Adaptation of synaptic connections to layer 2/3 pyramidal cells in rat visual cortex

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ABSTRACT

Neocortical synapses express differential dynamic properties. When activated at high frequencies, the amplitudes of the subsequent postsynaptic responses may increase or decrease, depending on the stimulation frequency and on the properties of that particular synapse. Changes in the synaptic dynamics can dramatically affect the communication between nerve cells. Motivated by this question, we studied dynamic properties at synapses to layer 2/3 pyramidal cells with intracellular recordings in slices of rat visual cortex. Synaptic responses were evoked by trains of test stimuli, which consisted of 10 pulses at different frequencies (5-40 Hz). Test stimulation was applied either without any adaptation (control) or 2 s after an adaptation stimulus, which consisted of 4 s stimulation of these same synapses at 10, 25 or 40 Hz. The synaptic parameters were then assessed from fitting the data with a model of synaptic dynamics. Our estimates of the synaptic parameters in control, without adaptation are broadly consistent with previous studies. Adaptation led to pronounced changes of synaptic transmission. After adaptation, the amplitude of the response to the first pulse in the test train decreased for several seconds and then recovered back to the control level with a time constant of 2-18 seconds. Analysis of the data with extended models, which include interaction between different pools of synaptic vesicles, suggests that the decrease of the response amplitude was due to a synergistic action of two factors, decrease of the release probability and depletion of the available transmitter. After a weak (10 Hz) adaptation, the decrease of the response amplitude was accompanied by and correlated with the decrease of the release probability. After a strong adaptation (25 or 40 Hz), the depletion of synaptic resources was the main cause for the reduced response amplitude. Adaptation also led to pronounced changes of the time constants of facilitation and recovery, however, these changes were not uniform in all synapses, and on the population level the only consistent and significant effect was an acceleration of the recovery after a strong adaptation. Taken together, our results suggest, that apart from decreasing the amplitude of postsynaptic responses, adaptation may produce synapse-specific effects, which could result in a kind of re-distribution of activity within neural networks.
INTRODUCTION

Brain function depends on synaptic plasticity at several different time scales. At the lower end of the scale (~100 ms) is short term plasticity, which has been studied in the rat neocortex for synaptic connections formed between different neuron types (Akaneya et al. 2003; Galarreta and Hestrin 1998; Jia et al. 2004; Markram et al. 1998; Petersen 2002; Reyes et al. 1998; Thomson 1997; Wang and Kaczmarek 1998). The strength of these synaptic connections is changed depending on the activity history of the particular synapse. Mechanisms of the short term synaptic changes include both release-dependent as well as release-independent components (reviewed in Zucker and Regehr 2002). Simple use dependent models have been very successfully applied for numerical characterization of the presynaptic component of facilitation and depression at these synapses. It has been shown that models with few degrees of freedom are able to capture essential parts of synaptic dynamics and can in turn be easily interpreted in terms of the depletion of transmitter vesicles after the release and their subsequent replenishment (Abbott et al. 1997; Tsodyks et al. 1998; Tsodyks and Markram 1997; Varela et al. 1997; Varela et al. 1999). The above studies characterized the dynamic behavior of synaptic connections after long periods of rest or during low activity levels, that is, essentially without taking into account the history of the high-frequency pre- or postsynaptic activity on the time scale of several seconds. However, it is well known that synaptic dynamics in the neocortex indeed can be altered by a number of manipulations, including induction of long-term plasticity (Markram and Tsodyks 1996; Volgushev et al. 1997) or sensory deprivation (Reyes and Sakmann 1999). On the short time scale of seconds to dozens of seconds, adaptation has been shown to depress responses at the thalamocortical synapses, but not at corticocortical synapses of rat somatosensory cortex (Chung et al. 2002). Thus, synaptic dynamics can be dramatically influenced by the activity history of that particular synapse on both, long-term and short-term scales.

The effect of synaptic dynamics on cortical information transmission has been investigated in a number of theoretical studies (Adorjan et al. 1999; Artun et al. 1998; Fuhrmann et al. 2002; Goldman et al. 2002). Depression of synaptic transmission in the afferent pathway was suggested as one of the mechanism contributing to various cortical phenomena, including nonlinear summation, temporal phase shifts, contrast saturation, contrast adaptation or cross-orientation suppression (Carandini et al. 2002; Chance et al. 1998). In particular, it has been proposed that contrast adaptation might be due to a slow form of synaptic depression (Chance et al. 1998) or a slow change in neurotransmitter release probability (Adorjan et al. 1999) at the thalamocortical synapses. Another study (Adorjan et al. 2000) implicated intracortical depression in the optimal coding strategy for the representation of complex stimuli. Motivated by these experimental and theoretical studies we investigated the effect of ‘adaptation’, which consisted of brief, 4 s intervals, of presynaptic activity on the dynamic characteristics of synaptic connections onto rat layer 2/3 pyramidal cells.

METHODS

The experimental procedures used in this study were in accordance with the guidelines published in the European Communities Council Directive (86/609/EEC, 1986) and were approved by the regional animal welfare committee (Arnsberg, Germany).

Slices

Slices of the visual cortex were prepared as described in detail elsewhere (Volgushev et al. 2004). Wistar rats (P25-P35, Charles River GmbH, Suzfeld, Germany) were anaesthetised
with ether, decapitated and the brain was rapidly removed and put into an ice cold oxygenated
solution. 350-400 µm thick frontal slices of the visual cortex were cut with a vibrotome
(Leica, VT 1000S, Nussloch, Germany). After the cutting, the slices were let to recover in an
incubator for at least one hour at room temperature. The solution used during the preparation
of the slices had the same ionic composition as the recording medium (see below), except for
L-glutamine.

Electrophysiological recordings

For recordings, a slice was put into a submerged chamber. The perfusion medium contained
(in mM) 125 NaCl, 2.5 KCl, 2 CaCl₂, 1.5 MgCl₂, 1.25 NaH₂PO₄, 25 NaHCO₃, 25 D-glucose
and 0.5 L-glutamine, and was aerated with 95% O₂ and 5% CO₂ bubbles. All recordings were
made at 32-34°C. Patch-electrodes were filled with a solution containing (in mM) 127 K-
Gluconate, 20 KCl, 2 MgCl₂, 2 Na₂ATP, 10 HEPES, 0.1 EGTA and had a resistance of 3-7
MΩ. Whole-cell recordings were made from pyramidal neurones 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 Zieglgansberger 1990). Reliability of the
identification of the pyramidal cells has been proved in our previous work by labelling the
recorded cells with biocytin and morphological reconstruction (Volgushev et al. 2000).
Recordings were made with Axoclamp-2A (Axon Instruments) in voltage-clamp mode at
holding potential between -75 mV and -85 mV, which was kept constant for the length of
recording from one cell. 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). We used low intensity of the stimulation, which was set to produce small postsynaptic
responses (excitatory postsynaptic currents, EPSCs) without failures. 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.

Chemicals

The following chemicals were obtained from Sigma (Deisenhofen, Germany): biocytin,
EGTA, HEPES, K-Gluconate, L-Glutamine, Na₂ATP. The remaining chemicals were from

Modelling use-dependent synaptic dynamics

To assess parameters of synaptic transmission, we fitted the excitatory postsynaptic currents
(EPSCs), evoked by repetitive stimulation at different frequencies with a phenomenological
model of synaptic transmission (Abbott et al. 1997; Markram et al. 1998; Tsodyks et al. 1998;
Tsodyks and Markram 1997). According to the model, a synapse contains a store R of
immediately releasable vesicles, the resource. When an action potential arrives at the
presynaptic terminal, it leads to a release of a fraction U of this store, and at the same time, to
a temporal increase of the release probability U by a certain amount. The utilisation U in the
model has physiological meaning of release probability. In this manuscript we will use U in
the equations describing the use-dependent model, as in the original account of the model
(Tsodyks and Markram 1997), and p to denote release probability in the binomial release
model. The released vesicles are replenished with the time constant τ_rec, and release
facilitation decays with the time constant τ_fac, both processes are exponential. The released
transmitter evokes a current in the postsynaptic neuron that is proportional to the number of released vesicles by a factor \( g \) (\( I = g R U \)). Thus our model equations describing the synaptic dynamics are

\[
\frac{dR}{dt} = \frac{1-R}{\tau_{rec}} - UR \delta(t - t_{sp}) \tag{1}
\]
\[
\frac{dU}{dt} = \frac{U_0 - U}{\tau_{fac}} + U_0 (1-U) \delta(t - t_{sp}) \tag{2}
\]

where \( U_0 \) is the utilization of resources \( R \) at very low frequency of stimulation and \( t_{sp} \) the time of a presynaptic spike. The peak synaptic current is then given by

\[
I_{peak} = gR(t_{sp})U(t_{sp}). \tag{3}
\]

Equations 1 and 2 can be cast into iterative expressions for \( R \) and \( U \) immediately before the arrival of the \((n+1)\)-th spike, which depend only on the values for \( R \) and \( U \) immediately before the arrival of the \( n \)-th spike and on the time interval \( \Delta t \) between the \( n \)-th and \((n+1)\)-th spikes

\[
R_{n+1} = 1 - \exp(-\Delta t / \tau_{rec}) + R_n (1 - U_n) \exp(-\Delta t / \tau_{rec}) \tag{4}
\]
\[
U_{n+1} = U_n (1 - U_0) \exp(-\Delta t / \tau_{fac}) + U_0 \tag{5}
\]

When fitting the synaptic responses, we initially assumed that between successive applications of the test stimuli the resources \( R_1 \) are fully recovered, that is \( R_1 = 1 \). While this assumption holds for the stimulation without adaptation, as indicated by the stable amplitude of the responses to the first pulses in each train (see results), it does not hold for the stimuli applied after adaptation.

**Binomial release model**

In addition to assessing the release parameters from the response dynamics, we estimated changes in the release probability with the use of quantal analysis (Korn and Faber 1991; Redman 1990; Tarczy-Hornoch et al. 1999). The binomial model of release assumes that all \( n \) release sites contributing to the postsynaptically recorded \( EPSC \) (i) have the same release probability \( p \), (ii) release neurotransmitter independently from each other, (iii) have the synaptic vesicles of identical size, and (iv) upon arrival of an action potential to the presynapse, release either none or exactly one vesicle, which produces a postsynaptic effect of a quantal size \( q \). The expectation (\( \bar{EPSC} \)) and the standard deviation (\( \text{std}(EPSC) \)) of the evoked \( EPSC \) is then given by

\[
\bar{EPSC} = q \cdot np \tag{6}
\]
\[
\text{std}(EPSC) = q \cdot (np(1-p))^{1/2}. \tag{7}
\]

The coefficient of variation (\( CV \)) is

\[
\frac{1}{CV^2} = \frac{np}{1-p} \tag{8}
\]

which is independent of the quantal size \( q \) of synaptic vesicles. This in turn leads to the expression

\[
p = \frac{1}{CV^2 n + 1} \tag{9}
\]

for the release probability \( p \), which still depends on the unknown number of release sites \( n \).

Under the reasonable assumption that \( n \) does not change after an adapting stimulus a change of release probability \( p \) can be estimated. While some of the above assumptions are not necessarily always correct, the inverse coefficient of correlation is often considered as one of
the indicators of changes of release probability, and may be used in combination with other approaches (Faber and Korn 1991; Voronin 1993).

**Stimulus protocol and data analysis**

We have assessed parameters of synaptic transmission and their changes after an adaptation by fitting the phenomenological model of synaptic dynamics (Abbott et al. 1997; Tsodyks et al. 1998) to the postsynaptic responses evoked by stimuli at different frequencies. Our experimental protocol is schematically illustrated in Fig. 1. Test stimuli were applied in trains of 10 pulses at 5, 10, 20 or 40 Hz, either after an adapting stimulation or without adaptation. The response of a cell to one train of test stimuli is referred to as one sweep. For adaptation of synapses, we used the same stimuli as in the test trains, but applied them for 4s at 10, 25 or 40 Hz. Thus the higher frequencies led also to the higher number of adapting stimuli. The adaptation was followed by a 2s interval without stimulation, before a train of test stimuli was applied. In one experiment, we applied test stimuli at 3-4 different frequencies without adaptation and after an adaptation, either with only one frequency or with one of the two different adapting frequencies. Different combinations of the above test and adaptation stimuli were presented intermingled, once in 75-90 s. The stimuli at two stimulation sites (Fig. 1A) were applied in an interleaved manner. Simulations, performed prior to the beginning of electrophysiological recordings, demonstrated that synaptic parameters can be assessed from the responses to 3-4 test frequencies (data not shown). Therefore, for the further analysis we used synaptic connections for which, responses to at least 5 presentations of 3 different test frequencies, without adaptation and after at least one adapting frequency, were collected. Out of 56 synaptic connections, which fulfilled these requirements, 26 could be characterized for two different adaptation frequencies. The amplitudes of excitatory postsynaptic currents (EPSC) were measured as the difference between the mean current within two windows of 1-5 ms width, one positioned immediately before the response and another one around the peak of the averaged EPSC or on the last portion of the rising slope (Volgushev et al. 2000). For each synaptic connection, the responses obtained with one given set of the adapting and test frequency were averaged and then normalized to the response evoked by the first stimulus in the test train. Our model equations 4 and 5 were then fitted via a least squares method to these normalised responses, obtained with all available test frequencies. Specifically, we were looking for the parameters \( U, \tau_{rec} \) and \( \tau_{fac} \) in equations 4 and 5 that led to the best match between model prediction and measured averaged test responses in a least-square sense. As a fitting routine we used a Gauss-Newton method provided by the function nlinfit of the statistics toolbox of Matlab\textsuperscript{TM} (Mathworks, Natick, MA). The maximum number of iterations was set to 500, the termination tolerance for the estimated coefficients as well as the residual sum of squares was chosen as \( 10^{-6} \). The Fig. 2A illustrates the averaged response traces (A1) and normalized EPSC amplitudes together with the best fit for one synaptic connection in the control condition (A2). To estimate the quality of the fits across the sample, we also calculated the root-mean-square (rms) error for each fit. Figs. 2B, show the distribution of the rms error per pulse over all synaptic connections in the control (Fig. 2B1) and for all adaptation conditions and control (Fig. 2B2). The mean and median rms error per stimulus for the whole sample were 0.11 and 0.10 respectively, thus a response to a single stimulus could be predicted by the model with an average error of 11%. The rms error is a measure of the quality of the fit, and it shows how well the data are represented by the model. However, it does not by itself say anything about the reliability of the estimation of the free model parameters \( U, \tau_{rec} \) and \( \tau_{fac} \) in equations 4 and 5.
In order to assess this reliability we have used two approaches. In the first approach, we have performed a series of simulations. For a set of combinations of parameters $U, \tau_{\text{rec}}$, and $\tau_{\text{fac}}$, we simulated the EPSC responses given by equations 4 and 5 and then added to each EPSC a Gaussian noise with a coefficient of variation $CV = 0.3$, which is similar to the values found in the experiments. As in the experiments we averaged 5 traces of simulated responses for each test frequency. Then we fitted the optimal parameters $U, \tau_{\text{rec}}$ and $\tau_{\text{fac}}$ to these noisy EPSC responses and compared them to the true $U, \tau_{\text{rec}}$ and $\tau_{\text{fac}}$ of the noiseless model synapse. The whole procedure was repeated 100 times for each set of synaptic parameters. The results of these simulations showed, that estimation of the release probability $U$ is highly reliable, with a median deviation of less than 7% from the true value of the $U$, regardless of the absolute values of the true release probability and facilitation time constant. In addition, the deviation of the estimated $U$ from the true value decreased rapidly with increasing the recovery time constant used in the simulations. Estimation of the facilitation time constant appeared to be less reliable, with a a peak deviation of up to 30% from the true value in the cases in which a combination of the high true release probability, long facilitation time constant, and short recovery time constant was used. The error in estimation of the facilitation time constant decreased rapidly with increasing the true recovery time constant. Estimation of the recovery time constant showed the strongest dependence on the initial settings used for the simulation of synaptic responses. For the set of true values of the release probability $U > 0.2$ and recovery time constants shorter than 1.5 s, the deviation of the estimated $\tau_{\text{rec}}$ from its true value remained below 15%. With the decreasing values of simulated release probability ($U < 0.2$), the median deviation of the estimated recovery time constant from the true value increased, but stayed below 35%. For the simulated synaptic responses with the combination of low $U$, long $\tau_{\text{rec}}$ and short $\tau_{\text{fac}}$, the estimation of the recovery time constant became unreliable, with deviation from the true value increasing to over 75%. In that latter parameter regime did not only the deviation from the true value increase but we observed a number of cases with ‘diverging’ ($> 10^3$ s) recovery time constant as the optimal fit to the data. Thus, for synapses with a long recovery process ($\tau_{\text{rec}} > 3-5$ s) and a low release probability ($U < 0.2$) we frequently (1% - 25% of all runs) could not attribute a finite recovery process to the synapse based on the optimal root-mean-square fit. Reversing the argument, if our recovery time constant estimate diverged, it was likely that the true recovery time constant was larger than 3s and the true release probability smaller than 0.2. Since under conditions of our experimental protocol the recovery processes lasting longer than 3s could not reliably resolved, all ‘diverging’ recovery time constants in the following will be regarded as $\tau_{\text{rec}} > 3$s.

In the second approach we investigated how variable are the estimations of synaptic parameters from repetitive measurements at the same synapse. We therefore increased the number of repetitions of test stimuli, and recorded 10 to 12 sweeps of responses to each test frequency, in the control condition and after an adaptation. For each of the 7 synaptic connections recorded with this protocol, we composed 20 random subsets of data, each subset including 5 randomly chosen sweeps of responses to each test frequency. It should be noted, that different random subsets are not mutually independent and therefore the following procedure gives only a rough estimate of the true $CV$ of the assessment of synaptic parameters by the model. These random subsets of sweeps were processed as described above and the optimal parameters $U, \tau_{\text{rec}}$ and $\tau_{\text{fac}}$ were estimated. The quality of these fits was not different from the rest of the sample, as indicated by the similar values of the mean rms errors (0.11 vs. 0.11 for the rest of the sample). Next, we calculated the coefficient of variation ($CV$) for the estimated $U, \tau_{\text{rec}}$ and $\tau_{\text{fac}}$ for each of the 7 synaptic connections. The $CV$ gives an estimate of the reproducibility of the convergence of the model to the same set of optimal synaptic parameter values, when different subsets of data from the same synapse are used. The lower
the CV, the higher is the reproducibility of convergence, and thus the reliability of the estimation. The mean CV for the estimated release probability $U$, the recovery time constant $\tau_{rec}$ and the facilitation time constant $\tau_{fac}$ in control were $\overline{CV}_{U}^{NoAdap} = 0.10$, $\overline{CV}_{\tau_{rec}}^{NoAdap} = 0.13$ and $\overline{CV}_{\tau_{fac}}^{NoAdap} = 0.40$, and after an adaptation the mean CV were $\overline{CV}_{U}^{Adap} = 0.15$, $\overline{CV}_{\tau_{rec}}^{Adap} = 0.17$ and $\overline{CV}_{\tau_{fac}}^{Adap} = 0.46$.

Taken together, the results of this analysis show that our protocol gives a reliable estimation of the release probability $U$, with a low variability of the assessments obtained from the repeated measurements. The recovery time constant $\tau_{rec}$ could also be reliably estimated in the above sample, but we expect from our theoretical analysis that this reliability would decrease substantially if the synapses had longer recovery time constants. The estimate of the facilitation time constant is less reliable and varies even between different measurements at the same synaptic connection.

**RESULTS**

*Heterogeneity of synaptic properties in control, without previous adaptation*

The dynamic parameters in the control condition without preceding adaptation were highly heterogeneous across the investigated synaptic connections. The distributions of the release probability $U$, the recovery time constant $\tau_{rec}$ and the facilitation time constant $\tau_{fac}$ over the population of 56 synaptic connections are shown in Fig 3. The release probability at these synapses varied between 0.04 and 0.57, with predominance of low values (Fig. 3A). The average release probability was $\overline{U} = 0.21$ (median: 0.17; std: 0.12). The distribution of the recovery time constant covered a wide range between 85 ms and more than 3000 ms, with most of the values below 1500 ms (39 out of 56, 70%), but 14 values (25%) larger than 3000ms. The facilitation time constant was on average $\overline{\tau_{fac}} = 32.9$ ms (median: 14.9 ms; std: 49.7 ms; range: 1 ms – 278.6 ms). Altogether, the assessed values of these three synaptic parameters, as well as their large heterogeneity are in line with previous studies of synaptic characteristics in rat visual cortex (Varela et al. 1997) or somatosensory cortex (Markram et al. 1998). The three synaptic parameters were not independent, but some of them were correlated. A negative correlation has been found between the release probability and the recovery time constant ($r = -0.47; p < 0.0003$; F-statistic) and a positive correlation between the release probability and the facilitation time constant ($r = 0.59; p < 2 \cdot 10^{-6}$). Thus, synapses with higher release probability had shorter recovery time constants, and longer facilitation. No significant correlation was found between the facilitation and recovery time constants, $\tau_{rec}$ and $\tau_{fac}$.

*Changes in synaptic transmission after adaptation*

Adaptation led to marked changes in synaptic transmission. The most prominent effect, consistently observed after adaptation with any of the 3 frequencies (10, 25 or 40 Hz) was a reduction of the amplitude of the response to the first stimulus in the test train (EPSC$_1$). The synaptic dynamics and assessed parameters of synaptic transmission expressed differential changes after a weak (10 HZ) and strong (25 or 40 Hz) adaptation. In the following we will
first consider the $EPSC_1$ amplitude changes, and then describe separately changes in synaptic parameters after weak and strong adaptation.

**Decrease of EPSC$_1$ amplitude after adaptation**

A typical example of the effect of a 10 Hz adaptation on synaptic transmission is illustrated in Fig. 4. The amplitude of the $EPSC_1$ decreased after the adaptation to about 65% of the control value. The $EPSC_1$ amplitude reduction is clearly seen in the averaged response traces (compare Fig. 4, A1 and B2) and is highly significant ($p < 2 \cdot 10^{-5}$; Wilcoxon non-paired test). The reduction of the $EPSC_1$ after adaptation was typical for our sample, and occurred in the majority of synaptic connections. In the scatter plot 4C, where the amplitude of the $EPSC_1$ after the 10 Hz adaptation is plotted against the $EPSC_1$ amplitude in the control condition, most of the points are located below the main diagonal. On average, 10 Hz adaptation led to a reduction of the $EPSC_1$ amplitude to 75.5% of the control value (median: 77.3%; std: 28.3; range: 32.4 to 164.6%; $p < 9 \cdot 10^{-5}$; Wilcoxon paired test). Stronger adaptation with 25 or 40 Hz led to a yet stronger decrease of the $EPSC_1$ amplitude (Fig. 5A). After 25 Hz adaptation the first response amplitude dropped to 65.6% (median: 63.0%; std: 23.3; range: 32.4 to 148.2%; $p < 3 \cdot 10^{-4}$) and after a 40 Hz adaptation to 53.5% (median: 54.9%; std: 23.5; range: 9.3 to 93.5%; $p < 3 \cdot 10^{-6}$).

In most of the cases, we recorded synaptic responses in the control condition and after adaptation with one of the 3 frequencies, therefore the effects of adaptation with different frequencies are compared on the sample basis. To verify the relation between the adaptation strength and the degree of reduction of the $EPSC_1$ amplitude, we performed control experiments, in which 2 different adaptation frequencies were used. Data from 26 synaptic connections studied in this way are presented in Fig. 5B. In the scatter plot, the change in the averaged $EPSC_1$ amplitude after a strong adaptation is plotted against the $EPSC_1$ amplitude change after a weak adaptation. In all but one connection the $EPSC_1$ amplitude decreased more after a strong (25 Hz and 40 Hz, ordinate in Fig. 5B) than after weak (10 Hz, abscissa in Fig. 5B) adaptation. The median difference over the recorded population is $\text{median}(\Delta EPSC_{1}^{40,10}) = \text{median}(EPSC_{1}^{40} / EPSC_{1}^{0} - EPSC_{1}^{10} / EPSC_{1}^{0}) = -0.24$

(mean: -0.31; std: 0.25; range: -0.89 to 0.06; $p < 4 \cdot 10^{-6}$; Wilcoxon paired test) for a comparison between 10 Hz and 40 Hz adaptation. Thus, at any given synapse, a stronger (higher frequency) adapting stimulation indeed led to a stronger reduction of the $EPSC_1$ amplitude.

**Recovery of single EPSCs after adaptation**

The decrease of the $EPSC_1$ amplitude after adaptation was short-lasting and reversible, and the response amplitude recovered to the control value before the next test stimulus was applied (in 75-90 seconds). To investigate the time course of the recovery of the adaptation-induced decrease of the $EPSC_1$, we performed an additional series of experiments, in which seven test pulses were applied at a low frequency (0.2 Hz) starting 2s after the end of the adaptation (Fig. 6A1). In the example shown in Fig. 6A, adaptation with 10 Hz lead to only a moderate increase of the response amplitude, but adaptation with 40 Hz led to a marked decrease of the $EPSC$ amplitude (Fig. 6 A2). After both, 10 Hz or 40 Hz adaptation, the response amplitude recovered to the control value after 10-20 s. In order to quantify this recovery process we fitted a single exponential to the normalized $EPSC$ amplitude responses:

$$EPSC(t) = (EPSC_0 - EPSC_\infty) \cdot \exp(-t / \tau) + EPSC_\infty$$
The three free parameters are the initial \( EPSC_0 \) amplitude response that would have been observed immediately after the adaptation stimulus, the control level \( EPSC_0 \), and the time constant \( \tau \) of this recovery process. When fitting the equation (10) to the data, the \( EPSC_0 \) was constrained to be larger than 0. Fig. 6B shows the relationship between the decrease of the \( EPSC_1 \) amplitude after adaptation to 25 Hz or 40 Hz and the optimal fit of the recovery time constant \( \tau \). The average time constant of the recovery was \( \tau = 7.1s \) (median: 5.2s; std: 5.9s; range: 1.8s – 18s), the correlation coefficient between the decrease of the \( EPSC_1 \) and the time constant \( \tau \) was \( r = -0.6 \) (\( p < 0.11 \); F-statistics).

### Synaptic changes after weak (10Hz) adaptation

We assessed changes of the synaptic parameters after a weak adaptation with 10 Hz frequency relative to control in 29 synaptic connections. The fitting of control data and of the responses recorded after the adaptation was of comparable, in both cases high, quality. This is illustrated in the scatter in Fig. 4D, where the rms errors of the fits of control and adaptation data are plotted against each other. No significant difference was found in the median error between the fits of the responses recorded in control (median: 0.09) and after 10 Hz (median: 0.10) adaptation (\( p > 0.3 \); Wilcoxon non-paired test).

In the example in Fig. 4, the control responses (Fig 4A) were optimally fitted with \( U = 0.17, \tau_{rec} = 500.5 \text{ ms}, \text{ and } \tau_{fac} = 1 \text{ ms} \). After the adaptation, the best fit was obtained with \( U = 0.09, \tau_{rec} = 629.9 \text{ ms} \) and \( \tau_{fac} = 11.7 \text{ ms} \) (Fig. 4B). In this example, the decrease in the release probability \( U^{10}/U^0 = 0.53 \) can reasonably well account for the reduction of the amplitude of the \( EPSC_1 \) (mean: 0.64). A decrease of the release probability after a 10 Hz adaptation was typical for our sample, as illustrated in Fig. 7A, in which for each synaptic connection, the release probability after the adaptation is plotted against the control value. A statistical analysis reveals a highly significant decrease of \( U \) in the population of measured connections (median\( \Delta U \) = median\( (U^0 - U^{10}) = 0.030 \); mean: 0.039; std: 0.060; range: -0.05 to 0.2; \( p < 2 \cdot 10^{-3} \); Wilcoxon paired test). Moreover, the change in the release probability was significantly albeit weakly correlated with the change of \( EPSC_1 \) amplitude after the adaptation (Fig. 7F, correlation coefficient: \( r = 0.45 \); \( p < 0.02 \); F-statistic). These observations, which rely on the assessment of the release probability from the response dynamics, are corroborated by an independent estimation of the release probability changes with the coefficient of variation method. After the adaptation, the inverse squared coefficient of variation (\( CV^{-2} \)) of the \( EPSC_1 \) amplitude decreased significantly (\( p < 0.04 \); Wilcoxon paired test), which is indicative of the decreased release probability (Fig. 7D). A significant correlation between the change in the \( CV^{-2} \) and the change in the \( EPSC_1 \) amplitude (\( r = 0.66 \); \( p < 1 \cdot 10^{-4} \); F-statistic, Fig. 7E) lends further support to the conclusion that the reduction of the \( EPSC_1 \) amplitude after the adaptation is at least partially due to the decrease of the release probability. However, since the above correlations are weak, and for some synapses changes in \( EPSC_1 \) and \( U \) or \( p \) clearly do not go hand in hand, other factors might have contributed to the decrease of the response amplitude after an adaptation. This topic will be elaborated further later in this text.

Other parameters of synaptic transmission, the time constants of recovery, \( \tau_{rec} \), and facilitation, \( \tau_{fac} \), did not show consistent changes on the population level. It should be noted here, that at some synaptic connections the best fit for recovery time constant was out of the range of its reliable estimation. The estimated recovery time constant was longer than 3 s in both, control conditions and after 10 Hz adaptation in 4 synaptic connections. In 3 more
synapses, the estimated $\tau_{\text{rec}}$ was longer than 3 seconds in control, and in 5 other synapses $\tau_{\text{rec}}$ became longer than 3 seconds after 10 Hz adaptation. All these cases were excluded from the population analysis. For the remaining subpopulation of synaptic connections, in which the estimation of $\tau_{\text{rec}}$ was reliable ($\tau_{\text{rec}} < 3s$) both before and after 10 Hz adaptation (Fig. 7B), no significant changes of the recovery time constant were found:

$$\text{median}(\Delta \tau_{\text{rec}}) = \text{median}(\tau_{\text{rec}}^0 - \tau_{\text{rec}}^{10}) = -7.9 \text{ ms}; \text{mean: 75.2 ms; std: 450 ms; range: -783 to 1092 ms; } p > 0.6.$$  

The facilitation time constant $\tau_{\text{fac}}$ also did not show significant changes on the population level (Fig. 7C, $\text{median}(\Delta \tau_{\text{fac}}) = 0.5 \text{ ms; mean: 13.9 ms; std: 34.5 ms; range: -21.7 to 108.3 ms; } p > 0.15$).

Despite the absence of a unidirectional trend in changes of facilitation and recovery time constants on the population level, individual connections often show a very different behaviour before and after the adaptation, and alter $\tau_{\text{rec}}$ and/or $\tau_{\text{fac}}$ quite dramatically. In Fig. 7B,C, in which the values of $\tau_{\text{rec}}$ and $\tau_{\text{fac}}$ after the adaptation are plotted against the control values, such cases are represented by points, which are located well away from the main diagonal. Some connections were only weakly depressing in the control, but displayed strong depression after an adaptation, or vice versa, as indicated by the increase or decrease of the recovery time constant, respectively. The facilitation time constant expressed most heterogeneous changes, whereby synaptic connections may be subdivided in 3 distinct groups with respect to the change of facilitation time constant (Fig. 7C). In most of the connections the $\tau_{\text{fac}}$ changes little (data points around main diagonal in Fig. 7C, filled circles), but in some it changes from about 1 ms, which corresponds to almost purely depressing synapses, to 10-20 ms, making synapses facilitating, or the other way round (data points next to the axes in Fig. 7C, open symbols). To figure out, if these groups exhibit special characteristics with regard to other parameters, we have segregated synaptic connections into three groups, one group that increases and one that decreases the facilitation time constant by more than a factor of six and one group in between (Fig. 7C, different symbols).

The separation of synaptic connections into these three subgroups neither revealed any group-specific pattern of parameter changes (Fig. 7 A-F), nor affected the significance of changes. We then related changes in each synaptic parameter after an adaptation to either changes in other parameters or to their values in the control. From all possible combinations, the only significant correlation was found between the change in release probability $U$ and the change in the recovery time constant $\tau_{\text{rec}}$ ($r = -0.38; p < 0.06; F$ statistic). Thus a decrease of the release probability after an adaptation was often accompanied by longer recovery of the resources at the presynapse.

**Synaptic changes after strong (25 Hz or 40 Hz) adaptation**

Changes of parameters of synaptic transmission after 25 Hz adaptation were assessed in 24 connections, and after 40 Hz adaptation in 29 synaptic connections. The quality of the fits of the responses after 25 Hz adaptation was similar to the quality of fitting the control data (Fig. 8D1), and the median rms error in the two data sets showed no significant difference (median: 0.11; $p > 0.25$; Wilcoxon non-paired test). Fitting of the responses recorded after 40 Hz adaptation was slightly inferior (Fig. 8D2) as indicated by a slightly higher median rms error (median: 0.13; $p < 0.02$).

Figs. 8A,B illustrate a typical example of synaptic responses in the control and their change after 25 Hz adaptation. Parameters of the optimal fits for this synaptic connection were $U^0 = 0.27$, $\tau_{\text{rec}}^0 = 839.4 \text{ ms}$ and $\tau_{\text{fac}}^0 = 18.6 \text{ ms}$ for the control condition and $U^{25} = 0.26$,
The amplitude of the \( EPSC_1 \) decreased from 0.15 pA in control to 0.09 pA after an adaptation (\( p < 3 \times 10^{-5} \)). Thus, although the first response amplitude clearly decreased after the adaptation, no accompanying reduction of the release probability was detected by the model. This situation was typical for the effects of strong adaptation with either 25 or 40 Hz stimulation (Fig. 9A,B). Despite significant decrease of the \( EPSC_1 \) amplitude to 66% and 54% of the control, (25 Hz and 40 Hz adaptation respectively, see above), no significant changes of the release probability over the population were found for either 25 Hz or 40 Hz adaptation (25 Hz adaptation: median(\( U_0 - U^{10} \)) = 0.004; mean: -0.011; std: 0.055; range -0.122 to 0.074; \( p > 0.4 \); 40 Hz adaptation: median: -0.029; mean: -0.064; std: 0.197; range: -0.764 to 0.210; \( p > 0.1 \)). Furthermore, there was no significant correlation between the \( EPSC_1 \) amplitude changes and the release probability changes after strong adaptation (Fig. 9I,J). In contrast to the results of assessment of release probability by fitting response dynamics, the coefficient of variation method indicated a decrease of the release probability after strong adaptation (Fig. 9G,H). The decrease in the median \( CV^{-2} \) was significant after both 25 Hz and 40 Hz adapting stimuli (25 Hz: \( p < 6.7 \times 10^{-4} \); 40 Hz: \( p < 3.8 \times 10^{-4} \); Wilcoxon paired test). Moreover, the changes in the \( CV^{-2} \) and the changes in the \( EPSC_1 \) amplitude after strong adaptation were significantly correlated (25 Hz: \( r = 0.58 \); \( p < 0.003 \); F-statistic, Fig. 8K; 40 Hz: \( r = 0.39 \); \( p < 0.03 \)). When comparing the results of the two methods, however, it is important to note that the coefficient of variation method relies on stronger assumptions. Strong adaptation with 25 Hz or 40 Hz led to a significant increase of the rate of recovery of synaptic resources (Fig. 9C,D). For the subpopulation of synapses, in which the recovery time constant was within the range of reliable estimation (\( \tau_{rec} < 3s \)) both in the control and after the adaptation, the mean decrease of the recovery time constant after 25 Hz adaptation was 285.3 ms (median 333.7 ms; std: 293.3 ms; range: -256 to 967 ms; \( p < 0.003 \)). After an adaptation with 40 Hz, the mean decrease of the recovery time constant was \( mean(\tau_{rec}^0 - \tau_{rec}^{10}) = 289 \) ms (median: 143.6 ms; std: 534 ms; range: -588 to 1699 ms; \( p < 0.008 \)). Those synapses, for which optimal fits were obtained with recovery time constants longer than 3 s, were excluded from the calculation of the above statistics. However, the higher frequency of occurrence of such synapses in control conditions than after an adaptation (7 in control, 2 after 25 Hz adaptation; 7 in control, 3 after 40 Hz adaptation) also points at shortening of the recovery time after a strong adaptation and thus reinforces the above conclusion. For the facilitation time constant \( \tau_{fac} \) no statistically significant changes on the population level for strong adaptation with either frequency were found (25 Hz adaptation: median: 0 s; mean: -33 ms; std: 201 ms; range: -959.9 to 109.1 ms; \( p > 0.6 \); 40 Hz adaptation: median: -14.7 ms; mean: -66.1 ms; std: 219.7 ms; range: -286.8 to 39.8 ms, \( p > 0.05 \)). Similar to the effects of weak adaptation, we observed three distinct subsets of connections with respect to change of the facilitation time constant. As after the weak adaptation, the connections with extreme changes of the facilitation did not express any specific pattern of changes of the other parameters after a strong adaptation, and their omission did not alter the significance of the parameter changes after adaptation. Analysis of the relation between changes of different parameters, reveal the only significant correlation between the change in release probability and the change in the facilitation time constant, which were positively correlated after both 25 Hz and 40 Hz adaptation (\( r = 0.48 ; p < 0.02 \) and \( r = 0.72 ; p < 3 \times 10^{-5} \); respectively, F-statistic). This positive correlation is, however, difficult to interpret since neither of these two parameters alone showed significant changes after the
strong adaptation. The change in any parameter after strong adaptation stimulus did not correlate significantly with any synaptic parameter in the control state.

To summarise, our analysis revealed the following changes in synaptic transmission after adaptation. (i) Both, weak and strong adaptation led to a significant decrease of the $EPSC_1$ amplitude, the stronger adaptation leading to a stronger decrease of the response amplitude. This decrease was short-lasting, and response amplitude recovered to the control level with a time constants 2s and 18s. (ii) After weak adaptation the decrease of the $EPSC_1$ was accompanied by and correlated with the decrease in release probability, and $CV^2$, although some synapses clearly deviated from this rule. (iii) After strong adaptation, despite an even stronger reduction of the $EPSC_1$ amplitude, no change in release probability was revealed from the fits of synaptic dynamics, however, a significant decrease of $CV^2$ was still observed. (iv) Both, weak and strong adaptation had very heterogeneous effects on facilitation and recovery time constants, and thus on the synaptic dynamics. But the only significant change on the population level was a decrease of the recovery time constant after strong adaptation.

**Model extension**

Inconsistency between the reduction of the $EPSC_1$ amplitude and the absence of a detected decrease of the release probability after strong adaptation implies, that other factors, not accounted for by the simple phenomenological model of synaptic dynamics were in play. One obvious candidate mechanism here is depletion of the resources, which are not completely recovered after an adaptation. The observed recovery of the depressed $EPSC_1$ amplitude to the control level with a time constant of several (2 to 18) seconds corresponds to the suggested time course of refilling of a ready-to-release pool of synaptic vesicles (Sudhof 2000; Zucker and Regehr 2002). In order to investigate if inclusion of that additional, slow recovery process may influence our estimations of the release parameters, we have extended the original three-parameter model. As a first step, we have included as an additional parameter the amount of resources, available at the beginning of the test train ($R_1$):

$$\frac{dR}{dt} = \frac{R_1 - R}{\tau_{rec}} - UR\delta(t - t_p)$$  \hspace{1cm} (11)

For fitting the control responses this parameter was set to 1, but for the responses after adaptation all 4 parameters ($R_1$, $U$, $\tau_{fac}$, $\tau_{rec}$) were optimized to get the best fit of the data. The best fits of this 4-parameter model for $U$, $\tau_{fac}$, $\tau_{rec}$ did not differ much from the best fits obtained with the original, 3-parameter model. Although the 4-parameter model did give better fits of the data, as indicated by the decrease of the rms error by 6.9% on the average (median: 5.8%), the optimal values for the release probability, facilitation and recovery time constants were not significantly different from the respective values obtained with the 3-parameter model. The average differences were, for estimations of the release probability $U$, 5.8% (median: 2.1%), for the recovery time constant, $\tau_{rec}$, 1.1% (median: 1.3%), and for the facilitation time constant, $\tau_{fac}$, 15.1% (median: 1.5%).

As the next step, we introduced a second recovery process, which describes slow recovery of the maximal amount of available resources after an adaptation. This maximal amount of resources, $R_{max}(t)$ recovers to the limit $R_{max}$ with a longer recovery time constant $\tau_{max}$. As initial conditions, we set $R_{max}(0) = R_1$, and $R_{max} > R_1$. This extended model is thus described by the following equations:
\[
\frac{dR}{dt} = \frac{R_{\text{max}}(t) - R}{\tau_{\text{rec}}} - UR\delta(t - t_{\text{sp}})
\]
(12)

\[
\frac{dR_{\text{max}}}{dt} = \frac{R_{\text{max}} - R_{\text{max}}(t)}{\tau_{\text{max}}}
\]
(13)

\[
\frac{dU}{dt} = \frac{U_0 - U}{\tau_{\text{fac}}} + U_0(1-U)\delta(t - t_{\text{sp}})
\]
(14)

In addition to the free parameters \(U, \tau_{\text{rec}}\) and \(\tau_{\text{fac}}\) from equations 4 and 5, three additional parameters \(R_1, R_{\text{max}}\) and \(\tau_{\text{max}}\) have to be estimated in equations 12 to 14. Since fitting all 6 parameters cannot be done unambiguously, we have fixed the \(\tau_{\text{max}} = 7\text{s}\), which corresponds to the average recovery time constant of the \(\text{EPSC}\) amplitude after the adaptation, measured experimentally. This extended 5-parameter model captured the changes of synaptic responses and their dynamics after both, weak and a strong adaptation. The results of fitting the responses after the weak adaptation (10 Hz), showed a significant decrease of the release probability, which was correlated with the decrease of the \(\text{EPSC}_1\) amplitude \((r = 0.39, p < 0.06, F\text{-statistics})\). Stronger correlations were found between the decrease of the \(\text{EPSC}_1\) amplitude on the one hand, and the decrease of the available resources \(R_1\) \((r = 0.47, p < 0.009)\) or decrease of the product of \(U\) and \(R_1\) \((r = 0.99, p < 10^{-10})\). Interestingly, after the strong adaptation, the extended 5-parameter model still did not reveal a change of the release probability, or a correlation between the release probability change and the \(\text{EPSC}_1\) amplitude decrease \((r = -0.07, p > 0.6\) for 25 Hz; \(r = -0.13, p > 0.3\) for 40 Hz adaptation). However, the decrease of the \(\text{EPSC}_1\) amplitude was significantly correlated with the decrease of estimated resources by the time of application of the first stimulus, \(R_1\) \((r = 0.70, p < 2 \cdot 10^{-4}\) for 25 Hz and \(r = 0.61, p < 6 \cdot 10^{-4}\) for 40 Hz adaptation).

Furthermore, the optimal values for \(U, \tau_{\text{fac}}, \tau_{\text{rec}}\) estimated with the extended 5-parameter model did not differ strongly from the estimations obtained with the 3-parameter model. The average differences were, for estimations of the release probability \(U\), 5.9\% \((\text{median: } 2.1\%)\), for the recovery time constant, \(\tau_{\text{rec}}\), 2.2\% \((\text{median: } 1.3\%)\), and for the facilitation time constant, \(\tau_{\text{fac}}\), 15.7\% \((\text{median: } 1.5\%)\). Moreover, inclusion of the second recovery process did not yield superior fits as compared to the 4-parameter model.

Taken together, comparison of the assessment of parameters of synaptic transmission and their changes after an adaptation with the help of the original 3-parameter model and extended, 4- and 5- parameter models allows to draw the following conclusions. First, the estimation of \(U, \tau_{\text{fac}}\) and \(\tau_{\text{rec}}\) is robust, since extensions of the model did not lead to notable changes of the estimates of these three basic parameters. Thus, the slow recovery of the \(\text{EPSC}_1\) after an adaptation did not exert significant influence on estimation of the parameters from responses to brief trains of test stimuli. Second, extended models allowed to quantify the contribution of the resource depletion to the adaptation induced changes of synaptic transmission. Finally, the extended models suggest differential contribution of the changes in release probability and resource depletion to the response changes after adaptation with different frequencies.

**DISCUSSION**

The results of our study of the effects of adaptation on synaptic transmission in the visual cortex can be summarized as follows. First, adaptation consistently led to a decrease of the amplitude of the postsynaptic response, stronger adaptation leading to a more pronounced
reduction of the response amplitude. This reduction recovered on a time scale of several seconds back to the control level. Second, two possible mechanisms of the response amplitude reduction, decrease of the release probability and decrease of the available resources, were differentially involved in the effects of adaptation with 10, 25 or 40 Hz. Third, adaptation led to heterogeneous changes of dynamics at different synapses, the only consistent and significant effects on the population level being a decrease of the release probability after a weak adaptation and an acceleration of the recovery after a strong adaptation.

Estimation of parameters of synaptic transmission in the neocortex

Before discussing the effects of adaptation on synaptic transmission and dynamics, we shall compare our assessments of the synaptic parameters to the published data. We have recorded small excitatory postsynaptic currents, evoked with the stimulation intensity set just high enough to produce responses without failures. Such weak stimuli, even if recorded in current-clamp mode, evoke postsynaptic responses which are well below the threshold of activation of voltage-gated conductances. Since we have used the same weak intensity of stimulation for both the test trains and the adaptation, we consider as highly unlikely the possibility of contribution of voltage clamp errors to our results. We have used a modification of a phenomenological model of synaptic transmission (Tsodyks and Markram 1997; Varela et al. 1997) for fitting the synaptic responses, evoked by stimulation with a set of test frequencies. At synaptic connections between layer 5 pyramidal cells in somatosensory cortex, earlier studies reported values for the mean recovery time constants of about 810 ms (Markram et al. 1998; Tsodyks and Markram 1997) and 760 ms (Fuhrmann et al. 2004). Applying a modified phenomenological model for the analysis of synaptic connections in rat barrel cortex, Finnerty et al reported mean recovery time constants of 480 to 1190 ms, depending on the group of recorded cells, the developmental history of an animal and experimental conditions (Finnerty and Connors 2000; Finnerty et al. 1999). The above results were obtained on synaptic connections involving single axons or few presynaptic fibers. In a complementary approach, which exploited both intracellularly recorded postsynaptic responses and field potentials, the use of dynamic models for larger populations of synapses has been validated (Varela et al. 1997). Further, the authors demonstrated the usefulness of models of dynamic synapses in prediction of cellular responses to more complex patterns of prolonged stimulation. Varela et al have found, that recovery from the depression could be best described by a bi-exponential process with time constants of several hundreds of ms and several, about 7 to 9, seconds (Varela et al. 1997). Sparse published data on time constants of facilitation at neocortical synapses show that at synapses between excitatory cells they are usually about a hundred of ms (Markram et al. 1998; Varela et al. 1997) but at synapses, which are formed by pyramidal cells onto interneurons, facilitation of release may last up to several hundreds of ms, making these synaptic contacts highly susceptible for temporal summation (Markram et al. 1998). In the visual cortex synapses in control, we have found recovery time constants in the range of hundreds of ms, with most recovery time constants below 1.5s, and facilitation time constants in the range from several ms to about 300 ms, with the mean of 33 ms. Application of an adapting stimulation revealed an additional, slower recovery process with the time constant of several seconds (mean 7.1 s). These estimations are in good agreement with the above data. Release probability at neocortical synapses is highly heterogeneous, the values reported so far covering almost the whole possible range. At synapses between layer 5 pyramidal cells in rat somatosensory cortex, possible values of release probability were between 0.025 and 0.9 (Markram et al. 1997). In the barrel cortex, at synaptic connections between layer 4 cells the release probability was found to be between 0.125 and 0.9 (Feldmeyer et al. 1999). One study reported an exceptionally high release probability, averaged 0.8, at synapses formed by layer 4
stellate cells onto layers 2-3 pyramidal neurons in the barrel cortex (Silver et al. 2003). Our recent study of release probability at synaptic connections to layer 2-3 pyramidal cells in rat visual cortex with the use of MK-801, an open-channel blocker of the NMDA-receptor gated channels, revealed a skewed distribution of release probabilities, with predominance of values below 0.2, and an average of 0.17 (Volgushev et al. 2004). In the present study, we found similar values of release probability in control, with the average of 0.21 and median of 0.17. Given the high degree of heterogeneity of synaptic connections in the neocortex, where even synapses formed by the same axon onto different postsynaptic cells may express differential dynamic properties (Markram et al. 1998; Reyes et al. 1998; Thomson and Deuchars 1994), the above comparisons show, that our assessments of parameters of synaptic transmission in control, without adapting stimulation, are in good agreement with the data published so far. Reliability of our estimations of synaptic parameters is further substantiated by the low errors of the fits, which were of comparable range for fits of the data obtained under different conditions of stimulation with and without adaptation, and by the fact that an extension of the model by additional parameters did not lead to a notable change of the optimal release probability, facilitation and depression time constants. Taken together, these results allow us to conclude, that our method of estimation of these three basic characteristics of synaptic transmission is reliable and can be exploited for assessment of changes of synaptic transmission after an adaptation.

Changes of synaptic transmission after adaptation

In the whole organism, adaptation is expressed as a reduction of the response amplitude. Recent in vivo study directly related adaptation of the responses to repetitive sensory stimulation, to the changes of synaptic responses, evoked with electric stimulation (Chung et al. 2002). The authors demonstrated that in rat barrel cortex, adaptation to repetitive whisker stimulation is indeed accompanied by the reduction of the amplitude of the postsynaptic potentials, evoked by electric stimulation of the thalamus. Thus, the reduction of the response amplitude to repetitive activation of the synapses, either in vivo by sensory stimulation, or in vitro by applying electric shocks, does serve as a mechanism of adaptation. Our results show, that this mechanism might also be involved in adaptation in the visual system, specifically at synapses in the visual cortex, where we have observed reduction of the amplitude of postsynaptic responses after an adapting stimulation. Possible changes of two parameters of the presynaptic release machinery may underlie the reduction of response amplitude after an adaptation: a decrease of the release probability and a decrease of the available synaptic vesicles, or resources. Evidence in support of the reduced release probability as one of the reasons for the response amplitude decrease includes the results of our analysis of synaptic dynamics after 10 Hz adaptation, and estimations of the changes of the release probability with the coefficient of variation method. Supportive evidence comes also from the recent study in somatosensory cortex, in which authors report the decrease of the EPSC amplitude after a train of 20 pulses (Fuhrmann et al. 2004). The authors found that 600 ms after the adapting train, the response amplitude decreased to 43-84% of the control, depending on the adapting frequency and temperature. The decrease of the response amplitude was accompanied by the decrease of the release probability, as indicated by the decrease of the inverse coefficient of variation and an increase of the skew of the distribution of response amplitudes (Fuhrmann et al. 2004). The similarity between the results obtained in the visual cortex and in the barrel cortex is further stressed by the similar magnitudes of the response reduction, and by the similar dependence of the amplitude reduction on the adaptation strength. In both, our data and results of Fuhrmann et al. the depression of responses was stronger after an adaptation with higher frequency. However, we observed a decrease of the
release probability in association with the reduction of the response amplitude only after 10 Hz adaptation, but not after 25 or 40 Hz, while the other study reported a decrease of the release probability after both, 10 Hz and 20 Hz adaptation. This apparent inconsistency might well be explained by the different adaptation protocols. We have adapted synapses with 4s trains at 10, 25 or 40 Hz, while Fuhrmann et al used trains of 20 pulses at 10 or 20 Hz. One possible effect of our adaptation with 25 or 40 Hz for 4s may be a kind of augmentation, which is typical for synapses in different parts of the brain including the neocortex (e.g. (Castro-Alamanacos and Connors 1997; Fuhrmann et al. 2004)); see (Thomson and Deuchars 1994; Zucker and Regehr 2002)) for review). The short-term increase of the release probability, associated with the augmentation, could have counteracted an adaptation-evoked suppression of the release.

One further presynaptic mechanism, which could be responsible for the decrease of the amplitude of postsynaptic responses after an adaptation, is the depletion of a ready releasable pool of synaptic vesicles. During synaptic transmission, the vesicles are released from the immediately releasable pool, which is refilled from the pool of readily releasable vesicles (see (Sudhof 2000; Zucker and Regehr 2002) for review). At low rates of presynaptic activity, the size of the larger readily releasable pool does not change substantially, and the recovery is limited by the speed of the vesicle transfer from the readily releasable pool to the immediately releasable pool. This process occurs with a time constant of several hundreds of milliseconds. At high rates of presynaptic activity, the readily releasable pool also becomes depleted and recovery is now limited by the slow process of replenishment of the readily releasable pool, which occurs with a time constant of several seconds. These two recovery processes are expressed as depression of synaptic transmission with two different, rapid and slow, time courses. Previous studies revealed a slow form of depression, which recovers with a time constant of seconds to tens of seconds also at neocortical synapses (Fuhrmann et al. 2004; Varela et al. 1997). Possible mechanism underlying this form of depression could be the slow replenishment of the readily releasable pool of synaptic vesicles, as suggested by the similarity of the time course of the slowly recovered depression at neocortical synapses, and the replenishment of the readily releasable pool at synapses in other structures (Sudhof 2000; Zucker and Regehr 2002). Our analysis of results with extended models, which took into account this slow recovery process, showed that the relative contribution of the depletion of vesicle pools to the decrease of the response amplitude increases with the adaptation strength. After a weak, 10 Hz adaptation, the reduction of the response amplitude could be accounted for by the reduced release probability, with little contribution of the vesicle depletion. In contrast to that, strong adaptation with 25 or 40 Hz, led to a significant depletion of the synaptic vesicles, which became the main factor of the response amplitude reduction. Moreover, this analysis demonstrates, that the effects of adaptation on synaptic transmission could not be faithfully described by a simple, three-parameter model. Only more complex models, in which interaction between different pools of synaptic vesicles is taken into account, are capable to capture the main features of the response changes after an adaptation. Notably, the time course of the vesicle exchange between different pools is itself a dynamic variable, since it can be accelerated by the high frequency presynaptic firing. This had been demonstrated first for the calyx of Held synapses (Wang and Kaczmarek 1998), and recent study provides evidence for activity dependent acceleration of the vesicle recovery at the synapses in somatosensory cortex (Fuhrmann et al. 2004). Our results on the consistent decrease of the recovery time constant after strong adaptation suggest that a similar acceleration of the vesicle trafficking between different pools might occur also at synapses in the visual cortex. However, further specific experiments are required to clarify the precise time course of these processes at neocortical synapses.
Our study revealed highly heterogeneous changes of dynamic properties of different synaptic connections after an adaptation. Although adaptation led to changes of the transmission in most of the synapses, the effects vary considerably from one synaptic connection to the other. On the population level, only the decrease of the release probability after a weak adaptation and acceleration of the recovery after a strong adaptation reached significance level. In other cases, synaptic parameters could change even in the opposite directions at different synapses, which resulted in the absence of significant changes in the averaged data. For example, adaptation led to an almost complete disappearance of facilitation at some synapses, but at other synapses, which did not show facilitation in control, it may become apparent after an adaptation. Therefore, apart from the decrease of the amplitude of postsynaptic responses, adaptation may produce also cell-specific or synapse-specific effects, which may be averaged out on the population level, but could nevertheless result in a kind of re-distribution of activity within neural networks. A possible role, which these subtle tunings of network activity may play in sensory adaptation and, more generally in sensory processing, remains to be clarified.

ACKNOWLEDGEMENTS

We are grateful to Pavel Balaban for participation in some of the experiments, Christa Tacke for technical assistance and Ulf Eysel for the support of this project. Supported by the Deutsche Forschungsgemeinschaft (DFG), SFB 509 TP A5 (to M.V), SFB 618, and by the BMBF, 10025304 Projekt B3 (O.B and K.O).

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Fig. 1. Recording situation and experimental protocol. A: positioning of the stimulation (S1 and S2) and recording electrodes in a slice of the rat visual cortex. B: The cartoon illustrates the protocol of stimulation at one of the sites. Stimulation was applied once every 80 sec, and consisted of a train of 10 test stimuli at frequencies of 5, 10, 20 or 40 Hz, either preceded by adapting stimulation (expanded on top right), or without adaptation (expanded on top left). Adapting stimulation consisted of a 4 s train of the stimuli of the same strength, applied at 10, 25 or 40 Hz. After the adaptation, a 2 s interval was set before application of the test stimuli. Stimulation at different frequencies, with or without adaptation was intermingled, as indicated. Test stimulation was applied in alternation at the two different stimulation sites.

Fig. 2. Synaptic responses evoked by stimulation at different frequencies and results of a fit to the model of synaptic transmission described by eqs. (4,5).
A1: Amplitudes of the EPSCs (ordinate) and after a 10 Hz adaptation (ordinate). Each trace is an average of 5 individual responses.
A2: Amplitudes of the EPSCs from A1, normalized to the amplitude of the response to the first pulse in each train, and plotted against the sequential number of the stimulus in a train (filled circles). Solid lines show the optimal fit of responses evoked by all 4 test frequencies. Optimal parameters for this synaptic connection were: release probability, $U = 0.57$, facilitation time constant 291.5 ms, recovery time constant 356.2 ms.
B, B1: distribution of the root-mean-square (rms) error of the fits of control responses (without adaptation), pooled over all 56 synaptic connections; B2: rms error of all fits pooled over all 56 synaptic connections and all available adaptation frequencies (n=138).

Fig. 3. Distributions of the parameters of best fits of the responses in the control condition, without adaptation. Data for n=56 synaptic connections. A: release probability $U$. B: recovery time constant $\tau_{rec}$. C: facilitation time constant $\tau_{fac}$.

Fig. 4. Synaptic responses and their dynamics in the control condition (A) and after a 10 Hz adaptation (B).
A1, B2: from top to the bottom: EPSCs evoked in a layer 2/3 pyramidal cell in rat visual cortex by test stimuli applied at 10, 20 and 40 Hz. Each trace is an average of 5 individual responses. Insets show superposition of responses to each of the stimuli in the train, grey bars are windows for amplitude measurement.
A2, B1: Amplitudes of the EPSCs from A1 and B2, normalized to the amplitude of the response to the first pulse in each train, and plotted against the stimulus number in a train (filled circles). Solid lines show the optimal fits using eqs. (4,5). Optimal parameters of the fits were: without adaptation (A): release probability, $U = 0.17$, facilitation time constant 1 ms, recovery time constant 481.7 ms. After the adaptation (B): release probability, $U = 0.09$, facilitation time constant 11.7 ms, recovery time constant 578.9 ms.
C: Scatter plot showing the relation between the amplitude of responses to the first pulse in a train in control (abscissa) and after a 10 Hz adaptation (ordinate). Each point represents data for one synaptic connection (n=29).
D: Relation between the root mean square (rms) errors of the fits of the responses in the control condition (abscissa) and after a 10 Hz adaptation (ordinate).

Fig. 5. Changes of the amplitude of the response to the first test stimulus in a train after adaptation to different frequencies. A: The EPSC$_{1}$ amplitude after an adaptation (ordinate) in percent of the response amplitude in the control conditions, plotted against the frequency of the adapting stimulation (abscissa). Solid and dotted lines are regression line and 95% confidence intervals. Each point represents data for one synaptic connection and one adaptation. N=29 for 10 Hz adaptation, n=24 for 25 Hz adaptation, n=29 for 40 Hz adaptation.
B: scatter plot of the EPSC$_{1}$ amplitude changes after adaptation with 25 Hz (open squares, n=3) or 40 Hz (solid dots, n=23), plotted against the EPSC$_{1}$ amplitude change after 10 Hz adaptation at the same synapses (abscissa).

Fig. 6. Recovery of single EPSCs after an adaptation stimulus.
A1: EPSCs evoked in a layer 2/3 pyramidal cell in rat visual cortex by a stimulus applied at 0.2 Hz starting 2s after an adaptation stimulus of 10 Hz (top) or 40 Hz (bottom). Each trace is an average of 10 individual responses. Small positive deflection at the beginning of each response is stimulus artefact. Grey bars are windows for amplitude measurement.
A2: Amplitudes of the EPSCs from A1, normalized to the amplitude of the response in the control in percent and plotted against the time after the adaptation. The arrow indicates the time of the end of the adapting train.
**B:** The EPSC$_1$ amplitude after an adaptation (ordinate) with 25 Hz (open squares) and 40 Hz (filled circles) in percent of the response amplitude in the control conditions, plotted against the time constant of an exponential fit to the recovery of EPSC amplitude (abscissa).

**Fig. 7.** Change of synaptic parameters after a weak (10 Hz) adaptation.

**A-C:** release probability $U$ (**A**), recovery time constant $\tau_{\text{rec}}$ (**B**) and facilitation time constant $\tau_{\text{fac}}$ (**C**), estimated by fitting responses to stimulation with different frequencies (using eqs. 4,5), in the control condition (abscissa) and after 10 Hz adaptation (ordinate). Note the double logarithmic scale in **C**. Data for synapses, at which the facilitation time constant after adaptation changed by less than a factor of 6 are shown as filled circles, for synapses at which facilitation time constant decreased by more than a factor 6 are shown as open diamonds, and for synapses at which facilitation time constant increased by more than a factor 6 are shown with open squares.

**D:** squared inversed coefficient of variation ($CV^{-2}$) of the EPSC$_1$ amplitude after the 10 Hz adaptation (ordinate) plotted against the $CV^2$ of the EPSC$_1$ in the control (abscissa) **E:** change of the amplitude of the EPSC$_1$ (ordinate) plotted against the change of the of the $CV^2$ of the EPSC$_1$, in percent of control values. **F:** change of the amplitude of the EPSC$_1$ (ordinate) plotted against change in the release probability $U$ (abscissa) in percent of control values. In **A-F**, data for synapses, at which the facilitation time constant after adaptation changed by less than a factor of 6 are shown as filled circles, for synapses at which facilitation time constant decreased by more than a factor 6 are shown as open diamonds, and for synapses at which facilitation time constant increased by more than a factor 6 are shown with open squares.

**Fig. 8.** Synaptic responses and their dynamics in control (**A**) and after a 25 Hz adaptation (**B**).

**A1, B2:** from top to the bottom: EPSCs evoked in a layer 2/3 pyramidal cell in rat visual cortex by the test stimuli applied at 5, 10 and 20 Hz. Each trace is an average of 5 individual responses. Insets show superposition of responses to each of the stimuli in the train, grey bars are windows for amplitude measurement.

**A2, B1:** Amplitudes of the EPSCs from **A1** and **B2**, normalized to the amplitude of the response to the first pulse in each train, and plotted against the stimulus number in a train (filled circles). Solid lines show the optimal fits according to eqs. (4,5). Optimal parameters of the fits were, without adaptation (**A**): release probability, $U = 0.27$, facilitation time constant 19.1 ms, recovery time constant 814.2 ms. After the adaptation (**B**): release probability, $U = 0.26$, facilitation time constant 1 ms, recovery time constant 541.9 ms.

**C1, C2:** EPSC$_1$ amplitude after a 25 Hz adaptation (**C1**, $n=23$, ordinate) and after a 40 Hz adaptation (**C2**, $n=29$, ordinate), plotted against control values (abscissa).

**D1, D2:** The root mean square (rms) errors of the fits of the responses after a 25 Hz adaptation (**D1**, $n=23$) and after a 40 Hz adaptation (**D2**, $n=29$), plotted against the rms errors of the fits for the control responses.

**Fig. 9.** Change of synaptic parameters after a strong adaptation (25 Hz or 40 Hz).

On top of each graph the adaptation frequency is shown in a box. Number of synapses recorded were $n=23$ for 25 Hz adaptation, $n=29$ for 40 Hz adaptation. In **A-H**, values after adaptation (ordinate) are plotted against control values (abscissa). In **A-L** data for synapses, at which facilitation time constant after an adaptation changed by less than a factor of 5 are shown as filled circles, for those with a more than 5x decrease of the facilitation time constant as open diamonds, and for synapses with a more than 5x increase of the facilitation time constant as open squares. In **I-L**, changes are given in percent of control.

**A, B:** release probability.

**C, D:** recovery time constant.

**E, F:** facilitation time constant.

**G, H:** squared inversed coefficient of variation ($CV^{-2}$) of the EPSC$_1$ amplitude.

**I-L:** change of the amplitude of EPSC$_1$ after an adaptation, plotted against changes of release probability ($LJ$) and $CV^2$ of the EPSC$_1$ amplitude ($KL$). The out of scale values in $J$ were: (205.1, 59.8), (384.8, 9.3), (206.2, 79.1), (222.2, 56.6), (817.9, 33.2), (412.3, 48.35).
Figure 1
Figure 2
Figure 3
Figure 4
Figure 5
Figure 6
Figure 7
Figure 8
Figure 9