NeuroReport 10, 3869–3874 (1999)

NMDA receptor blockade prevents LTD, but not LTP induction by intracellular tetanization

Marina Chistiakova,[1,2] Pavel Balaban,[2] Ulf T. Eysel[1] and Maxim Volgushev[1,2,CA]

[1]Department of Neurophysiology, Ruhr-University Bochum, MA4/149, D-44801 Bochum, Germany; [2]Institute of Higher Nervous Activity and Neurophysiology RAS, 117865 Moscow, Russia

[CA,1]Corresponding Author and Address

INTRACELLULAR tetanization, the activation of a postsynaptic cell without concomitant presynaptic stimulation, was applied to layer II/III pyramidal cells in slices of rat visual cortex. In standard extracellular medium, intracellular tetanization led to LTP (21 of 43 inputs) or LTD (14 of 43 inputs), the direction of the amplitude change depending on initial paired-pulse facilitation (PPF) ratio: inputs with high initial PPF ratio were usually potentiated, and inputs with initially low PPF were most often depressed or did not change. When applied during blockade of NMDA receptors (50 μM APV), intracellular tetanization failed to induce LTD, but was still capable of inducing LTP (14 of 26 inputs). Although LTP could occur in inputs with both, low and high initial PPF ratio, the correlation between the amplitude change and initial PPF ratio remained: potentiation was stronger in inputs with initially higher PPF. These data suggest that intracellular tetanization activated simultaneously NMDA receptor-dependent LTD mechanisms and NMDA receptor-independent LTP mechanisms, the final change of synaptic gain depending on their balance. *NeuroReport* 10:3869–3874
© 1999 Lippincott Williams & Wilkins.

Key words: Intracellular tetanization; Long-term depression (LTD); Long-term potentiation (LTP); NMDA receptors; Paired-pulse facilitation (PPF); Rat; Synaptic plasticity; Visual cortex

Introduction

Long-term potentiation (LTP) and long-term depression (LTD) of synaptic transmission in the hippocampus and neocortex can be induced with a number of different induction protocols. It had been demonstrated recently that even purely postsynaptic challenges which do not include presynaptic stimulation, for example depolarizing pulses applied to a postsynaptic cell [1–3] or photolysis of pre-loaded Ca^{2+} cages inside a cell [4] are able of induction of both LTP and LTD. These potentiation and depression expressed mutual interaction with synaptic changes induced with conventional protocols, suggesting involvement of at least some common mechanisms [1,4,5]. Several forms of long-term plasticity induced by afferent tetanization depend on activation of NMDA receptors [6,7]. In this study we examined whether induction of LTP and LTD by intracellular tetanization is affected by NMDA receptor blockade.

Materials and Methods

Slices (350 μm) of the visual cortex of 4- to 6-week-old rats were prepared by conventional methods [8] and investigated under submerged conditions at 30°C. Perfusion medium contained (in mM) 125 NaCl, 2.5 KCl, 2 $CaCl_2$, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 1.5 $MgCl_2$ and 25 D-glucose bubbled with 95% O_2 and 5% CO_2. Intracellular recordings were obtained with glass microelectrodes (70–100 MΩ) filled with 2.5 M potassium acetate, in some experiments with addition of biocytin (1%, Sigma). Electrical stimuli (1–20 μA, 40–100 μs) were applied as paired pulses at an interval of 50 ms through bipolar tungsten electrodes positioned laterally (layers I–II) and below (layer IV) the recording site. Stimulation intensity was set just above threshold for the elicitation of an EPSP to minimize the number of activated presynaptic fibres and was kept constant throughout the experiment. In the first series of experiments after a control testing period of 20–40 min, intracellular tetanization was applied without presynaptic stimulation. Tetani consisted of three trains (1/min) of 10 bursts (1/s) of 20 depolarizing pulses (0.5–1.8 nA, 10 ms, 50 Hz). Current was adjusted so that the first 2–5 pulses in a burst evoked spikes. A typical response of a cortical cell to one burst of intracellular pulses is illustrated in Fig. 1. In the second series of experiments the same procedure was applied under conditions of NMDA receptors blockade with 50 μM D-APV (Tocris Cookson Ltd).

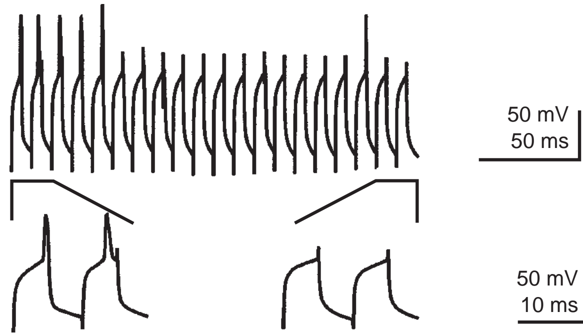

FIG. 1. Response of a cortical cell to one burst of depolarizing pulses (1.1 nA, 10 ms, 50 Hz) Intracellular tetanization consisted of 3 trains (1/min) of 10 (1/s) such bursts. Lower traces show parts of responses at expanded time scale, as indicated.

After amplification (Axoclamp 2A) the data were fed into computer (PC-486; Digidata-1200; PClamp software, Axon instruments). The EPSP amplitudes were measured as a difference between the membrane potential within two 1–3 ms windows, one positioned immediately before the EPSP, and the other one over the last third of the initial slope of averaged response, or covering the peak. Only cells without significant drift of EPSP amplitude during the control period (15–30 min before the tetanization) and with stable membrane potential more negative than −70 mV were subject to analysis. Paired-pulse facilitation was calculated as the ratio between the mean amplitude of responses evoked by the second and the first stimulus in paired-pulse paradigm. Amplitude changes were assessed from responses to the first stimulus in a pair. For correlation analysis peak post-tetanic changes, measured over a 5–10 min period when amplitude changes were maximal, starting 10–50 min post-tetanus, were used [9]. The t-test and Mann–Whitney test were used to evaluate the significance of differences. We accepted differences as significant at $p < 0.05$, unless otherwise specified.

Results

Intracellular recordings with sharp electrodes were made from 39 layer II/III cells in slices of rat visual cortex. During the course of that and related studies about 30 cells were labelled with biocytin. All these cells were identified morphologically as pyramidal cells, allowing us to conclude that under our recording conditions pyramidal cells were selectively sampled.

Synaptic responses were evoked in recorded cells with two pairs of bipolar stimulating electrodes, positioned below (layer IV) and aside (layer II/III) of the recording electrode. Responses from both stimulation sites were recorded in 30 cells and from only one of the sites in the remaining nine neurones.

Intracellular tetanization, which consisted of three trains of bursts of short depolarizing pulses, was applied to the postsynaptic cell without concomitant stimulation of the synaptic inputs and led to long-lasting changes of synaptic transmission [2,8].

In the first series of experiments the effect of intracellular tetanization on synaptic transmission was studied in a control medium. In an example shown in Fig. 2A, the response amplitude increased after the intracellular tetanization, on average to 155% of the pretetanic value. In the other example, the response amplitude decreased moderately, but clearly, after intracellular tetanization, on average to 79% (Fig. 2B). Altogether, long-term changes of synaptic transmission were observed in 35 of 43 inputs studied in this series. Potentiation was observed in 21 inputs, depression in 14 inputs, and the response amplitude did not change significantly in the remaining eight inputs.

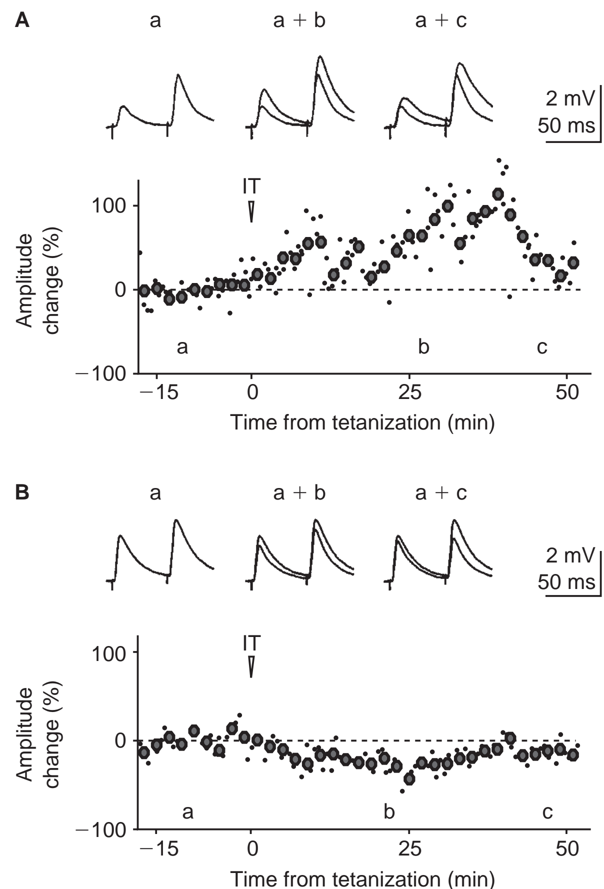

FIG. 2. Changes of synaptic transmission induced by intracellular tetanization in control experiments. (**A**) LTP induced by IT in the input with high initial PPF ratio (2.52). Averaged responses ($n = 50$) evoked by paired stimulation before (a) and after (a + b; a + c) tetanization, as indicated. Time course of the amplitude changes of averaged responses ($n = 5$ for points; $n = 10$ for circles) evoked by the first stimulus in a pair before (a) and after (b, c) intracellular tetanization. The arrowhead marked IT indicates the onset of the intracellular tetanization. In this and the following figures response amplitudes were normalized to the control (16–20 min before tetanization). (**B**) LTD induced by ICT in another input with low initial PPF ratio (1.09). Conventions as in (A).

Difference of the effects of intracellular tetanization on synaptic transmission in the two examples in Fig. 2 was correlated with the initial state of the presynaptic release mechanisms, assessed with paired-pulse stimulations [10]. Inputs with high initial PPF had a tendency to be potentiated (as in Fig. 2A, initial PPF = 2.52), and inputs with initially low PPF were most often depressed (as in Fig. 2B, initial PPF = 1.09) or did not change. For the whole sample, there was a highly significant correlation between initial PPF ratio on the one side and the direction and magnitude of the amplitude changes on the other (Fig. 3A, $n = 43$, $p < 0.001$). Separation of a sample into two subgroups of a nearly equal size reveals a marked difference of the intracellular tetanization effects. The net effect of intracellular tetanization on 22 inputs with PPF > 1.3 was potentiation. During the period 15–45 min after intracellular tetanization the averaged amplitudes of these inputs were $136 \pm 18\%$ of the pretetanic control. Peak potentiation in these inputs was on average $171 \pm 20\%$. In the remaining 21 inputs, which had initial PPF < 1.3, intracellular tetanization produced an opposite net effect. These responses were depressed on average to $83 \pm 8\%$ of the control amplitude. The difference between the effect of intracellular tetanization on inputs with initially high PPF and on the inputs with initially low PPF was highly significant ($p < 0.001$). When only those inputs which underwent potentiation were considered, a significant correlation was found between initial PPF ratio and the magnitude of potentiation ($r = 0.54$, $p < 0.05$, $n = 21$). Thus, the direction of the amplitude changes induced by intracellular tetanization depends on initial PPF, and potentiation was stronger in inputs with higher initial PPF.

In the second series of experiments the same postsynaptic challenge, intracellular tetanization, was applied under conditions of NMDA receptor

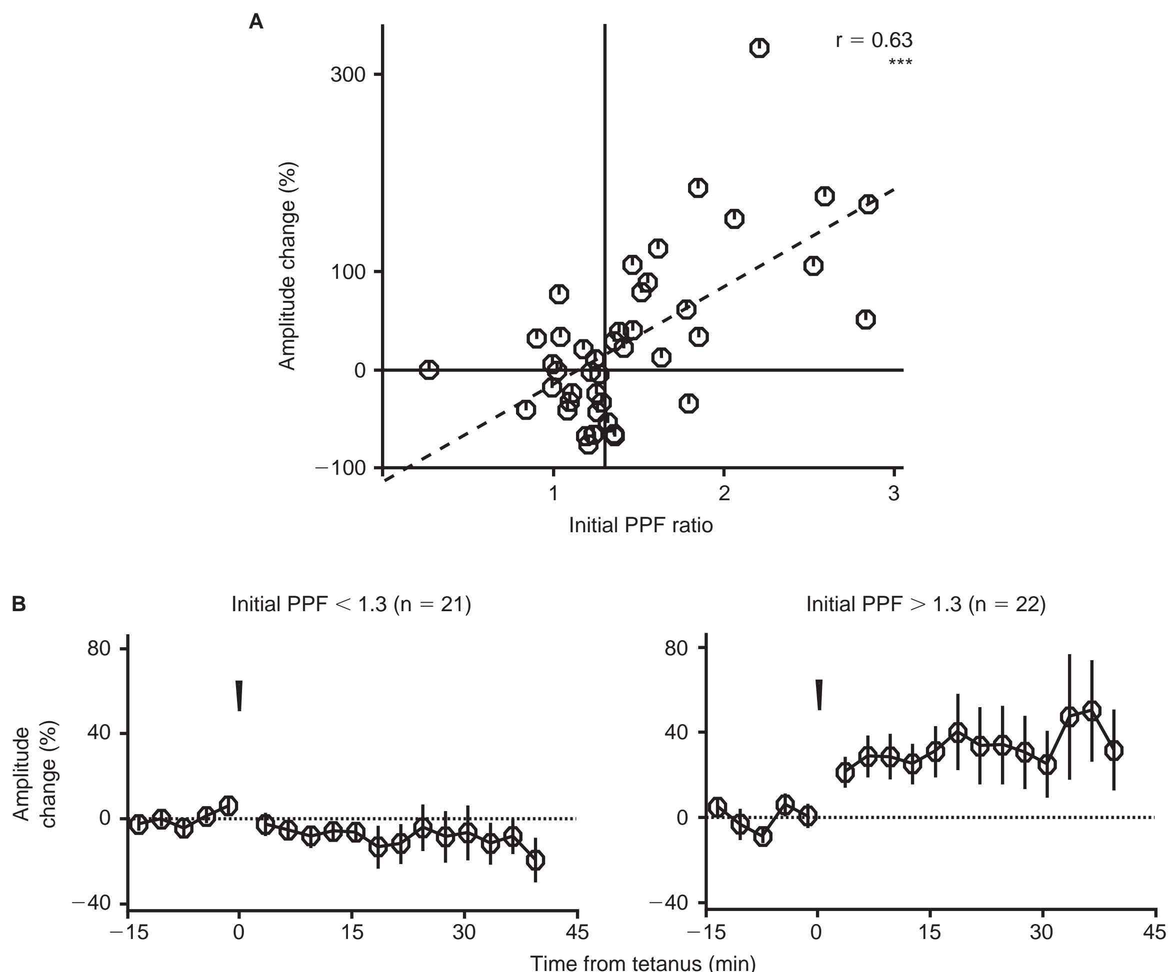

FIG. 3. Dependence of post-tetanic changes induced by intracellular tetanization on initial PPF ratio in control experiments. (**A**) Correlation between post-tetanic response amplitude changes (ordinate) and initial PPF ratios (abscissa) for 43 inputs. Thin vertical line (PPF = 1.3) divides a sample in two parts of nearly equal size ($n = 21$ and $n = 22$). Interrupted line shows a linear regression. *** $p < 0.001$. (**B**) Time course of the amplitude changes after intracellular tetanization for inputs with low (< 1.3, left) and with high (> 1.3, right) initial PPF ratios.

blockade (50 μM APV in medium throughout the experiments). In a case shown in Fig. 4A intracellular tetanization led to significant and long-lasting potentiation of EPSP amplitude to 136% in the input with initially high PPF (1.46). In another input, with a low PPF ratio (1.13), intracellular tetanization also led to a significant ($p < 0.01$) increase of the response amplitude, albeit weak (to 116% of the control). Under NMDA receptor blockade, intracellular tetanization induced potentiation in 14 of 26 inputs, but never led to significant depression of synaptic transmission. This can be clearly seen in Fig. 5A, where points are located above, but not significantly below the zero ordinate (compare with Fig. 3A). Despite this absence of depression, a significant correlation between the amplitude change and the initial PPF ratio remained: inputs with higher initial PPF showed greater increase of the response amplitude after intracellular tetanization.

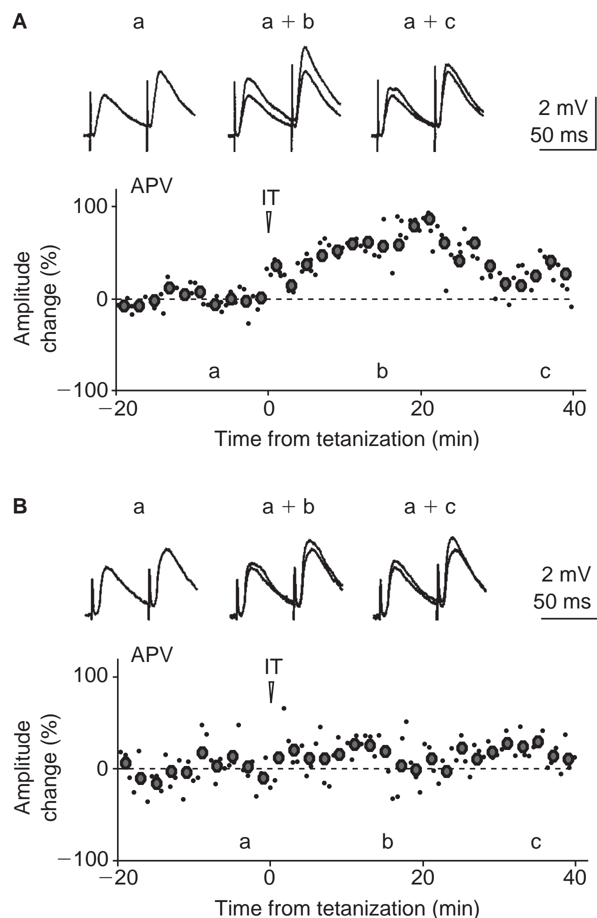

FIG. 4. Changes of synaptic transmission induced by intracellular tetanization during NMDA receptor blockade with 50 μm D-APV. (**A**) Strong LTP induced by IT in the input with high initial PPF ratio (1.46). Averaged responses ($n=50$) evoked by paired stimulation before (a) and after (a + b; a + c) tetanization, as indicated. Time course of the amplitude changes of averaged responses ($n=5$ for points; $n=10$ for circles) evoked by the first stimulus in a pair before (a) and after (b, c) intracellular tetanization. The arrowhead marked IT indicates the onset of the intracellular tetanization. (**B**) Weak LTP induced by intracellular tetanization in the input with low initial PPF ratio (1.13) under NMDA receptor blockade. Conventions as an (A).

Segregation of the data in two groups using the same criterion as in the first series of experiments (initial PPF < 1.3 or > 1.3) further illustrated the relation between initial PPF and the magnitude of potentiation. In 12 inputs with initial PPF > 1.3, potentiation was stronger than in the remaining 14 inputs with initial PPF < 1.3 ($129 \pm 13\%$ *vs* $117 \pm 6\%$). Further, this segregation revealed a marked difference between the two groups in respect to an impact of NMDA receptor blockade on synaptic changes induced by intracellular tetanization (Fig. 5B). Inputs with initially high PPF were potentiated by intracellular tetanization, both in the control medium and under the blockade of NMDA receptors. In contrast, effect of intracellular tetanization on the inputs with initially low PPF was essentially reversed by NMDA receptor blockade. When applied in control conditions intracellular tetanization led to a decrease of response amplitude in these inputs to $83 \pm 8\%$ of the pretetanic value. Under the blockade of NMDA receptors intracellular tetanization induced an increase of response amplitude to $117 \pm 6\%$. The difference between these two effects was significant ($p < 0.01$).

Thus, when the NMDA receptors are blocked intracellular tetanization is not capable of inducing depression of synaptic transmission but is still capable of inducing potentiation. Both with and without NMDA receptor blockade, the magnitude of potentiation depended on the initial PPF, being higher in inputs with high PPF.

Discussion

Our data demonstrate that induction of LTD by intracellular tetanization depends on activation of NMDA receptors and is abolished by their blockade. In contrast, induction of LTP by the same protocol was not eliminated by blockade of NMDA receptors, therefore the mechanisms which led to LTP in our experimental situation, were NMDA receptor independent.

These findings are unexpected since our induction protocol, intracellular tetanization, was applied without presynaptic stimulation and thus could not lead to synaptic activation of NMDA receptors. The observed NMDA receptor dependence of LTD induction could be accounted for by the following considerations. If to presume that some minor amount of background glutamate is present in a slice, then, it could be bound to some of the NMDA receptors due to their extremely high affinity [11]. These receptors, despite being occupied by the

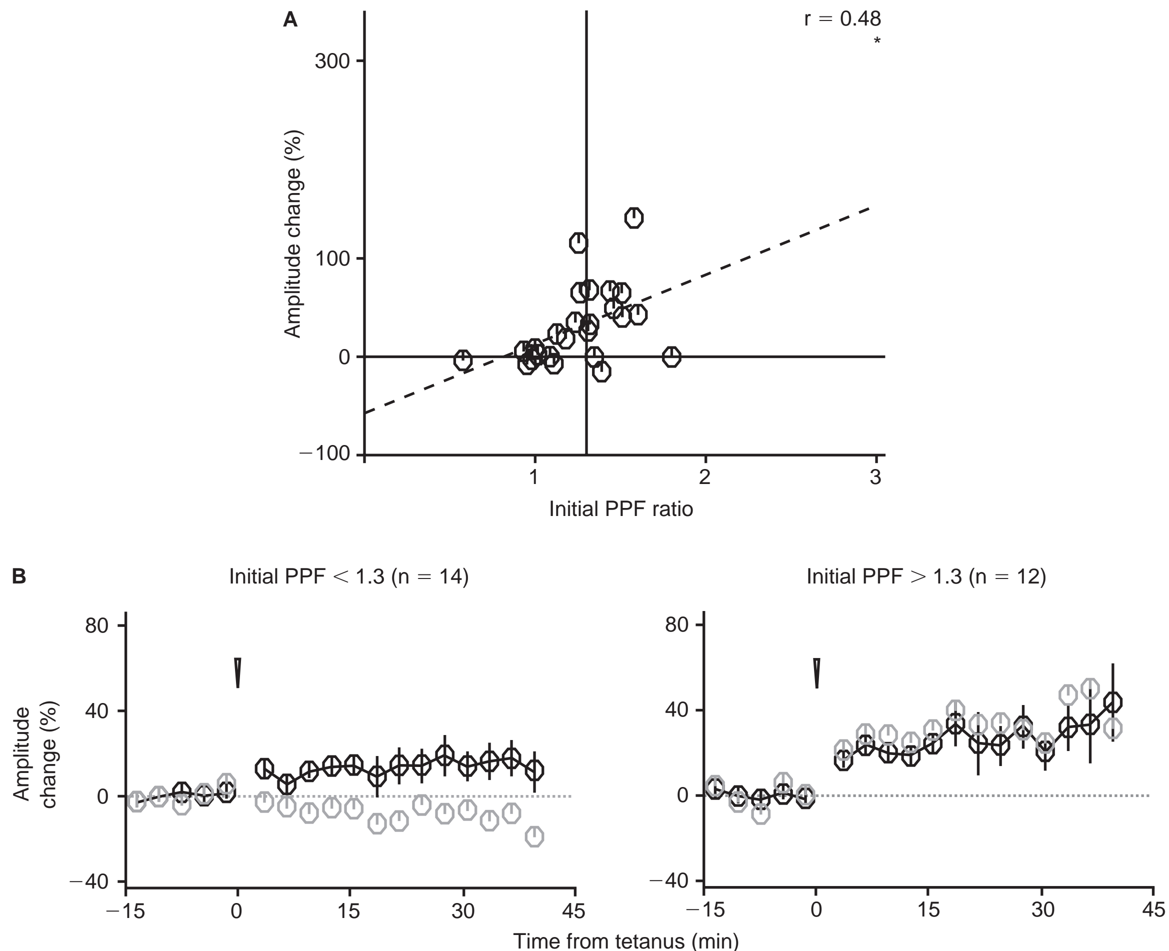

FIG. 5. Dependence of post-tetanic changes of synaptic transmission induced by intracellular tetanization during blockade of NMDA receptors with 50 μm D-APV. (**A**) Correlation between post-tetanic amplitude changes of EPSP1 (ordinate) and initial PPF ratios (abscissa) for 26 inputs studied under 50 μm D-APV. $^{*}p < 0.05$. (**B**) Time course of the amplitude changes after intracellular tetanization for inputs with low (< 1.3, left) and with high (> 1.3, right) initial PPF ratios, studied under 50 μm D-APV. Data obtained in control solution are shown for comparison (unconnected symbols without error bars).

agonist, do not affect properties of a cell membrane at rest, since the channels are blocked by magnesium [12]. During intracellular tetanization, the magnesium block is removed, and calcium can enter the cell through these NMDA receptor gated channels (probably only a small part of the total number). This local Ca^{2+} influx through the NMDA channels, in combination with $[Ca^{2+}]$ raise caused by other mechanisms, could be just enough to reach the threshold of activation for mechanisms which are responsible for NMDA dependent LTD induced by afferent tetanization. A small number of the NMDA receptors which can be occupied by the low level of background glutamate might be not sufficient to let in enough calcium for reaching the threshold of NMDA dependent LTP, since that threshold might be higher [13]. The fact that predominantly inputs with low initial PPF ratio are depressed is consistent with this scheme. Lower initial PPF ratio is indicative of higher release probability at these synapses [10]. Higher release probability, in turn, increases the possibility of spontaneous release, and therefore of the presence of glutamate at these synapses.

Our results provide further support for the conjecture that mechanisms of both potentiation and depression are activated by the same challenge, and that the final result depends on their balance [8,14,15]. In the group of inputs with low initial PPF, intracellular tetanization led to depression under control conditions. If only the mechanisms responsible for depression were activated at these synapses, the inputs should have remained unchanged when intracellular tetanization was applied during NMDA receptor blockade. However, under blockade of NMDA receptors the intracellular tetanization led to potentiation, indicating that potentiation mechanisms were activated at these same synapses by that same challenge. These data are, in a sense, complementary to the recent finding that when the mechanisms responsible for potentiation

are blocked, a pairing protocol leads to a depression rather than to a potentiation of synaptic transmission in the dentate gyrus [15]. They are also compatible with results obtained in different brain structures, showing that a balance between potentiation and depression, and thus the final effect of a particular plasticity inducing protocol, can be altered by pharmacological means or by recently induced synaptic changes [4,5,15–18].

The blockade of mechanisms responsible for depression has isolated the effect of potentiation mechanisms. This allowed to reveal a gradual nature of both predispositions for potentiation and of activation of mechanisms of potentiation. Potentiation occurred more often, and was of a higher magnitude, in the inputs with a high initial PPF.

Conclusion

The results of the present study demonstrate that induction of LTD by a purely postsynaptic challenge, in the form of intracellular tetanization, is abolished by NMDA receptor blockade. Under control conditions inputs with a low initial PPF ratio were susceptible for LTD. Since lower PPF is indicative of higher release probability, spontaneous release of glutamate should be higher at these synapses. This amount of background glutamate might support enough Ca^{2+} surge through NMDA receptors during intracellular tetanization, to activate NMDA dependent mechanisms of LTD.

In contrast, NMDA receptor blockade did not prevent induction of LTP by intracellular tetanization. This data suggest that mechanisms of both potentiation and depression can be activated simultaneously by the same procedure, and the final change of synaptic gain depends on their balance.

References

1. Kuhnt U, Kleschevnikov AM and Voronin LL. *Neurosci Res Commun* **14**, 115–123 (1994).
2. Volgushev M, Voronin LL, Chistiakova M *et al. NeuroReport* **5**, 2069–2072 (1994).
3. Christofi G, Nowicky AV, Bolsover SR *et al. J Neurophysiol* **69**, 219–229 (1993).
4. Neveu D and Zucker RS. *Neuron* **16**, 619–629 (1996).
5. Volgushev M, Mittman T, Chistiakova M *et al. Neuroscience* **93**, 1227–1232 (1999).
6. Bliss TV and Collingridge GL. *Nature* **361**, 31–39 (1993).
7. Malenka RC and Nicoll RA. *Trends Neurosci* **16**, 521–527 (1993).
8. Volgushev M, Voronin LL, Chistiakova M *et al. Eur J Neurosci* **9**, 1656–1665 (1997).
9. Markram H and Tsodyks M. *Nature* **382**, 807–810 (1996).
10. Zucker RS. *Annu Rev Neurosci* **12**, 13–31 (1989).
11. Clements JD and Westbrook GL. *Neuron* **7**, 605–613 (1991).
12. Nowak L, Bregestovski P, Ascher P *et al. Nature* **307**, 462–465 (1984).
13. Artola A, Bröher S and Singer W. *Nature* **347**, 69–72 (1990).
14. Li H, Weiss SRB, Chuang DM, Post RM *et al. J Neurosci* **18**, 1662–1670 (1998).
15. Wang Y, Wu JQ, Rowan MJ *et al. J Neurophysiology* **78**, 2569–2573 (1997).
16. Stäubli U and Chun D. *Learn Mem* **3**, 96–105 (1996).
17. Wagner JJ and Alger BE. *J Neurosci* **15**, 1577–1586 (1995).
18. Cohen AS, Raymond CR and Abraham WC. *Hippocampus* **8**,160–170 (1998).

ACKNOWLEDGEMENTS: We are grateful to Mrs Christa Schlauss for excellent technical assistance. This work was partially supported by the Deutsche Forschungsgemeinschaft (Ey 8/23 and SFB 509 TP A5).

Received 10 August 1999; accepted 6 October, 1999