude of the responses to both the first and second stimulus that persisted for the whole recording period, lasting > 30 min post-tetanus (Fig. 1B). On average, the depression of the first EPSC 30 min after tetanization was

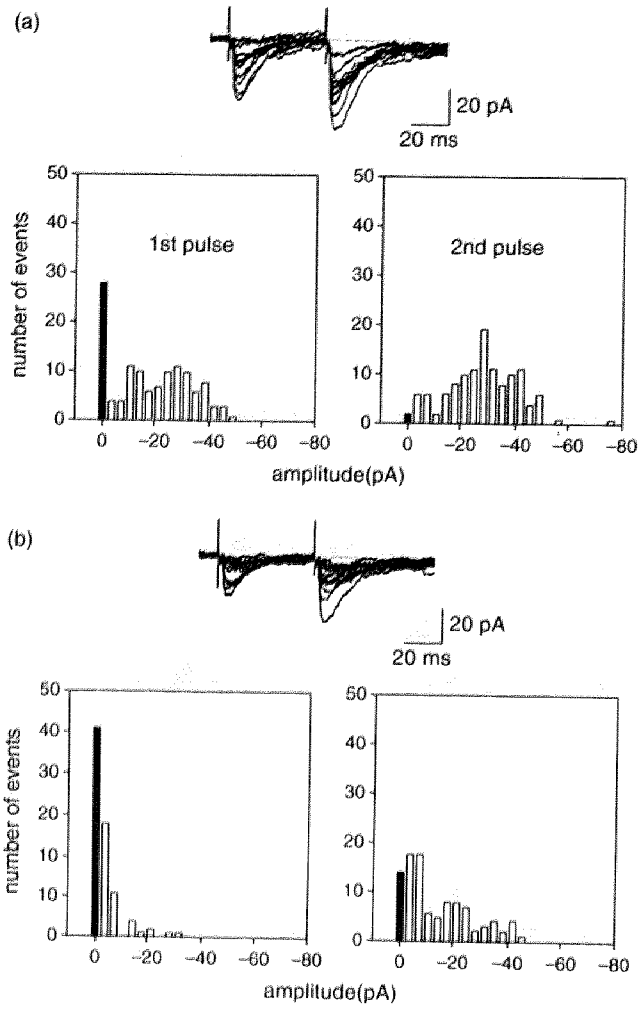

FIG. 2. Amplitude distribution histograms of EPSCs evoked by paired-pulse stimulation before (**A**) and after (**B**) tetanization of the mossy fibres in a P8 animal. In each graph, the number of events was 100, taken in 27 min epochs. Bin width was 3.2 pA. Black columns represent the number of failures. In the insets above the graphs, samples of 10 consecutive responses before and are tetanization are superimposed.

25 ± 5.6% ($n = 6$). However, the paired-pulse facilitation ratio was not affected by the train (1.80 before the tetanus; 1.75 40 min after the tetanus). LTD was produced only when the HFS train was delivered in current clamp conditions, thus allowing the postsynaptic neurone to depolarize. When CA3 pyramidal neurones were voltage-clamped at −70 mV during the tetanus, LTD did not occur. In two cells only a short-term depression (lasting < 20 min) of EPSC amplitude was observed. Figure 2A shows the amplitude distribution histogram of 100 consecutive responses (taken at the frequency of 0.06 Hz in 27 min epochs, before and after tetanization of the afferent pathway) to the first or second stimulus. The black columns represent the number of failures. It is clear from the graphs that, in control conditions when a stimulation intensity just above threshold was used, a fluctuation in amplitude of both the first and the second EPSC was present. The graphs also show a drastic reduction in the number of failures in response to the second stimulus, and an increased number of larger amplitude events, as expected for a presynaptic phenomenon such as the paired-pulse facilitation. After the tetanus, the amplitude distribution histogram of the first response was characterized by a significant ($p < 0.05$, Student's t-test) increase in the number of failures (161 ± 39%) and a marked reduction of larger amplitude events while the amplitude distribution histogram of the second response was

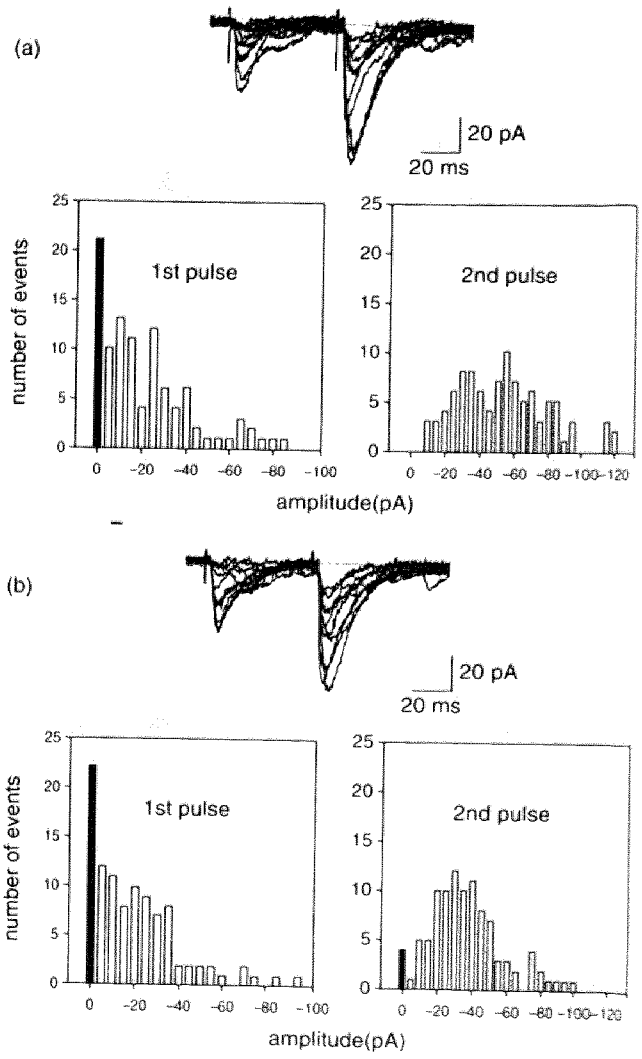

FIG. 3. Amplitude distribution histograms of EPSCs (recorded with a patch pipette containing 20 mM of BAPTA) evoked by paired-pulse stimulation before (**A**) and after (**B**) tetanization of the mossy fibres in a P11. In each graph, the number of events was 100 taken in 27 min epochs. Bin width was 4 pA. Black columns represent the number of failures. In the insets above the graphs, samples of 10 consecutive responses before and after tetanization are superimposed. With intracellular BAPTA, LTD was blocked but paired-pulse facilitation was still present.

characterized by an increase of larger amplitude events with no significant ($p > 0.5$) changes in the relative number of failures (7% in control conditions and 26% after the HFS train; Fig. 2B). Similar effects were seen in four cells. To determine whether LTD induction requires a rise in postsynaptic calcium concentration, cells were loaded with the calcium chelator BAPTA (20 mM). In these conditions tetanization of the mossy fibres failed to induce LTD (Fig. 3). The distribution amplitude of EPSCs evoked by the first or second pulse was unchanged after tetanization. In three patches, the mean peak EPSC evoked by the first pulse was 16.9 ± 5.2 pA or 16.4 ± 4.8 pA before or after tetanization, respectively (the average includes also the failures). Moreover, no significant ($p > 0.5$) change in the number of failures to the first stimulus was detected after HFS (20.6% or 21.7% before or after tetanization, respectively). As in control conditions, BAPTA caused a potentiation of the amplitude of the EPSC elicited by the second pulse, associated with a reduction in the number of failures.

Discussion

The present data clearly show that induction of LTD at the mossy fibre–CA3 synapse during a critical period of postnatal development involves a postsynaptic mechanism, and that a rise in intracellular calcium is essential for this phenomenon. In previous experiments with extracellular field potentials we have emphasized the role of calcium in the genesis of LTD.[3] However in that study it was not possible to specify whether calcium changes occurred at the pre- or postsynaptic level. Our experiments with BAPTA confirm a role for postsynaptic calcium in LTD induction. A rise in intracellular calcium can be achieved through activation of ligand- or voltage-gated calcium channels or through release from intracellular stores following activation of metabotropic receptors. Among ligand-gated channels, the NMDA receptor is of special interest due to its high permeability to calcium.[9] We can exclude the participation of NMDA receptors in LTD induction, since the afferent pathway was tetanized in the presence of the selective NMDA receptor antagonist CPP. A rise in intracellular calcium was achieved during membrane depolarization, as suggested by the fact that LTD was blocked when the postsynaptic cell was voltage clamped at –70 mV. A rise in intracellular calcium following mGluR activation may also contribute to the induction of LTD as demonstrated for LTD occurring at the CA3–CA1 synapse during the first postnatal week.[4] In a previous unpublished study, using extracellular field potentials, tetanization of the mossy fibres in the presence of the selective mGluR antagonist MCPG (1 mM for 20 min, $n = 12$) was still able to induce LTD, thus ruling out the possibility that activation of mGluRs contributes to LTD induction. Further evidence in favour of a postsynaptic mechanism for LTD induction is provided by the observations that a similar paired-pulse facilitation ratio was present before and after LTD, and also, that after the HFS train the coefficient of variation significantly ($p < 0.05$) increased from 0.36 ± 0.03 to 0.59 ± 0.14, $n = 3$ (but see ref. 10 for reservations about this method). Arguing against a postsynaptic mechanism is the unexpected finding that LTD was associated with an increase in the relative proportion of failures when a minimal stimulation was used to activate a small number of synapses. According to the classical probabilistic theory of transmitter release, this effect has been attributed to a presynaptic mechanism[11] (when an action potential fails to invade the presynaptic terminal). However, this effect can be also interpreted as a failure of postsynaptic reception. As recently suggested for LTP or LTD, a decrease or increase in the number of failures may reflect activation or inactivation of previous silent or active synapses, with up- or down-regulation of AMPA receptors.[12–15] A quantal analysis of spontaneous and evoked EPSCs, precluded in the present experiments by the low number of events, will help to further elucidate this problem. Finally we cannot excluded the possibility that induction of LTD is predominantly a postsynaptic phenomenon but that its expression occurs mainly at the presynaptic site.

Conclusions

This study clearly demonstrates that induction of LTD at the mossy fibre–CA3 synapse during a critical period of postnatal development is a postsynaptic phenomenon that involves changes in intracellular calcium in the postsynaptic cell. Tetanization of the mossy fibre tract would depolarize the postsynaptic membrane and cause a rise in intracellular calcium through activation of voltage-dependent calcium channels. Functional changes in synaptic connections, mainly at the mossy fibre level,[16] may regulate calcium fluxes and consequently the level of protein phosphorylation, leading to the development of LTD or LTP, respectively.

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References

1. Bear ME and Malenka RC. *Curr Opin Neurobiol* **4**, 389–399 (1994).
2. Dudek SM and Bear MF. *J Neurosci* **13**, 2910–2918 (1993).
3. Battistin T and Cherubini E. *Eur J Neurosci* **6**, 1750–1755 (1994).
4. Bolshakov VY and Siegelbaum SA. *Science* **264**, 1148–1152 (1994).
5. Hamill OP, Marty A, Neher E *et al. Plügers Arch* **391**, 85–100 (1981).
6. Zalutsky RA and Nicoll RA. *Science* **248**, 1619–1624 (1990).
7. Jonas P, Major G and Sakmann B. *J Physiol* **472**, 615–663 (1993).
8. Zucker RS. *Rev Neurosci* **12**, 13–31 (1989).
9. Mayer ML and Westbrook GL. *J Physiol* **394**, 501–527 (1987).
10. Faber DS and Korn H. *Biophys J* **60**, 1288–1294 (1991).
11. Katz B. *The Release of Neural Transmitter Substances*. Liverpool: Liverpool University Press, 1969.
12. Edwards F. *Nature* **350**, 271–272 (1991).
13. Kullmann DM. *Neuron* **12**, 1111–1120 (1994).
14. Malinow R. *Science* **266**, 1195–1196 (1994).
15. Liao D, Hessler NA and Malinow R. *Nature* **375**, 400–404 (1995).
16. Gaiarsa JL, Beadoin M and Ben Ari Y. *J Comp Neurol* **321**, 612–625 (1992).

General Summary

The whole cell configuration of the patch clamp technique was used to study the mechanisms of induction of long term depression (LTD) at the mossy fibre–CA3 synapse between postnatal days 6 and 13. Low intensity stimulation of the mossy fibre tract elicited EPSCs that fluctuated in amplitude from trial to trial and were associated with a 10–30% failure rate. When two stimuli were applied 50 ms apart a facilitation of the response to the second one, associated with a reduction in the number of failures, was observed. One or two HFS trains produced a LTD of the response to the first and second stimulus without affecting the paired pulse facilitation ratio. In cells loaded with BAPTA, the same HFS train failed to induce LTD but did not modify the paired pulse facilitation ratio. These data suggest that LTD induction occurs at the postsynaptic site.