

Probability of Transmitter Release at Neocortical Synapses at Different Temperatures

Maxim Volgushev,^{1,2} Igor Kudryashov,² Marina Chistiakova,^{1,2,3} Mikhail Mukovski,¹ Johannes Niesmann,¹ and Ulf T. Eysel¹

¹Department of Neurophysiology, Faculty of Medicine, Ruhr-University Bochum, D-44780 Bochum, Germany; ²Institute of Higher Nervous Activity and Neurophysiology RAS, 117865 Moscow, Russia; ³Department of Electrical Engineering and Computer Science, Technical University of Berlin, 10587 Berlin, Germany

Submitted 5 December 2003; accepted in final form 28 February 2004

Volgushev, Maxim, Igor Kudryashov, Marina Chistiakova, Mikhail Mukovski, Johannes Niesmann, and Ulf T. Eysel. Probability of transmitter release at neocortical synapses at different temperatures. *J Neurophysiol* 92: 212–220, 2004. First published March 3, 2004; 10.1152/jn.01166.2003. The probability of transmitter release at synaptic terminals is one of the key characteristics of communication between nerve cells because it determines both the strength and dynamic properties of synaptic connections. To assess the distribution of the release probabilities at excitatory synapses on supragranular pyramidal cells in rat visual cortex, we have used the MK-801, a blocker of the open *N*-methyl-D-aspartate (NMDA) receptor-gated channels. With this method, the release probability can be calculated from the time course of the blockade of NMDA-receptor mediated postsynaptic currents in the presence of MK-801. At temperatures $>32^{\circ}\text{C}$, the distribution of release probabilities covered the range from 0.05 to 0.43 [mean: 0.171 ± 0.012 (SE), $n = 65$], being skewed toward low values. When estimated at room temperature ($22\text{--}25^{\circ}\text{C}$), the release probabilities were significantly lower (mean: 0.123 ± 0.009 , $n = 54$), and almost the whole distribution was restricted to values <0.2 . Furthermore, warming from room temperature to $>32^{\circ}\text{C}$ led to a pronounced overshooting increase of the release probability. Taken together, the results of the present study show that release probabilities at synapses formed onto layer 2/3 pyramidal cells in the visual cortex vary significantly, but values >0.3 are rare and the results obtained either at room or variable temperature differ significantly from those made under conditions of constant temperature in the physiological range.

INTRODUCTION

Knowledge about the probability of transmitter release and its changes at neocortical synapses is a necessary step toward understanding information processing by neural networks in sensory areas. Data available for the synaptic connections in the neocortex show that the release probability of synapses at neurons of similar morphological type are more uniform than the release probability of synapses at cells of different morphology (see Thomson and Deuchars 1994 for review; Markram et al. 1998; Reyes et al. 1998; Tsodyks and Markram 1997). These estimates of the release probability at neocortical synapses, as well as more numerous data on synaptic transmission in the hippocampus (e.g., Allen and Stevens 1994; Bolshakov and Siegelbaum 1995; Stricker et al. 1996; Voronin et al. 1992), were obtained by use of statistical analysis of the amplitude fluctuations of the postsynaptic responses. Another

method, which allows estimation of the release probability, is based on the irreversible block of the *N*-methyl-D-aspartate (NMDA) receptor-gated channels by MK-801 (Huettner and Bean 1988). Because the MK-801 blocks only open channels, the rate of the blockade of the NMDA receptor-mediated synaptic responses depends on the release probability. This method has been successfully applied to calculate release probabilities at synapses in cultures of hippocampal cells and in slices of the hippocampus (Hessler et al. 1993; Huang and Stevens 1997; Rosenmund et al. 1993).

Several lines of evidence suggest that the results of extensive studies of synaptic transmission in the hippocampus may not be directly applicable to the neocortex. First, the short-term plasticity, as studied with evoked field potentials, differs in the hippocampus and the neocortex (Castro-Alamancos and Connors 1997), indicating that release probabilities may be different too. Second, many of the earlier studies on release probability in the hippocampus were performed at room temperature. It has been argued that release probability at hippocampal synapses may be the same at room temperature and at temperatures $>30^{\circ}\text{C}$ (Allen and Stevens 1994). However, this is definitely not the case for the neocortical synapses, for which low (room) temperature has been shown to depress the release of transmitter and to increase the failure rate (Hardingham and Larkman 1998). This conclusion is supported by the results of our studies (Volgushev et al. 2000a,b), which demonstrated that basic membrane properties, spike generation, and synaptic transmission in the neocortex are dramatically different at room temperature and in the physiological temperature range. Finally, target specificity of the properties of synaptic connections in the neocortex (Markram et al. 1998; Reyes et al. 1998; Thomson and Deuchars 1994; Tsodyks and Markram 1997) necessitate separate investigation of the synapses formed at different classes of postsynaptic target cells. Therefore we set out to examine the release probability at glutamatergic synaptic inputs to layer II–III pyramidal cells in the visual cortex, using the MK-801 method. Our aims were, first, to assess the distribution of probabilities of transmitter release and, second, to compare the release probabilities at different temperatures.

Address for reprint requests and other correspondence: M. Volgushev, Ruhr-University Bochum, Dept. of Neurophysiology, MA 4/149, D-44780 Bochum, Germany (E-mail: maxim@neurop.ruhr-uni-bochum.de).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

METHODS

Slices

Slices of the visual cortex of P23–P40 Wistar rats (Charles River GmbH, Suzfeld, Germany) were prepared as described elsewhere (Volgushev et al. 2000a,b). The rats were anesthetized with ether and decapitated, and the brain was rapidly removed and put into an ice-cold oxygenated solution. Frontal slices (350–400 μm thick) of the visual cortex were cut with a vibrotome (TSE, Kronberg, Germany). After ≥ 1 h recovery in an incubator at room temperature a slice was placed in the recording chamber.

Recording and data analysis

Recordings were made with the slices in submerged conditions. The perfusion medium contained (in mM) 125 NaCl, 2.5 KCl, 2 CaCl₂, 1.5 MgCl₂, 1.25 NaH₂PO₄, 25 NaHCO₃, 25 D-glucose, 0.5 L-glutamine, and 0.001 glycine and was aerated with 95% O₂-5% CO₂ bubbles. Temperature in the recording chamber was varied within a range of 20–36°C and monitored with a thermocouple positioned close to the slice, 2–3 mm from the recording site. During the recording, temperature was either held constant throughout the experiment, or, when changed from ~ 22 –24°C to >33 °C, left for several minutes to reach the equilibrium state. Calibrations found that, under these conditions, the temperature measured in the bath close to the slice was within 0.4°C of the within slice temperature.

Patch-electrodes were filled with a solution containing (in mM) 127 K-gluconate, 20 KCl, 2 MgCl₂, 2 Na₂ATP, 10 HEPES, and 0.1 EGTA and had a resistance of 3–7 M Ω . Whole cell recordings were made from pyramidal neurons in layers II–III in slices of rat visual cortex. Pyramidal cells were selected under visual control using Nomarski optics and infrared videomicroscopy (Dodt and Zieglgänsberger 1990; Stuart et al. 1993) with Axoclamp-2A (Axon Instruments). Reliability of the identification of the pyramidal cells has been proved in our previous work by labeling the recorded cells with biocytin and morphological reconstruction (Volgushev et al. 2000a,b). Synaptic responses were evoked by electric shocks applied through bipolar stimulation electrodes located 0.5–1.5 mm below or lateral to the recording site (Fig. 1). The stimulation intensity was set to produce small responses without failures. To isolate NMDA receptor-mediated

currents, 6,7-Dinitroquinoxaline-2,3-dione, DNQX, 5 μM) and picrotoxin (50 μM) were added to the medium, and cells were held at -50 to -55 mV during the recording.

The electrode signal was digitized at 10 kHz and fed into a computer (PC-486; Digidata 1200 interface and pCLAMP software, Axon Instruments). Data were processed off-line using custom written programs. For statistical analysis we used Kolmogorov-Smirnov and Wilcoxon-Mann-Whitney test.

The probability of transmitter release was calculated from the rate of exponential decay of the amplitude of the consequent evoked NMDA receptor-mediated currents in the presence of an MK-801, a blocker of open NMDA receptor-gated channels (Huettner and Bean 1988), as following (Hessler et al. 1993; Huang and Stevens 1997; Rosenmund et al. 1993).

From equations

$$R_i = R_0 \times \text{Exp}(-i/\tau)$$

$$\tau = -1/\ln(1 - F)$$

$$F = p \times \text{FB}$$

follows that

$$p = (1 - \text{Exp}(-1/\tau))/\text{FB} \quad (1)$$

where R is response amplitude; R_0 is the amplitude of the unblocked response; i is stimulus number; F is fraction of all available NMDA-channels blocked in one trial; τ is decay constant; p is release probability; and FB, is fraction of NMDA channels blocked at a synapse, which released transmitter.

The decay constant of the response amplitude blockade was calculated from the plots of the response amplitude against stimulus number.

Chemicals

The chemicals were obtained from the following sources. Sigma (Deisenhofen, Germany): biocytin, EGTA, HEPES, K-gluconate, L-glutamine, Na₂ATP, tetrodotoxin, DNQX, picrotoxin; Tocris Cookson (Bristol, UK): DNQX, MK-801 maleate. The remaining chemicals were from J.T. Baker B.V., Deventer, Holland.

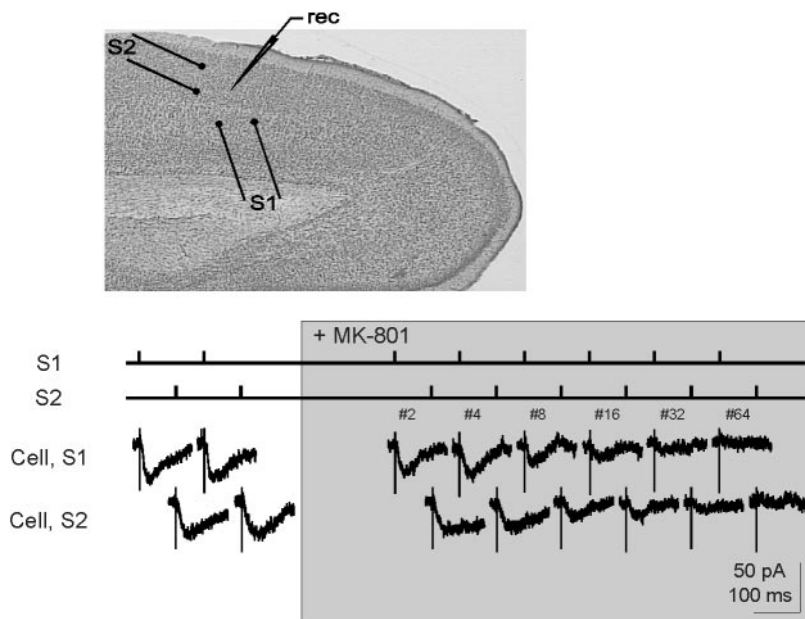


FIG. 1. Recording situation and experimental protocol. *Inset* (top): positioning of the stimulation (S1 and S2) and recording electrodes in a slice of the rat visual cortex. Test stimuli were applied in alternation at 2 stimulation sites once in 15–20 s. After recording control responses test stimulation was stopped and a blocker of open *N*-methyl-D-aspartate (NMDA) channel, MK-801, was added to the recording medium at final concentration of 20–80 μM . After 7–10 min of wash-in, test stimulation was resumed. Pharmacologically isolated NMDA receptor-mediated responses of a cell to test stimuli (cell, S1 and Cell, S2) before and after wash in of the MK-801 are shown. Sequential number of the response after application of MK-801 is indicated above the traces.

RESULTS

Experimental protocol and estimation of the fraction of channels blocked during a single excitatory postsynaptic current

We have used the MK-801 method (Hessler et al. 1993; Huang and Stevens 1997; Rosenmund et al. 1993) to calculate the release probability at synaptic connections to layer II–III pyramidal cells in rat visual cortex. Our basic experimental protocol is illustrated in Fig. 1. Test stimuli that evoked small NMDA receptor-mediated excitatory postsynaptic currents (EPSCs) in the recording cell were applied in alternation at two stimulation sites (S1 and S2 in Fig. 1). After recording stable control responses for 5–10 min, test stimulation was stopped and an irreversible blocker of open NMDA channels, MK-801, was added to the recording medium at a final concentration of 20–80 μM . After 7–10 min of wash-in to let the MK-801 penetrate the slice and spread evenly, test stimulation was resumed.

In the presence of MK-801 the test EPSCs changed in two ways. First the amplitude of the responses to consequently applied stimuli progressively decreased (Fig. 1). The decrease of the response amplitude was fitted with a mono-exponential function and the decay constant was calculated (see following text). Second, the shape of individual EPSCs changed. Each individual EPSC decayed faster than in the control medium (Fig. 2A), reflecting the dynamics of the block of the open NMDA receptor-gated channels by the MK-801 during the response (Huettner and Bean 1988). This process can be simplistically described with the following kinetic model (Huang and Stevens 1997; Rosenmund et al. 1993). In control medium, when glutamate is bound to the NMDA receptor, the channel gated by that receptor could be in open or closed state, and

glutamate could unbind from the receptor (Fig. 2, *inset*). The EPSC kinetics depends on the dynamic balance among these states. When MK-801 is present in the medium, it blocks irreversibly some of the open channels, thus accelerating the EPSC decay. Despite its simplicity, this model describes the EPSC kinetics reasonably well, and it has been applied successfully for calculating the fraction of blocked channels (FB) in response to single stimuli in different preparations (Huang and Stevens 1997; Rosenmund et al. 1993). We have used the same model and calculated the FB as follows. The four-state model was fitted to the recorded EPSCs in two steps. First, we fitted the EPSC recorded in control medium with the model consisting of three states: unbound, closed, open. With these parameters determined and fixed, an additional state (blocked) was added to the model, and EPSCs in the presence of MK-801 were fitted. Thus all parameters describing the first three states (unbound, closed, open) were the same for the both procedures. From these fits, the fraction of channels, which are blocked during a single response, was calculated as

$$\text{FB} = K_{ob}/(K_{ob} + K_{oc})$$

In theory, if the preceding considerations are correct, the FB must depend on the concentration of MK-801. Our estimations of the FB on the basis of the data obtained with different concentrations show that this was indeed the case (Fig. 2B), and thus the formalization of the MK-801 method holds for our experimental conditions. Both the range of the FB values (from 29 to 45%) and their dependence on the concentration of MK-801 correspond closely to those reported for hippocampal synapses in slices (Hessler et al. 1993; Huang and Stevens 1997). Because one of the aims of this study was the comparison of the release probabilities at different temperatures, we

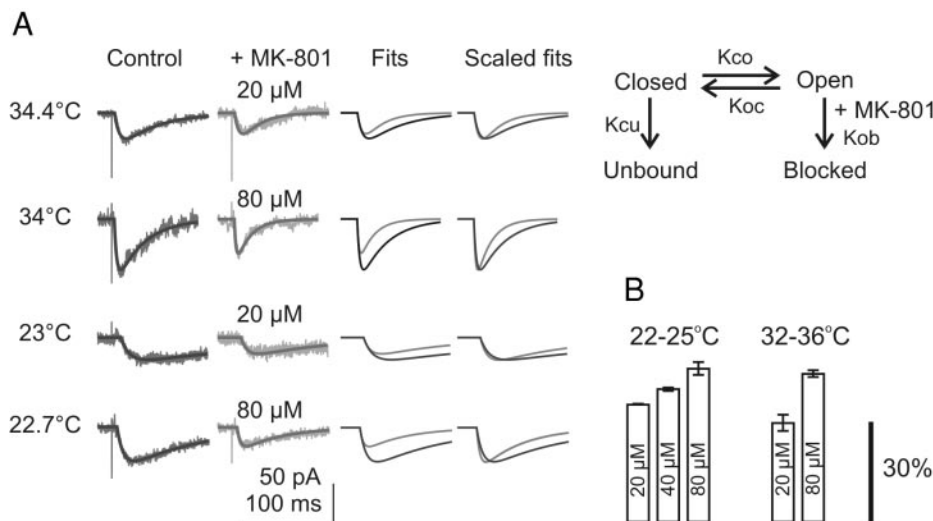


FIG. 2. Estimation of the fraction of NMDA receptor-gated channels, which was blocked during a single response. *A*: averaged NMDA receptor-mediated EPSCs recorded before (control) and after application of MK-801 and their fits. Note the faster decay of the responses after application of MK-801. Recording temperature and concentration of MK-801 is indicated for each experiment. Fraction of the open NMDA receptor-gated channels blocked during a single response, calculated from the results of fitting the shown examples, was (from top to the bottom): 0.34; 0.42; 0.32; 0.48. *Inset*: a simplistic 4-state kinetic scheme (Huang and Stevens 1997). In control medium, during a response to synaptic stimulation, an NMDA receptor-channel complex can be in 1 of the 3 states (unbound, bound and closed, bound and open). In the medium with MK-801, some open channels are irreversibly blocked. Coefficients next to the arrows are rates of transition between the states. *B*: estimated fraction of open NMDA channels blocked during a single response. Data for different concentrations of MK-801, at room temperature (22–25°C) and at temperature >32°C. MK-801 concentration is indicated within each bar.

have also estimated the FB at two temperature ranges. FB values obtained at different temperatures but with the same MK-801 concentrations were similar at room temperature and at temperatures $>32^{\circ}\text{C}$ (Fig. 2B). This could be due to the compensation of an increased rate of kinetics of the biochemical reactions by a decrease of the channel open times at higher temperature (Silver et al. 1996; Wyllie et al. 1993).

We have determined the FB from the change of the EPSC kinetics in the presence of MK-801 for different concentrations of the MK-801 and at two temperature ranges. In each group, which contained the data obtained at given MK-801 concentration and temperature, the FB has been determined for several EPSCs (4–7 cases in each group), and the average FB value was calculated for that group. These group averages were then used in calculations of the release probability from the data obtained with the respective MK-801 concentration and temperature.

Release probability at $32\text{--}36^{\circ}\text{C}$

Figure 3 shows two typical examples of the MK-801 blocking function at temperatures $>32^{\circ}\text{C}$. The amplitude of EPSCs remained stable in the control but decayed gradually after MK-801 was washed in to the final concentration of $20\ \mu\text{M}$. After $\sim 50\text{--}60$ stimuli in the presence of the MK-801, the EPSCs were blocked completely (black, Fig. 3, A1 and B1). The complete blockade of the responses shows that the EPSCs were mediated by the NMDA receptor-gated channels.

For the responses illustrated in Fig. 3A, the decrease of the response amplitude was best approximated with a mono-exponential function with a decay constant of $\tau = 25.1$ (Fig. 3A2). Using the τ and the respective FB value in the equation [1], the release probability at this synaptic connection is $P = 0.13$. In another example (Fig. 3B) the blockade of the EPSC amplitude occurred faster, with the decay constant $\tau = 14.8$, which corresponds to a release probability of $P = 0.23$.

Altogether we have analyzed 65 synaptic connections at temperatures $>32^{\circ}\text{C}$. In these connections, the constant of the response decay in the presence of MK-801 varied from 7.6 to 64.7. This corresponds to a range of release probabilities from 0.05 to 0.43. On average, the release probability at these synapses was 0.171 ± 0.012 (mean \pm SE, $n = 65$), with a median of 0.148.

Release probability at room temperature ($22\text{--}25^{\circ}\text{C}$)

Because many of the earlier studies on synaptic transmission were performed at room temperature, we repeated, for the purpose of comparison, the experiments described above but at room temperature ($22\text{--}25^{\circ}\text{C}$). Typical examples of the MK-801 blocking functions are shown in Fig. 4. In the Fig. 4A, the blockade of the NMDA receptor-mediated postsynaptic responses occurred very slowly with a decay constant $\tau = 49.4$. This corresponds to a probability of transmitter release $P = 0.06$. In the synaptic connection in the other example (Fig. 4B), the blockade occurred faster with the decay constant $\tau = 19.7$, which corresponds to a release probability $P = 0.14$. Similar to experiments performed at higher temperatures, the synaptic responses were completely blocked by the MK-801 (Fig. 4, A2 and B2), showing that the responses were mediated by NMDA receptor-gated channels.

In 54 synaptic connections studied with that protocol at room temperature, the estimated release probability varied from 0.04 to 0.33, with a mean of 0.123 ± 0.009 ($n = 54$) and a median of 0.111. These values are significantly lower than the release probability measured at temperatures $>32^{\circ}\text{C}$ ($P = 0.036$, Kolmogorov-Smirnov test; $P < 0.001$ Mann-Whitney test).

Potential of synaptic transmission by temperature increase

In the next series of experiments, we have checked whether the probability of transmitter release at a synapse increases on warming of the slice from room temperature to temperatures $>32^{\circ}\text{C}$. We recorded synaptic responses at room temperature, then stopped the test stimulation and washed in the MK-801. During the 7- to 10-min period of washing in the blocker, we

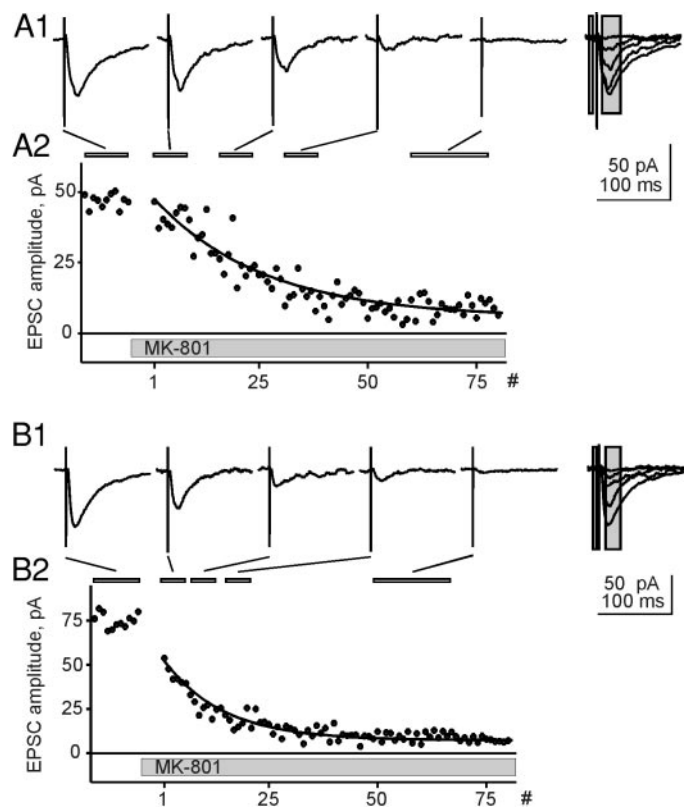


FIG. 3. Release probability at $32\text{--}36^{\circ}\text{C}$. Two representative examples show inputs with release probability of 0.13 (A) and 0.23 (B). A1: NMDA receptor-mediated EPSCs in a cortical cell recorded before (leftmost trace) and in different periods after bath application of MK-801 at final concentration of $20\ \mu\text{M}$. On the rightmost panel the responses are superimposed. Response amplitude was measured as the difference between integral currents calculated in the 2 measuring windows, which are shown as shaded rectangles on the superimposed response traces. A2: amplitudes of the consecutive responses plotted against stimulus number. Number 1 corresponds to the 1st response recorded in the medium with MK-801. MK-801 was washed in for 7 min without stimulation to allow for a uniform distribution of the blocker in a slice. Bars indicate responses used to calculate averaged traces shown in A1. The decay of the response amplitude with application of MK-801 was approximated with a mono-exponential function with $\tau = 25.1$ (black line). This corresponds to the release probability $P = 0.13$ (see METHODS). B, 1 and 2: NMDA receptor-mediated responses and time course of their block by MK-801 in another cell. The decay of the EPSC amplitude is approximated by a mono-exponential function with $\tau = 14.8$, which corresponds to the release probability $P = 0.23$. Other conventions as in A.

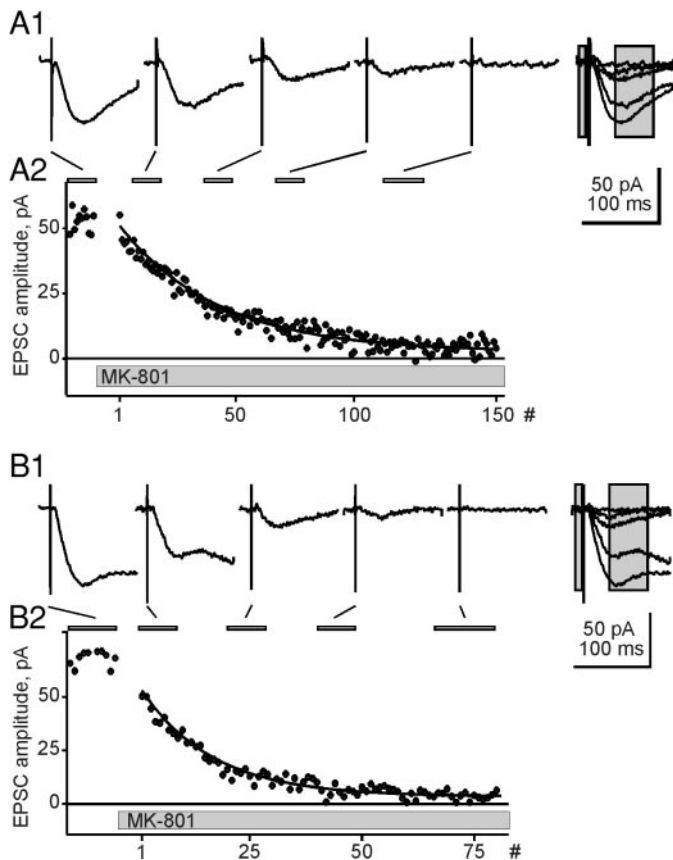


FIG. 4. Release probability at room temperature (22–25°C). Two representative examples show inputs with release probability of 0.06 (A) and 0.14 (B). A, 1 and 2: NMDA receptor-mediated responses and time course of their block by MK-801. MK-801 was washed in the recording chamber for 7 min without stimulation. The decay of the EPSC amplitude is approximated by a mono-exponential function with $\tau = 49.4$, which corresponds to the release probability $P = 0.06$. Note that abscissa scaling is twice as large as in other figures. B, 1 and 2: NMDA receptor-mediated responses and time course of their block by MK-801 in another cell. The decay of the EPSC amplitude is approximated by a mono-exponential function with $\tau = 19.7$, which corresponds to the release probability $P = 0.14$. Other conventions in A and B as in Fig. 3.

increased the temperature in the recording chamber to 33–34°C. When the test stimulation was started again, the amplitude of first EPSCs had increased dramatically, but the responses were blocked very rapidly by the MK-801 (Fig. 5A). In the example shown in Fig. 5A, the decay constant of the response blockade was $\tau = 11.3$, which corresponds to a release probability of $P = 0.29$. An enhanced response and its rapid block by MK-801 was observed in all 17 experiments made with this protocol. The release probability in these experiments ranged from 0.21 to 0.67, with an average of 0.428 ± 0.012 ($n = 17$) and a median of 0.411. This is significantly higher than the release probability measured at constant temperature—either at room temperature or at 32–36°C ($P < 0.001$ for both comparisons).

To test whether the increase of the response amplitude is due solely to an increased probability of release at the same synapses or whether additional presynaptic fibers were recruited at temperatures $>32^\circ\text{C}$ as compared with room temperature, we did the following experiments. Recording was started at room temperature, and MK-801 was applied as usually. After the responses were blocked completely, temperature in the recording chamber was increased to 34°C. If new presynaptic fibers

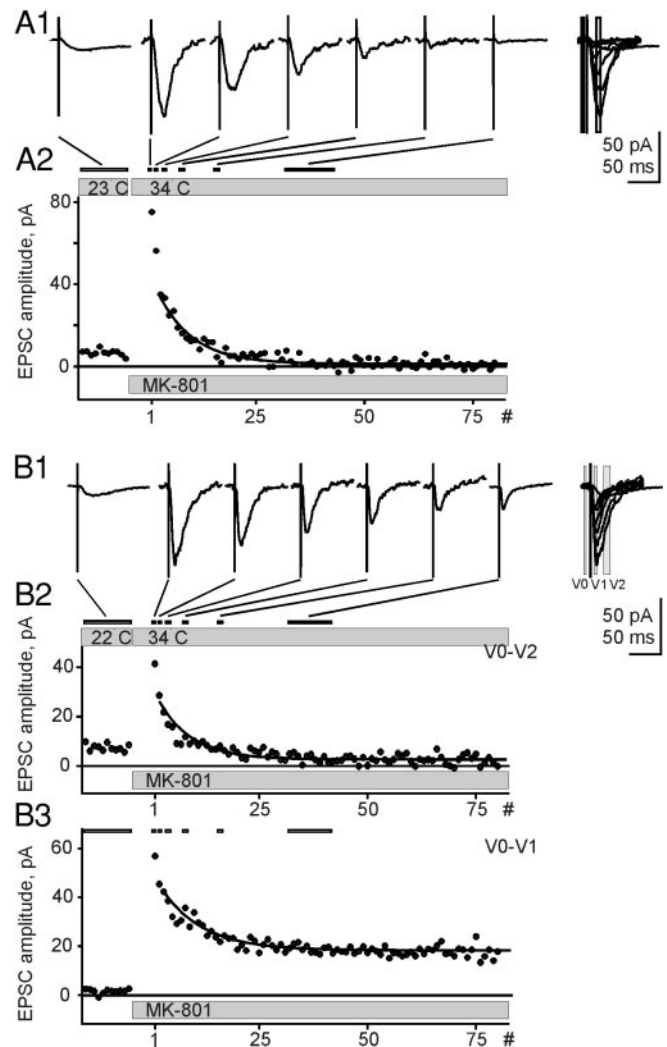


FIG. 5. Potentiation of synaptic transmission by temperature increase. A, 1 and 2: NMDA receptor-mediated postsynaptic currents and time course of their amplitude changes. After collecting control responses, test stimulation was stopped, MK-801 was applied in the recording medium and temperature in the recording chamber was increased from 23 to 34°C. Then after a 10-min interval, the test stimulation was resumed. In the presence of MK-801, the response amplitude decayed exponentially with $\tau = 11.3$, which corresponds to the release probability $P = 0.29$. B, 1–3: potentiation of the slow, NMDA receptor-mediated EPSC and fast, non-NMDA response by warming from room temperature (22°C) to 34°C. MK-801 was washed in and the temperature was increased during a 7-min interval without stimulation. B1: postsynaptic currents recorded in a cortical cell at 22°C during the control period and at 34°C in the presence of MK-801. The NMDA component of the response was measured as the difference between integral current in windows V0 and V2. The amplitude of the fast non-NMDA response component was measured as the difference between integral current in windows V0 and V1. In case of compound responses, the window for measuring the amplitude of fast non-NMDA responses was positioned around the response peak, ~ 2 –4 ms from the beginning of EPSC (V1). For measuring the NMDA receptor-mediated component, the measuring window was positioned ~ 20 –25 ms after the response latency (V2). The reference window (V0) was always positioned just before the response. B2: time course of the amplitude change of the NMDA receptor-mediated response component. The NMDA receptor-mediated component of the increased response was blocked in the presence of the MK-801 with a decay constant $\tau = 7.5$, which corresponds to the release probability $P = 0.43$. B3: time course of the amplitude change of the fast non-NMDA response component. Other conventions in A and B as in Fig. 3.

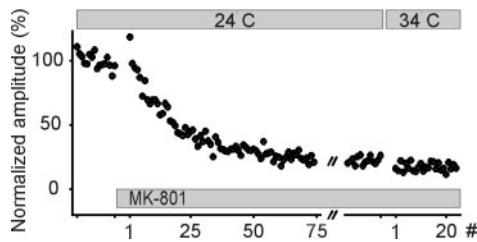


FIG. 6. Potentiation of synaptic transmission by temperature increase is not due to activation of additional presynaptic fibers. Averaged time course of the amplitude changes of the NMDA receptor-mediated postsynaptic currents. After the responses were blocked in the presence of MK-801, temperature in the recording chamber was increased to 34°C, as indicated. Note that raising the temperature did not lead to an increase of the response amplitude. Averaged data for 10 cells.

were activated at higher temperature, this should lead to the appearance of some responses after the warming. This was not the case in any of the 10 experiments conducted with this protocol, and there was no temporal increase of the response after warming evident in the averaged data (Fig. 6). Thus the increase of the EPSC amplitude on warming was due solely to an increased probability of transmitter release at those same synapses that contributed to the response at room temperature.

The dramatic enhancement of the release after the temperature increase was transient and lasted for minutes only. When measured 15–25 min after the warming, the release probability was significantly lower than just after the temperature change (0.229 ± 0.028 , $n = 8$ against 0.428 ± 0.012 , $n = 17$, $P = 0.021$) and close to that measured at temperatures $>32^\circ\text{C}$ without an acute warming. However, a residual moderate increase of release probability after warming cannot be excluded.

In some experiments, enhancement of the synaptic transmission due to the temperature increase led to appearance of fast non-NMDA responses. In the cell in Fig. 5, *B1–B3*, the late component of the increased response was blocked by MK-801 (Fig. 5, *B2*). However, a fast response component remained even after the late response component was blocked completely (Fig. 5*B1*, rightmost response) and the MK-801 effect on the response amplitude reached a plateau (Fig. 5*B*, 2 and 3). This fast response component could represent residual glutamatergic non-NMDA response, which was not blocked completely by 5 μM DNQX, or be of nonglutamatergic nature. Two reasons indicate, that this component was not

GABAergic. First, picrotoxin was always added to the recording medium throughout the experiment, and second, the recorded current was inward and not outward at holding potential between -50 and -55 mV.

We did not perform experiments with the opposite protocol, i.e., changing the temperature from 32–36°C to room temperature for the following reason. At room temperature, the release probabilities at the low end of the distribution (see following text, values of 0.04–0.06) already reach the limit of detectability. Therefore even if an “undershoot” decrease of the release probability on temperature decrease occurred, it might have remained undetected.

Summary data: comparison of the transmitter release probability at temperatures $>32^\circ\text{C}$ and at room temperature

Comparison of the time course of the blockade of the NMDA receptor-mediated synaptic responses in the presence of 20 μM MK-801 at different temperatures shows that the blockade occurred faster at temperatures $>32^\circ\text{C}$ than at room temperature. The averaged decay constant of the response blockade was significantly higher at room temperature than at temperatures $>32^\circ\text{C}$ (34.12 ± 3.28 against 24.56 ± 1.7 , Kolmogorov-Smirnov test $P = 0.023$, Mann-Whitney test $P < 0.006$). The time course of the response blockade with these averaged decay constants is shown in Fig. 7*C*. The calculated probabilities of transmitter release were significantly higher in synaptic connections studied at $>32^\circ\text{C}$ than at room temperature (0.171 ± 0.012 , $n = 65$ against 0.123 ± 0.009 , $n = 54$; $P = 0.036$, Kolmogorov-Smirnov test; $P < 0.001$ Mann-Whitney test).

For further comparison of these two sets of data, we plotted the release probability against temperature in a scatter diagram (Fig. 7*A*) and the distributions of the release probabilities in the two groups (Fig. 7*B*). Both distributions start from similar minimal values of release probability of ~ 0.04 , and there is little if any difference between them at probability values < 0.1 . At room temperature, the release probability was typically < 0.2 . Only in 6/54 (11%) synaptic connections was $P > 0.2$, and in only 3/54 cases (5%) it was > 0.25 . At temperatures $>32^\circ\text{C}$, although the majority of the probability values were also < 0.2 , higher values were more often encountered. Release probabilities > 0.2 were found in 18/65 (28%), and $P > 0.25$ in

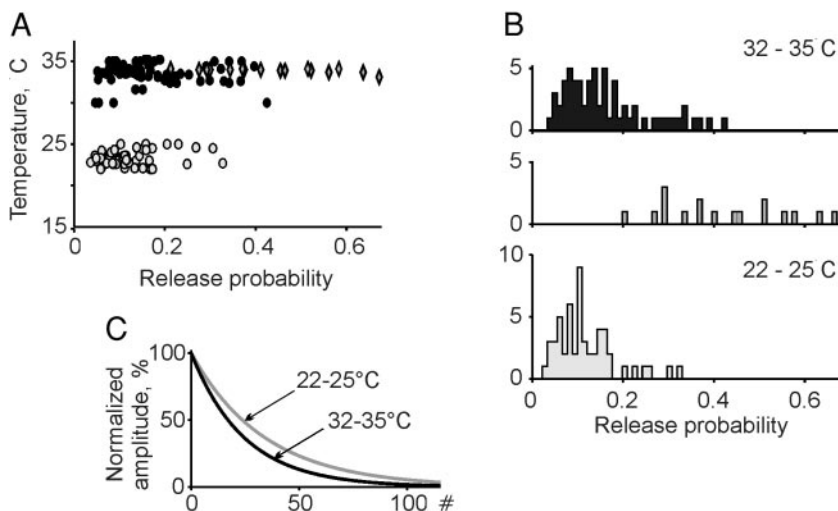


FIG. 7. Summary data: release probability at neocortical synapses at different temperatures. *A*: release probability (abscissa) plotted against recording temperature (ordinate). Diamond shaped symbols represent data obtained after increasing the temperature in the recording chamber from 22–25 to 32–36°C. *B*: distributions of release probabilities at neocortical synapses evaluated in 3 types of recording conditions. *Top*: data from slices held under constant temperature in the range 32–36°C throughout the recording (correspond to black circles in *A*). *Middle*: data from slices, in which control responses were recorded at room temperature (22–25°C), but on application of MK-801 temperature was increased to 32–35°C (correspond to diamond shaped symbols in *A*). *Bottom*: data recorded at room temperature (22–25°C), (correspond to gray circles in *A*). *C*: Averaged decay of the amplitude of the responses mediated by NMDA receptor-gated channels in the presence of 20 μM MK-801 at room temperature (22–25°C, gray) and at 32–36°C (black).

13/64 (20%) of the synaptic connections studied at $>32^{\circ}\text{C}$. Thus the main difference between the two temperature ranges is the presence of a substantial portion of synapses with higher release probability at $32\text{--}36^{\circ}\text{C}$.

Figure 7 demonstrates also a clear enhancement of the release probability after the temperature increase in the recording chamber from $23\text{--}24$ to $33\text{--}35^{\circ}\text{C}$ (diamonds in Fig. 7A and 7B, *middle*). The distribution of the release probabilities measured under these conditions is clearly shifted to the right as compared with any of the other two, room temperature and "warm" distributions.

DISCUSSION

The following conclusions can be drawn from our data. First, the probability of the transmitter release at glutamatergic synapses formed on supragranular pyramidal cells in the visual cortex is variable, and the distribution of release probabilities is skewed, with a predominance of values <0.2 . Second, at room temperature, the probability of the glutamate release is lower, and the distribution of probabilities is more compact than at temperatures $>32^{\circ}\text{C}$. Third, warming the recording chamber from room temperature to $>32^{\circ}\text{C}$ leads to a dramatic but transient increase of the release probability.

Technical considerations

Calculation of the release probability from the dynamics of response blockade by MK-801 has two potentially weak points: first, calculation of the fraction of receptors, which is blocked during one synaptic response (FB), and second, possible contribution of multiple release sites with nonuniform release probability to the synaptic responses.

In earlier studies, estimation of the fraction of the NMDA receptor channels, that is blocked during one synaptic response in the presence of MK-801 (FB), was made for synaptic transmission between hippocampal cells in culture (Rosenmund et al. 1993) and in slices (Hessler et al. 1993; Huang and Stevens 1997) at room temperature. It was suggested, on the basis of indirect evidence, that for the synapses in somatosensory cortex, the FB might be similar to that in the hippocampus (Castro-Alamancos and Connors 1997). Our results are in agreement with that conclusion and show that FB at neocortical synapses is indeed similar to that at synapses in hippocampal slices. We found that at room temperature and for different concentrations of the MK-801 the FB was in the range from 29 to 45%. Both, the range of the FB values as well as their dependence on the MK-801 concentration are similar to those reported earlier for hippocampal synapses (Hessler et al. 1993; Huang and Stevens 1997). At temperatures $>32^{\circ}\text{C}$, the FB values were in a similar range and also concentration dependent. Similarity of the FB values obtained in the two temperature ranges suggests that possible acceleration of the kinetics of the channel blockade with MK-801 at temperatures $>32^{\circ}\text{C}$ was compensated by the shorter channel open times. This is consistent with reports of temperature effects on NMDA receptor-gated channel open times in cerebellar granule cells: mean open time of $600\ \mu\text{s}$ at 24°C reduced almost by half to $350\ \mu\text{s}$ at 32°C (Silver et al. 1996; Wyllie et al. 1993). Further discussion and detailed evaluation of the FB estimation can be found in the earlier studies (Hessler et al. 1993; Huang and

Stevens 1997). The close correspondence of our results on FB estimation to those reported earlier by different groups (Hessler et al. 1993; Huang and Stevens 1997) shows reliability of the approach used.

Another potential concern with application of the MK-801 method is related to the stimulation of several synapses with different release probabilities. In fact, this problem is inevitably present in any electrophysiological experiment. Even with recording from pairs of synaptically connected cells the postsynaptic response is usually due to activation of several synapses with different release probabilities, since in the neocortex each presynaptic fiber makes several synaptic contacts on the postsynaptic cell (e.g., Deuchars et al. 1994; Markram et al. 1997). With our weak extracellular stimulation, a set of synapses originated from few presynaptic fibers was activated. We have evaluated the performance of the MK-801 method in a situation, when a set of synapses contributes to the response in a simulation study (Mukovski and Volgushev, unpublished results). When the release probabilities at the contributing synapses differ by $<20\%$ from each other, the MK-801 method gives as an estimated release probability, approximately the mean of the probability at contributing synapses. With the increasing difference between release probabilities at individual synapses, the estimated value shifts toward the highest release probability within the activated set of synapses. This shift is due to the fact that synapses contribute to the control response in proportion to their release probability. As a result, estimation of the release probability from the dynamics of the MK-801 blockade is dominated by the high probability synaptic contacts. In our experimental data, we did not find evidence for the cases, in which responses could have been composed by two groups of synapses with extremely different release probabilities (<0.05 and >0.5), with high contribution of the group of the low probability synapses. This is indicated by the fact that typically, after 60–70 stimuli in the presence of MK-801 the responses decreased to $<10\%$ of the initial amplitude. With a substantial ($>30\%$) contribution of synapses with low probability, the blockade should have been slower, and the residual response larger. Further arguments for the capability of the MK-801 method to detect underlying differences in the release probabilities on the basis of compound synaptic responses come from earlier studies, where it was applied in the situations which are known to change the probability of transmitter release. It was demonstrated, that addition of Cd^{2+} to the recording medium (Hessler et al. 1993), change of the extracellular Ca^{2+} concentration or stimulation with paired pulses (Huang and Stevens 1997) altered the dynamics of the MK-801 blockade in the expected way. Taken together, these data demonstrate that even when sets of synapses are stimulated, the MK-801 method provides a realistic estimation of the release probability and is suitable for detecting differences in release probability.

Distribution of release probabilities at neocortical synapses

Our study revealed a broad heterogeneity of the probabilities of transmitter release at glutamatergic synapses in connections to pyramidal cells in the supragranular layers of the rat visual cortex. The distribution of the release probabilities is skewed with a predominance of values <0.2 and only few values >0.3 .

Because no published data are available for direct comparison, we can relate our findings only to the results obtained from other structures and other synaptic connections. In the neocortex, few available estimations of the release probability in the somatosensory cortex show that the release probabilities, even at the synapses formed by the same axon, may vary considerably, and depend on the postsynaptic target (Markram et al. 1997, 1998; Reyes et al. 1998; Tsodyks and Markram 1997). A recent study reports very high release probability, ~ 0.8 on average, at synaptic connections between layer 4 stellate cells and layer 2/3 pyramidal neurons in the barrel cortex (Silver et al. 2003). At glutamatergic cortical synapses on CA1 pyramidal cells in the hippocampus, release probability has been estimated with several methods. The analysis of the response amplitude fluctuations with different variants of quantal analysis revealed release probabilities over the whole possible range, from well < 0.1 to almost 1 (e.g., Allen and Stevens 1994; Bolshakov and Siegelbaum 1995; Stricker et al. 1996; Voronin et al. 1992; but see Bekkers 1994; Redman 1990 for problems associated with the quantal analysis methods). Early studies of the release probability at hippocampal synapses using the MK-801 method led to the conclusion that the distribution of the release probabilities at hippocampal synapses in culture and in slices is bimodal, with one peak < 0.1 and the other one $\sim 0.35\text{--}0.55$ (Hessler et al. 1993; Rosenmund et al. 1993). The bimodal distribution of the release probabilities contradicts, however, the estimations obtained with quantal analysis and with measuring the rates of uptake and release of the fluorescent dye FM1-43, which binds to the membrane of synaptic vesicles. With that latter technique, Murthy et al. (1997) found a continuous distribution of release probabilities at individual autaptic synapses in the culture of hippocampal cells. In addition, Rosenmund et al. (1993) pointed out that their data and analysis did not exclude the possibility of a continuous distribution of the release probabilities. A re-examination of that issue in hippocampal slices showed that indeed, the MK-801 blocking functions are consistent with a continuum of release probabilities (Huang and Stevens 1997).

Our data show, that as in other structures studied so far, the release probability at synapses on pyramidal cells in the supragranular layer of the visual cortex vary considerably from one connection to the other. The distribution of the release probabilities is continuous covering the range of probabilities up to ~ 0.4 but with a strong predominance of low values < 0.2 . It should be noted that similarly to other methods based on the recording of synaptic responses, we might have underestimated the number of connections with very low release probabilities, such that the real distribution may be skewed further toward low values. We did not find synaptic connections with release probabilities > 0.5 , although earlier data on all-or-none EPSPs in the visual cortex were indicative of that possibility (Stratford et al. 1996; Volgushev et al. 1995). This apparent discrepancy could be resolved by the existence of a mechanism which synchronizes the release from several release sites (Volgushev et al. 1995). If that mechanism of release synchronization involves ephaptic feedback (Voronin et al. 1999), it might have been disrupted by the application of the AMPA-receptor antagonist DNQX, which was used in our experiments to isolate the NMDA receptor-gated component.

Release probability and recording temperature

We have demonstrated, that at room temperature the probability of transmitter release is lower, than at temperatures close to physiological range ($> 32^\circ\text{C}$). Thus data obtained at room temperature do not represent the whole range of the release probabilities found at higher temperatures. This conclusion is supported by the earlier data on the increase of the failure rate, decrease of the frequency of spontaneous miniature EPSPs, and changes of other indices of presynaptic release at low temperature (Hardingham and Larkman 1998). Higher release probability at higher temperature could be due to a faster influx of Ca^{2+} and thus a steeper raise of the Ca^{2+} concentration in the presynaptic fibers during an action potential (Sabatini and Regehr 1996). This alteration of the dynamics of the calcium influx may also explain a marked increase of the latency of synaptic responses at low temperature (Hardingham and Larkman 1998; Sabatini and Regehr 1996; Volgushev et al. 2000a). Taken together with the results of our previous studies on a pronounced temperature dependence of basic membrane properties, spike generation and synaptic transmission in the neocortex (Volgushev et al. 2000a,b), the present results stress that data gained at close to physiological temperatures are most relevant for drawing conclusions about synaptic function in vivo.

We did not find synaptic connections with release probabilities > 0.5 . This contrasts with a report by Hardingham and Larkman (1998), who analyzed statistics of amplitude distributions. The discrepancy could be at least partially due to a $\text{Ca}^{2+}/\text{Mg}^{2+}$ ratio almost half of that in the earlier study (2/1.5 as compared with 2.5/1 in the study by Hardingham and Larkman). This ratio is known to decrease release probability. Furthermore the studies differ considerably in their temperature regimes. We performed our main experiments under constant temperature—either high or low, because the block by MK-801 is irreversible and the release probability at a given synaptic connection can be calculated only once. In contrast, Hardingham and Larkman (1998) varied temperature during the recording session, which may have lead to potentiation of release on warming (see following text).

Interestingly, when temperature was increased acutely, within minutes, the release probability increased overproportionally. When estimated within few minutes of the temperature increase, the release probability values were significantly higher than those at similarly high temperatures that were held constant throughout the experiment. Fifteen to 25 min after the temperature increase, the release probability decreased again, approaching the expected range. This transient enhancement of the transmitter release could be due to a transient increase of the vesicle refilling rate and an overfilling of the readily releasable pool of vesicles on warming, as reported in other preparations (Dinkelacker et al. 2000; Pyott and Rosenmund 2002). Appearance in some cases after the warming of fast responses indicates that the temperature increase had indeed facilitated release but was not due to potentiation of postsynaptic NMDA receptor-gated channels. If a similar increase of the release probability occurs in the hippocampus, this finding has important implications for studies of long-term plasticity. In vivo studies, the transient increase of the release probability may be one of the factors responsible for the enhancement of the field potentials in the dentate gyrus, associated with

increased brain temperature (Andersen and Moser 1995; Erickson et al. 1996; Moser et al. 1993). Further, the warming-induced enhancement of the release points to possible mechanisms of an otherwise unexplained finding that in hippocampal slices, a transient warming may lead to potentiation of field potentials after the temperature is returned to the control level (Buldakova et al. 1995; Masino and Dunwiddie 2000). In this scenario, the potentiation by warming could be either induced during a short-lasting dramatic enhancement of the release on the acute warming or it may reflect a prolonged but moderate residual increase of the release probability.

ACKNOWLEDGMENTS

We are grateful to P. Balaban for comments on the earlier version of the paper, to M. Calford for comments and improving the English, and to C. Tacke for excellent technical assistance.

GRANTS

This work was supported by the Deutsche Forschungsgemeinschaft SFB 509 TP A5 and NATO LST.CLG.978859.

REFERENCES

- Allen C and Stevens CF. An evaluation of causes for unreliability of synaptic transmission. *Proc Natl Acad Sci USA* 91: 10380–10383, 1994.
- Andersen P and Moser, EI. Brain temperature and hippocampal function. *Hippocampus* 5: 491–498, 1995.
- Bekkers JM. Quantal analysis of synaptic transmission in the central nervous system. *Curr Opin Neurobiol* 4: 360–365, 1994.
- Bolshakov VY and Siegelbaum SA. Regulation of hippocampal transmitter release during development and long-term potentiation. *Science* 269: 1730–1734, 1995.
- Buldakova S, Dutova E, Ivlev F, and Weiss M. Temperature change-induced potentiation: a comparative study of facilitatory mechanisms in aged and young rat hippocampal slices. *Neuroscience* 68: 395–397, 1995.
- Castro-Alamancos MA and Connors BW. Distinct forms of short-term plasticity at excitatory synapses of hippocampus and neocortex. *Proc Natl Acad Sci USA* 94: 4161–4166, 1997.
- Deuchars J, West DC, and Thomson AM. Relationships between morphology and physiology of pyramid-pyramid single axon connections in rat neocortex in vitro. *J Physiol* 478.3: 423–435, 1994.
- Dinkelacker V, Voets T, Neher E, and Moser T. The readily releasable pool of vesicles in chromaffin cells is replenished in a temperature-dependent manner and transiently overfills at 37°C. *J Neuroscience* 20: 8377–8383, 2000.
- Dotz H-U and Zieglgänsberger W. Visualizing unstained neurons in living brain slices by infrared DIC videomicroscopy. *Brain Res* 537: 333–336, 1990.
- Erickson CA, Jung MW, McNaughton BL, and Barnes CA. Contribution of single-unit spike waveform changes to temperature-induced alterations in hippocampal population spikes. *Exp Brain Res* 107: 348–360, 1996.
- Hardingham NR and Larkman AU. The reliability of excitatory synaptic transmission in slices of rat visual cortex in vitro is temperature dependent. *J Physiol* 507: 249–256, 1998.
- Hessler NA, Shirke AM, and Malinow R. The probability of transmitter release at a mammalian central synapse. *Nature* 366: 569–572, 1993.
- Huang EP and Stevens CF. Estimating the distribution of synaptic reliabilities. *J Neurophysiol* 78: 2870–2880, 1997.
- Huettnner JE and Bean BP. Block of N-methyl-D-aspartate-activated current by the anticonvulsant MK-801: selective binding to open channels. *Proc Natl Acad Sci USA* 85: 1307–1311, 1988.
- Markram H. A network of tufted layer 5 pyramidal neurons. *Cereb Cortex* 7: 523–533, 1997.
- Markram H, Lubke J, Frotscher M, Roth A, and Sakmann B. Physiology and anatomy of synaptic connections between thick tufted pyramidal neurons in the developing rat neocortex. *J Physiol* 500.2: 409–440, 1997.
- Markram H, Wang Y, and Tsodyks M. Differential signaling via the same axon of neocortical pyramidal neurons. *Proc Natl Acad Sci USA* 95: 5323–5328, 1998.
- Masino SA and Dunwiddie TV. A transient increase in temperature induces persistent potentiation of synaptic transmission in rat hippocampal slices. *Neuroscience* 101: 907–912, 2000.
- Moser E, Mathiesen J, and Andersen P. Association between brain temperature and dentate field potentials in exploring and swimming rats. *Science* 259: 1324–1326, 1993.
- Murthy VN, Sejnowski TJ, and Stevens CF. Heterogeneous release properties of visualized individual hippocampal synapses. *Neuron* 18: 599–612, 1997.
- Plyott SJ and Rosenmund C. The effects of temperature on vesicular supply and release in autaptic cultures of rat and mouse hippocampal neurons. *J Physiol* 539.2: 523–535, 2002.
- Redman S. Quantal analysis of synaptic potentials in neurons of the central nervous system. *Physiol Rev* 70: 165–198, 1990.
- Reyes A, Lujan R, Rozov A, Burnashev N, Somogyi P, and Sakmann B. Target-cell-specific facilitation and depression in neocortical circuits. *Nature Neurosci* 1: 279–285, 1998.
- Rosenmund C, Clements JD, and Westbrook GL. Nonuniform probability of glutamate release at a hippocampal synapse. *Science* 262: 754–757, 1993.
- Sabatini BL and Regehr WG. Timing of neurotransmission at fast synapses in the mammalian brain. *Nature* 384: 170–172, 1996.
- Silver RA, Colquhoun D, Cull-Candy SG, and Edmonds B. Deactivation and desensitization of non-NMDA receptors in patches and the time course of EPSCs in rat cerebellar granule cells. *J Physiol* 493: 167–173, 1996.
- Silver RA, Lübke J, Sakmann B, and Feldmeyer D. High-probability unquantal transmission at excitatory synapses in barrel cortex. *Science* 302: 1981–1984, 2003.
- Stratford KJ, Tarczy-Hornoch K, Martin KAC, Bannister NJ, and Jack J. Excitatory synaptic inputs to spiny stellate cells in cat visual cortex. *Nature* 382: 258–261, 1996.
- Stricker C, Field AC, and Redman SJ. Statistical analysis of amplitude fluctuations in EPSCs evoked in rat CA1 pyramidal neurons in vitro. *J Physiol* 490: 419–441, 1996.
- Stuart GJ, Dodt HU, and Sakmann B. Patch-clamp recordings from the soma and dendrites of neurons in brain slices using infrared video microscopy. *Pflügers* 423: 511–518, 1993.
- Thomson AM and Deuchars J. Temporal and spatial properties of local circuits in neocortex. *Trends Neurosci* 17: 119–126, 1994.
- Tsodyks MV and Markram H. The neural code between neocortical pyramidal neurons depends on neurotransmitter release probability. *Proc Natl Acad Sci USA* 94: 719–723, 1997.
- Volgushev M, Vidyasagar TR, Chistiakova M, and Eysel UT. Synaptic transmission in the neocortex during reversible cooling. *Neuroscience* 98: 9–22, 2000a.
- Volgushev M, Vidyasagar TR, Chistiakova M, Yousef T, and Eysel UT. Membrane properties and spike generation in rat visual cortical cells during reversible cooling. *J Physiol* 522: 59–76, 2000b.
- Volgushev M, Voronin L, Chistiakova M, Artola A, and Singer W. All-or-none excitatory postsynaptic potentials in the rat visual cortex. *Eur J Neurosci* 7: 1751–1760, 1995.
- Voronin LL, Kuhnt U, Hess G, Gusev A, and Roschin V. Quantal parameters of “minimal” excitatory postsynaptic potentials in guinea pig hippocampal slices: binomial approach. *Exp Brain Res* 89: 248–264, 1992.
- Voronin LL, Volgushev M, Sokolov M, Kasyanov A, Chistiakova M, and Reymann KG. Evidence for an ephaptic feedback in cortical synapses: Postsynaptic hyperpolarization alters the number of response failures and quantal content. *Neuroscience* 92: 399–405, 1999.
- Wyllie DJ, Traynelis SF, and Cull-Candy SG. Evidence for more than one type of non-NMDA receptor in outside-out patches from cerebellar granule cells of the rat. *J Physiol* 463: 193–226, 1993.