Modeling Short-Term Synaptic Plasticity at the Calyx of Held Using In Vivo-Like Stimulation Patterns

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Hermann J, Grothe B, Klug A. Modeling short-term synaptic plasticity at the calyx of Held using in vivo-like stimulation patterns. *J Neurophysiol* 101: 20–30, 2009. First published October 29, 2008; doi:10.1152/jn.90243.2008. We measured synaptic responses to complex stimulus trains in the calyx of Held and used the data to test how well several vesicle-release models could capture the observed dynamics. We tested stimulation protocols consisting of Poisson-distributed activity with periodically changing mean frequencies, trains with constant inter spike intervals, and stimulus trains derived from in vivo responses to natural sounds. All stimuli were embedded in chronic background activity attempting to imitate the naturally occurring spontaneous activity in the auditory brain stem. We found that already the most basic model variant produced very good results, exhibiting very high correlation coefficients between the experimental data and the model predictions. None of the more complex model variants, which incorporated receptor desensitization, synaptic facilitation, and double-exponential recovery from depression, showed improved data-prediction matching accuracy. These findings are in contrast to previous modeling work performed in nonchronically active synapses, where the inclusion of additional physiological parameters into the modeling process tended to result in models with higher accuracy. Our findings suggest that the functional state of chronically active calyces may differ from the functional state of silent calyces and that this functional state of chronically active synapses can be described in relatively simple terms.

**INTRODUCTION**

The short-term dynamics of synaptic transmission under various activity levels have been modeled in a number of synapses (Abbott et al. 1997; Gundlach et al. 2007; Markram and Tsodyks 1996; Tsodyks et al. 1998), among them the calyx of Held in the auditory brain stem. Basic features of most of these models are the vesicle release from a readily releasable vesicle pool as well as a pool size-dependent vesicle recovery (Weis et al. 1999). Beyond these basic features, additional effects have been incorporated, for example, calcium-dependent facilitation (Varela et al. 1997) or postsynaptic receptor desensitization (Graham et al. 2004). These physiological effects of short-term plasticity have been demonstrated to shape synaptic transmission at the calyx of Held, thus the inclusion of them into the modeling process are supposed to yield more accurate models with higher predictive power, as was observed by those investigators.

However, the modeling studies mentioned in the preceding text were based on data recorded from in vitro brain slice preparations. In the case of the calyx of Held, these preparations lack the chronic background spontaneous activity that calyces experience in the intact brain (Irvine 1992; Kadner et al. 2006; Kopp-Scheinpflug et al. 2003; Smith et al. 1998; Sommer et al. 1993). This spontaneous activity is a common feature in the nervous system, and one of the hallmarks of neurons in the auditory brain stem. On average, it evokes firing rates of ~25 Hz at the calyx of Held in gerbils. Indeed, calyces in brain slice preparations lacking this chronic activity differ in a number of physiological properties of synaptic transmission, such as baseline synaptic amplitudes, depression during high-frequency stimulation, recovery from depression, or transmission fidelity (Hermann et al. 2007).

The focus of the present study was to find the model variant that would capture transmission at the calyx of Held during typical in vivo activity levels. Rather than including all known parameters of short-term plasticity into the model, our approach was to evaluate different models incorporating different physiological effects and to test how well each one of these variants could predict neural responses to the complex stimulation patterns used in the study. To imitate naturally occurring activity as closely as possible, all presented stimuli were embedded in chronic background activity resembling the spontaneous activity present in the intact brain. The embedded test stimuli incorporated a large amount of statistical complexity and, in some cases, were derived from auditory responses to sound clips. Different variations of the basic model were used to predict the synaptic currents produced by a given calyx of Held to these stimuli, and these predictions were compared with the neuron’s actual responses as determined through voltage-clamp recordings from gerbil brain slice preparations.

**METHODS**

**Slice preparation**

Coronal slices of brain stem were prepared from Mongolian gerbils (*Meriones unguiculatus*) aged 15–19 days old. Animals were briefly anesthetized by isoflurane inhalation (Isoflurane Curamed, Curamed Pharma) and decapitated. The brain stem was dissected out under ice-cold dissection Ringer [containing (in mM) 125 NaCl, 2.5 KCl, 1 MgCl2, 0.1 CaCl2, 25 glucose, 1.25 NaH2PO4, 25 NaHCO3, 0.4 ascorbic acid, 3 myo-inositol, and 2 pyruvic acid; all chemicals from Sigma]. Sections of 180–200 μm were cut with a vibratome (VT100S, Leica). Slices were transferred to an incubation chamber containing extracellular solution [ECS; containing (in mM) 125 NaCl, 2.5 KCl, 1 MgCl2, 2 CaCl2, 25 glucose, 1.25 NaH2PO4, 25 NaHCO3, 0.4 ascorbic acid, 3 myo-inositol, and 2 pyruvic acid; all chemicals from Sigma] and bubbled with 5% CO2-95% O2. Slices were incubated for 30 min at 37°C, after which the chamber was brought to room temperature. Recordings were obtained within 4–5 h of slicing.

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Whole cell recordings

All recordings were performed at 36–37°C. After incubation, slices were transferred to a recording chamber and continuously superfused with ECS at 3–4 ml/min through a gravity-fed perfusion system. Medial Nucleus of the trapezoid body (MNTB) neurons were viewed through a Zeiss Axioskop 2 FS microscope equipped with DIC optics and a ×40 water-immersion objective (Zeiss). Whole cell voltage-clamp recordings were made with an EPC 10 double amplifier (HEKA Instruments), the holding potential was −60 mV. Signals were filtered at 10 kHz and subsequently digitized at 50 kHz using Patchmaster Version 2.02 software (HEKA Instruments). Uncompensated series resistance was 9.7 ± 1.1 MΩ (mean ± SE) and was compensated to 1.8 ± 0.3 MΩ with a lag time of 10 μs. Potential changes in series resistance were monitored throughout the recordings and data collection was discontinued whenever uncompensated series resistance changed by >2 MΩ.

Patch pipettes were pulled from 1.5 mm borosilicate glass (Harvard Instruments) using a DMZ Universal Puller (Zeitz Instruments). Pipettes were filled with cesium-methanesulfonate-based solution [containing (in mM) 125 CsMeSO₃, 4.5 MgCl₂, 9 HEPES, 5 EGTA, P, 0.3 tris-GTP, and 1.5 CaCl₂, all chemicals from Sigma].

Strychnine hydrochloride (500 nM; Sigma) was added to the bath to block glycinergic inhibition, and 5 mM QX-314 (Alomone Labs) was added to the pipette fill to eliminate sodium currents.

Stimulation of synaptic inputs

Synaptic currents were elicited by midline stimulation of the calyceal input fiber bundle with a 5 MΩ bipolar stimulation electrode (matrix electrodes with 270 μm distance, Frederic Haer). Stimuli were 100-μs-long square pulses with a constant voltage 10 V above the stimulation threshold (10–40 V) delivered with a STG 2004 computer-controlled four-channel stimulator (Multichannel Systems) and a stimulation isolation unit (Iso-Flex, AMPI). Due to the long stimulation durations (≤10 min), some cells showed stimulation failures, i.e., they did not show an excitatory postsynaptic current (EPSC) and the time constant, τᵣ, to restrict the release probability to values smaller than 1. In between events it decayed with the characteristic time constant τᵣ back to its minimum value Pᵣᵢₘᵢₙ. For the second extension, synaptic facilitation was included (Varela et al. 1997). The release probability was raised for every synaptic event by the factor F·(1 − Pᵣᵢₘᵢₙ) to restrict the release probability to values smaller than 1. In between events it decayed with the characteristic time constant τᵣᵢ₈ to its minimum value Pᵣᵢₘᵢₙ. In the third variant an additional factor R(t) representing receptor desensitization was included (Graham et al. 2004).

Data analysis and vesicle release model

All data analyses were done with Igor Pro 5.05A (WaveMetrics) and Matlab 6.5 (The Mathworks). Unless stated otherwise, values are always provided with the standard error. To assess the short-term dynamics of synaptic currents, a vesicle release model was implemented and fitted to the experimental data. The model was first described independently by Tsodyks and Markram (1997) and Abbott et al. (1997) and has since been repeatedly used by various investigators. The model can be divided into two parts, the reduction of the pool of available neurotransmitter at the time point of a synaptic event and the recovery of the pool in between release events. The dynamically changing size of the pool is characterized by I_l(t) which is a measure of the synaptic current produced by all available neurotransmitter vesicles in the pool. The reduction is regarded as an instantaneous event described by

\[ I_{\text{after}} = I_{\text{before}} \cdot (1 - P_R) \]  \hspace{0.5cm} (1)

with a constant release probability Pᵣᵢ₈. The “after” and “before” subscripts refer to the immediate times before and after the arrival of the action potential. The recovery process follows the differential equation:

\[ \frac{dI}{dt} = \frac{I_0 - I}{\tau} \]  \hspace{0.5cm} (2)

where \( I_i \) is the synaptic current created by the amount of neurotransmitter vesicles in case of a fully recovered functional vesicle pool, and \( \tau \) is the recovery time constant. Solving the differential equation and combining it with Eq. 1 leads to an iterative form that can be used to calculate the amount of vesicles available at the synaptic event \( n \) based on values calculated for event \( n - 1 \) as follows

\[ I_n = I_0 - [I_0 - I_{n-1} \cdot (1 - P_R)] \cdot \exp\left(-\frac{(t_n - t_{n-1})}{\tau}\right) \]  \hspace{0.5cm} (3)

The synaptic current \( i_n \) is produced by a fraction of the available vesicles: \( i_n = P_R \cdot I_n \). This value was compared with the experimentally measured EPSC amplitudes. The model has three free parameters, namely \( P_R \), \( \tau \), and \( i_0 \). To calculate the optimal parameter set, the squared error

\[ \frac{1}{N} \sum_{n=1}^{N} \left( i_n \text{ (experiment)} - i_n \text{ (model)} \right)^2 \]  \hspace{0.5cm} (4)

was minimized using the downhill simplex method (Nelder and Mead 1965).

Model variants

To account for additional influences on short-term dynamics of synaptic currents, three variants of the vesicle release model were implemented. In the first variant, the single-exponential recovery was replaced by a double-exponential time course (Wang and Kaczmarek 1998)

\[ \frac{dI}{dt} = \frac{I_{\text{fast}} - I}{\tau_{\text{fast}}} + \frac{I_{\text{slow}} - I}{\tau_{\text{slow}}} \]  \hspace{0.5cm} (5)

For the second extension, synaptic facilitation was included (Varela et al. 1997). The release probability was raised for every synaptic event by the factor \( F \cdot (1 - P_R) \) to restrict the release probability to values smaller than 1. In between events it decayed with the characteristic time constant \( \tau_{\text{fac}} \) back to its minimum value \( P_{\text{min}} \)

\[ \frac{dP_R}{dt} = \frac{P_{\text{min}} - P_R}{\tau_{\text{fac}}} \]  \hspace{0.5cm} (6)

In the third variant an additional factor \( R(t) \) representing receptor desensitization was included (Graham et al. 2004).

\[ i_n = P_R \cdot I_n \cdot R(t) \]  \hspace{0.5cm} (7)

\( R(t) \) was raised for every synaptic event by the factor \( D \cdot I_0 \), where \( D \) defines the percentage of receptors that desensitize for each synaptic event. Between events, \( R(t) \) recovers with a characteristic time constant \( \tau_{\text{re}} \)

\[ \frac{dR}{dt} = -\frac{1 - R(t)}{\tau_{\text{re}}} \]  \hspace{0.5cm} (8)

Because the model variants affect distinct parts of the basic model, the extensions can also be combined. For all three variants and their combinations, the additional free parameters were included into the model optimization and the squared error was minimized similar to the simple model.

Model predictions

For stimulation patterns with regular inter pulse intervals, the steady-state amplitude \( I^* \) can be calculated as a function of the stimulation frequency, \( f \), and the time constant, \( \tau_A \), which describes the exponential time course from any initial amplitude to the steady
state. To calculate these two values, the iterative formula for \( I_n \) can be rewritten as

\[
I_n = a + b \cdot I_{n-1}
\]

with \( a \) and \( b \) representing the more complex terms from the original equation describing the basic model

\[
a = I_0 - I_o \cdot \exp \left( \frac{-\left( t_n - t_{n-1} \right)}{\tau} \right)
\]

\[
b = (1 - P_R) \cdot \exp \left( \frac{-\left( t_n - t_{n-1} \right)}{\tau} \right)
\]

The steady-state amplitude can be calculated by equating the currents at event \( n \) and event \( n - 1 \)

\[
I^* = \frac{a}{1 - b} = I_0 - \frac{I_o \cdot P_R}{\exp[1/(f-\tau)] - 1 + P_R}
\]

The interpulse interval \( t_n - t_{n-1} \) has been replaced with the inverse of the stimulation frequency \( 1/f \). Multiplying \( I^* \) with the release probability \( P_R \) gives an estimate for the steady-state EPSC which can be compared with experimental values.

To calculate the time constant \( \tau_A \), the deviation from the steady-state \( J_n \) is defined as follows

\[
J_n = I_n - I^*
\]

Now the resulting iterative equation \( J_n = b \cdot J_{n-1} \) can be solved in a noniterative way

\[
J_n = b^n \cdot J_0
\]

By replacing \( n \) with \( t/\Delta t \) and \( J \) with \( I \), one can obtain an explicit solution of this equation

\[
I(t) = \exp \left( \frac{\ln b}{\Delta t} \right) \cdot I_0
\]

The characteristic time constant can now be extracted from the exponent and results in

\[
\tau_A = -\frac{\Delta t}{\ln b} = \frac{\Delta t}{\ln(1 - P_R) - \Delta t/\tau} = \frac{1}{\Delta t - f^* \ln(1 - P_R)}
\]

RESULTS

The central goal of this study was to model the short-term synaptic dynamics at the calyx of Held in response to highly complex stimulation patterns. Furthermore, we wanted to investigate the synaptic dynamics under conditions mimicking the in vivo situation of chronically active synapses as closely as possible. We therefore embedded our various stimulation protocols in spontaneous background activity mimicking the naturally occurring spontaneous firing of cells in the auditory brain stem. As we have shown previously (Hermann et al. 2007), this activity causes several changes in the properties of synaptic transmission, mainly a significant decrease in synaptic strength and a decrease of synaptic depression in response to high-frequency stimulation. Here we used a Poisson distributed pulse train with a mean frequency of 20 Hz to simulate spontaneous background activity. Neurons were initially stimulated with this background activity for \( \geq 2 \) min to “condition” the synapses. During this conditioning period, the synapses reached a new steady state with significantly lower synaptic currents (Hermann et al. 2007). We term this condition the “in-vivo-like rested state” of the synapse because we assume that in vivo calyces of Held are in this state in the absence of any sound stimulation. All other stimulations used in the present study were embedded in this background activity, and the change between stimulus and background was gapless, guaranteeing a true embedding of the recorded stimulus. This experimental design attempts to imitate the in vivo situation, in which periods of sound stimulation are embedded in the naturally occurring spontaneous activity. The mean initial synaptic strength of all cells measured as the EPSC in voltage-clamp experiments was 12.2 \( \pm \) 1.2 nA (\( n = 16 \)). During the 2 min of conditioning with the 20-Hz Poisson train, this value dropped to 4.1 \( \pm \) 0.6 nA, corresponding to a decrease to 34% of the initial amplitude.

Poisson-distributed test trains

The first set of test stimuli consisted of Poisson distributed trains with varying mean frequencies. The stimulus consisted of 34 segments and had alternating periods of low and high activity levels as depicted in Fig. 1A. Each one of these 34 periods had a given mean frequency, which was applied for a brief time period and then changed at the beginning of the next segment of the stimulus train. The distribution of single events within these periods was Poisson with the exception that interpulse intervals \(< 1 \) ms were not used. The low activity periods had mean frequencies ranging from 5 to 50 Hz, whereas the high activity periods had mean frequencies between 100 and 350 Hz. The entire stimulus was 30 s long and consisted of 1,201 stimulations.

We had two motivations to choose this particular type of stimulation. First, this stimulation protocol is highly complex and is made of many very different sequences of inter pulse intervals. Therefore it is possible to obtain a lot of information about synaptic dynamics with this stimulus. Second, it covers the full range of frequencies experienced by MNTB cells in an intact brain. A normal Poisson train with a fixed mean frequency could, for example, not account for longer periods of low activity reflecting silence and at the same time mimic sound input that would result in high activity. Therefore a pattern with varying mean frequencies reflects naturally occurring auditory activity closer.

For each neuron, experiments were started by conditioning the synapse to background activity for \( \geq 2 \) min with the 20-Hz Poisson stimulus described in the preceding text. Next, the Poisson test train with varying mean frequencies described here was played to calyces, embedded into the 20-Hz background activity and repeated four times wherever possible. An example of a single voltage-clamp trace of recorded EPSCs is shown in Fig. 1B. EPSC amplitudes were extracted and the mean amplitudes were calculated from the four repetitions. The grey area in Fig. 1C indicates the maximum and minimum EPSC measured over the four repetitions.

Next, the EPSC amplitudes were modeled using the basic variant of our prediction model. The black line in Fig. 1C corresponds to the model prediction. The EPSC amplitudes predicted by the model were fit against the mean amplitudes calculated from the four repetitions. For most events, the prediction falls inside the grey area depicted in Fig. 1C,
indicating that the model prediction was in the range of the naturally occurring amplitude variations. To quantify the quality of our predictions, we calculated the correlation coefficient between the predicted and corresponding measured amplitudes. Figure 1D shows the correlation coefficients of all 16 neurons that were tested in this way. The majority of cells show a correlation coefficient of >0.9, indicating a very high fit quality. The mean correlation coefficient over the 16 cells was 0.918 ± 0.017, the median was 0.94.

For two example cells (indicated by “i” and “ii” in Fig. 1D), the quality of fits is further illustrated with a set of correlation plots, shown in Fig. 1E. The two plots on the left show a comparison between two consecutive recordings of the same stimulation pattern for each one of the two neurons. For a perfect match, i.e., perfect repeatability, all points would fall on the black diagonal line. The deviations from this line demonstrate the degree of variability between individual trials. For each neuron in our sample, we compared the EPSC amplitudes from the four individual stimulus repetitions to each other (6 comparisons per neuron) and calculated the average correlation coefficient for each neuron (0.603 ± 0.012 to 0.961 ± 0.002, n = 16). The average correlation coefficient for all 16 neurons was 0.880 ± 0.019.

The two middle plots in Fig. 1E show the comparison between single EPSC traces and corresponding model prediction for the two sample neurons. The distribution of points is very similar to the left plot. Among our sample of neurons, the average correlation coefficient obtained by correlating data from each one of the four single traces with the corresponding model prediction was 0.884 ± 0.018 (4 comparisons per neuron, value for individual cells varied between 0.749 ± 0.012 and 0.955 ± 0.004, n = 16). These values are almost identical to the average correlation coefficient obtained by correlating single traces with each other, suggesting that the accuracy of predictions provided by our model was near maximal and that the remaining inaccuracies in the predictions were mainly due to biological noise. The two plots on the right compare the mean EPSCs calculated over four repetitions to the corresponding model predictions. The spread of points is smaller than in the other cases. As stated in the preceding text, the average correlation coefficient obtained by comparing model predictions to the average EPSC amplitude was 0.918 ±
0.017 and thus higher than in the cases described in the preceding text. Presumably, by averaging over four repetitions, the biological variability was reduced partially, with the result that the model predictions improved somewhat. The remaining prediction errors might be reduced even further with more repetitions. These data suggest that overall, the predictions obtained with our relatively simple model were quite good, and errors were almost within the range of biological variation. However, note that some of the graphs in Fig. 1 reveal a very slight S-shaped distribution of points in both the single trace as well as the average trace fits. This S-shape was seen for most of the recorded cells and indicates a (small) systematic prediction error of the model, with an underestimation of small and very large amplitudes and an overestimation of medium amplitudes.

**Model variants**

Although the simple vesicle release model already provided a very accurate description of synaptic short-term dynamics at the chronically active calyx of Held, some systematic deviations remained. This may not be surprising, as a large number of effects of short-term plasticity have been shown to influence synaptic strength. Some of them were incorporated in previous models, for example synaptic facilitation caused by an increase in calcium concentration (Varela et al. 1997), or postsynaptic receptor desensitization (Graham et al. 2004). The single-exponential time course for vesicle recovery that we assume in receptor desensitization (Graham et al. 2004). The single-exponential time course for vesicle recovery that we assume in our basic model has long been replaced by a double-exponential recovery reflecting two populations of vesicles (Sakaba and Neher 2001; Wang and Kaczmarek 1998).

Therefore we wanted to investigate next how additional degrees of freedom introduced by new parameters improve the model predictions. We chose three extensions, namely a variant with a double-exponential recovery, one with added facilitation, and one which accounts for receptor desensitization (details see METHODS). Figure 2A shows a short clip of recorded EPSC amplitudes and corresponding predictions obtained with the different model variants. The experimental data, shown in black, is represented as the average of four repetitions of the same stimulus pattern recorded from a single cell. The predictions obtained with the basic model are shown in gray; the colored traces represent predictions obtained with the three model variants. Generally, the differences between the individual model predictions were rather small. To quantify these differences, we plotted the model predictions of the different variants against the experimentally measured values. Figure 2B shows these correlation graphs for the same cell as shown in A.

A summary of the correlation coefficients between predictions and experimental data of all 16 neurons tested with various models is shown in C. The data suggest that the model improvements do not yield better predictions as the correlation coefficients obtained with the various models are almost the same. Also the shape of the correlation plots does not change in any obvious way. Finally we tested combinations of model improvements. For example, we tested a combination of receptor desensitization and double-exponential recovery, which neither resulted in higher correlation coefficients, nor an elimination of the systematic deviations shown in Fig. 1E (data not shown).

In all variants of the model, the parameters were not restricted to certain ranges, and all parameters were optimized to generate a model output with the smallest possible squared error as defined earlier. This strategy yielded parameters, which did not change significantly in the four different scenar-
ios. Summary plots of these parameters, namely correlation coefficient, release probability, and recovery time constant are plotted in Fig. 2C. In case of the double-exponential recovery, the fast component of the recovery time course is plotted in Fig. 2C (right). The slow component is infinitely long in some cases (>10^10 s, 5 of 16 neurons) or has nearly the same value as the fast component (±1 SD, 8 of 16 neurons). In the remaining 3/16 neurons, the slow component assumed independent values. However, in these cases the combination of slow and associated fast component yielded almost identical results as the fast component by itself in the corresponding model variant with single-exponential recovery. It appears that in these cases, the number of degrees of freedom in the data were smaller than the number of parameters in the fit and therefore one particular situation could be described by several parameter combinations. All three possibilities lead to the conclusion that the slow component of the recovery was not necessary for an accurate description of the experimental data set. One reason may be that values for the slow component are typically in the range of several seconds (von Gersdorff et al. 1997; Wang and Kaczmarek 1998; Wu and Borst 1999), while the largest interpulse intervals in all our stimulation protocols are in the sub second range. For the model with variable release probability (i.e., the model including synaptic facilitation), the minimal release probability is plotted in Fig. 2C (middle). In this model variant, either the parameter increasing the release probability is infinitely small or the recovery time course of the changing release probability is infinitely fast, depending on the seed values used (16 of 16 cells). Again, both possibilities lead to the conclusion that a variable release probability is not important for accurate predictions with our modeling approach. Receptor desensitization is a postsynaptic effect and is therefore not influencing the basic model parameters but rather altering the output of the basic model. The time courses describing the receptor desensitization for the neurons are either in a range similar to the single-exponential recovery from depression and thus cannot be distinguished from it (14 of 16 neurons) or infinitely short (2 of 16 neurons). All the results described in the preceding text suggest that none of the model extensions yielded useful results and that the parameter space of the basic model variant could capture the variability in our data set best.

Both release probability and recovery time constant, as determined by our model, are very similar to values that have been obtained from experimental data. The release probability has been measured, for example in the rat MNTB (Schneggenburger et al. 1999; Wu and Borst 1999), and determined to be ∼0.2. The recovery time constant in the gerbil MNTB has been determined in our previous paper (Hermann et al. 2007) under similar experimental conditions and is plotted in Fig. 2C next to the modeling results for comparison. The close agreement of our modeling results with previous experimental results suggests that the parameters calculated by the model are the computational equivalents to the corresponding physiological phenomena.

**Stimulation with trains consisting of regularly spaced intervals**

One important feature of the firing pattern of auditory brain stem neurons is phase locking. For certain acoustic inputs, especially pure tones of low frequencies, cells fire with high temporal precision and phase lock to certain parts of the sound wave. This is especially true for MNTB neurons, which have a number of cellular specializations for temporal precision (Forsythe et al. 1998; Smith et al. 1998; Taschenberger and von Gersdorff 2000; Taschenberger et al. 2002; von Gersdorff and Borst 2002). Phase locking results in a very regular firing pattern not reflected by our Poisson distributed spike trains. Therefore we also tested the effects of regular spike trains with varying frequencies. Different frequencies were all tested in a single stimulus, which consisted of various segments with low and high frequencies. Each frequency segment consisted of 20 stimuli and was immediately followed by a segment of 20 stimuli at a different frequency (Fig. 3A). The different train frequencies were chosen to imitate sound stimuli at various frequencies. Within the train, each switch from a low to a high frequency was matched by a corresponding switch from a high to a low frequency. Note that the frequencies in Fig. 3A are plotted against stimulus number, not time. Figure 3B shows a short clip of the complete stimulus plotted against time (top), and a clip of the corresponding voltage-clamp trace from a sample cell (bottom). The segments marked by “i” and “ii” are shown at a magnified scale in Fig. 3, C and D (mean over 3 repetitions). Please note that Fig. 3C represents a high-frequency train (196 Hz), whereas D represents a low-frequency train (45 Hz) at a different time scale. For comparison, clips of the two trains plotted at the same time scale are shown in the gray insets of Fig. 3, C and D. To evaluate the data, the 20 EPSC amplitudes of each constant frequency period were extracted and plotted against time (Fig. 3, C and D, bottom). We then fitted the data points with a single-exponential function (solid line in C and D, bottom).

Besides mimicking phase-locked acoustic input, trains with regular stimulation patterns allow for the measurement of steady-state amplitudes and single-exponential time courses leading to these steady states. These two values are frequency dependent and characteristic for each synapse. In our case, predicting a neuron’s response to a train with regularly spaced intervals with the vesicle release model allowed us to not only test the accuracy of the predicted EPSC amplitudes but also the accuracy of the predicted steady-state amplitudes as well as the dynamics of adjusting to it. We did this by obtaining a data set with Poisson trains as well as a data set with regularly spaced trains from each cell that was tested with this protocol. The Poisson data were used to fit the basic model and calculate the model parameters as described in the preceding text. Then the obtained parameters were used to calculate predictions for the steady-state amplitude \( I^* \) as well as for the time course of adaptation to this steady state characterized by the time constant \( \tau_A \). These two values were then compared with the parameters for the exponential fits of the experimental data. The steady-state amplitude \( I^* \) corresponds to the offset of the exponential fit, and the time constant \( \tau_A \) corresponds to the characteristic time constant of the exponential fit. Figure 3E shows, for the same cell, the comparison of the steady-state amplitudes for various frequencies; the gray line corresponds to the model prediction, the black diamonds to the experimentally determined amplitudes. The time constants were plotted in a similar fashion in Fig. 3F for \( \pm 150 \) Hz. For lower frequencies, time constants were not determined for the following reason: The experimental approach used exponential
fits to determine the time constant, and thus the quality of the fit depended on a reasonable number of data points in the range of one time constant. The predicted time constants were very fast (<50 ms), therefore the transition to the new steady state happened for low frequencies within just a few events. Because of fluctuations in the measurements of EPSC amplitudes, the resulting error in the fitted time course was too large to allow any meaningful statements. To indicate this, time constants are not shown for frequencies <150 Hz (gray box in Fig. 3F).

The average differences between model predictions and measured EPSC amplitudes over our sample of five neurons are shown in Fig. 4. The absolute differences for the steady-state amplitude are plotted in Fig. 4A; deviations from zero indicate differences between calculated and measured EPSC amplitudes. Because steady-state amplitudes depend on the stimulation frequency and are larger for lower stimulation frequencies, absolute differences between measured and calculated amplitudes also tend to be larger for lower frequencies. Therefore we also calculated the normalized difference (Fig. 4B, solid square) and compared this difference to the coefficient of variation (CV) of the calculated steady-state amplitudes. The CV was calculated as the mean CV of the single cells, averaged over the sample of five neurons and is plotted in Fig. 4B (gray area). For all tested frequencies but one (196 Hz), the normalized difference between predicted and measured steady-state amplitude was smaller than one CV. Besides steady-state amplitudes, the model also predicted the neuron’s time course for reaching the steady-state amplitude. This calculated time constant was compared with the measured time constant in five neurons; the differences between the two are plotted in Fig. 4C. For the considered frequencies the model provided very accurate predictions.

Natural stimuli

Finally, we tested the performance of our model on “natural” input, i.e., with spike trains that represent acoustic stimuli of biological relevance. We obtained these spike trains through in vivo recordings from single units in the MNTB of the anesthetized gerbil. The in vivo recording procedures were identical to the ones used in our previous study (Hermann et al. 2007). The presented stimuli were vocalizations of owls, typical gerbil predators (Lay 1974). The spectrogram of a 2-s sound sample is depicted in Fig. 5A.

Using this approach to mimic natural input in a brain slice preparation has two caveats. The first one is the variability of firing patterns between different MNTB cells in vivo, mainly caused by the differential tuning between cells. As information about the neuron’s tuning is lost in a slice preparation, it is not possible to stimulate with one spike train associated with the

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FIG. 3. A: time course of the 2nd stimulation protocol, plotted against stimulus number not time. Each period consists of 20 stimulations with constant interpulse intervals corresponding to the indicated frequency. Frequencies are randomly distributed and range from 5 up to 300 Hz. B: a short clip of the top trace plotted against time for comparison to the raw data trace below. The raw data are the mean over 3 repetitions of a voltage clamp recording. C and D: detailed views of 2 sample constant frequency periods. Note that the traces are plotted against different time axes; the gray insets compare the initial parts of the same trains on identical time scales. The graphs below show the extracted EPSCs plotted as black diamonds and the corresponding model predictions plotted as gray diamonds. The black lines represent single-exponential fits to the data shown by the black diamonds. E: summary of the steady-state amplitudes for all frequencies. The black diamonds represent the offset of the single-exponential fit shown in C and D. F: summary plot of the characteristic time constants of adaptation to the new steady state. As in E, black diamonds represent the time constant of the single-exponential fit; the gray line shows the model prediction. The low-frequency part has been blanked, see text for further information.

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chose three traces as stimuli for in vitro experiments (Fig. 5). Recorded 10 repetitions of sound presentation and randomly to the offset of the exponential fit (i.e., measured steady-state amplitude) and predicted steady-state amplitude. B: the same difference as in A but normalized to the characteristic time constant of the exponential fit and the model prediction. Low frequencies were blanked; for details, see text.

“correct” frequency band. The second caveat arises from the fact that the in vivo recordings were conducted in the MNTB and not from globular bushy cells in the cochlear nucleus, which give rise to the calyx of Held. Also, we did not perform simultaneous pre- and postsynaptic recordings (Guinan and Li 1990; Kopp-Scheinpfug et al. 2003) and thus were unable to determine if our single units were calyces or postsynaptic principal neurons. With these caveats in mind, we stimulated calyceal input fibers with spike trains reflecting characteristics of natural sound evoked input. These spike trains have different characteristics than Poisson or regular stimulations and thereby present yet another class of stimulation pattern.

The spectrogram of the sample that we used to stimulate the cells in vivo is depicted in Fig. 5A. The barn owl call has pronounced temporal features with the main energy between 2 and 5 kHz. We chose data recorded from two in vivo units to stimulate cells in our slice preparation. The cells had best frequencies of 2,500 and 3,500 Hz, respectively, and showed a clear response to the stimulation. From each of these units, we recorded 10 repetitions of sound presentation and randomly chose three traces as stimuli for in vitro experiments (Fig. 5B). Thus a total of six spike trains were used to stimulate each neuron in vitro. An example of a voltage-clamp response recorded in vitro in response to the trace marked in Fig. 5B is shown in C. Similar to the other stimulation protocols, we extracted the EPSC amplitudes and compared them to the model prediction. The model was fitted using data from Poisson stimulations in the same cell, so the comparison between data and model prediction is based on parameters obtained with a different stimulation pattern. This was done to test if the model could also predict the synaptic strength in response to stimulation patterns not used for the parameter optimization. Figure 5D shows the comparison for the sample trace from C; the corresponding correlation coefficient is 0.94. The mean correlation coefficient over all stimulation patterns recorded in six neurons is 0.88 ± 0.03, slightly lower but statistically not different from the values obtained for the Poisson trains. Therefore we conclude that the model produced very good predictions for stimulation patterns based on naturally occurring input.

**Model performance in rested synapses and during the transition period**

The data presented in the preceding text suggest that a very simple vesicle release model is sufficient for describing the release dynamics at the calyx of Held. This result is inconsistent with findings of previous modeling studies, which have shown that simple models are not sufficient to accurately describe transmission at this synapse (Graham et al. 2004; Weis et al. 1999). However, one major difference between this and previous studies is that in our case synapses were tested only after they had been stimulated for prolonged periods of...
time while previous studies tested and modeled the condition of rested synapses. To determine if and how key parameters of transmission might differ between the rested and active state, we analyzed synaptic responses during the conditioning period where neurons were shifted from a rested to a chronically active state. We determined changes in correlation coefficient, and the three model parameters (pool size, release probability, and recovery time constant) at the beginning of the conditioning period, i.e., when synapses were still rested, and during the following 2 min of conditioning with the 20-Hz Poisson train. This was done by fitting the parameters of the same simple release model to 10-s periods of Poisson responses, i.e., to analysis windows of on average 200 synaptic responses (gray box in Fig. 6A). Fitting the responses contained in one such analysis window to the model resulted in one set of numbers for the four parameters shown in Fig. 6, B–E. The analysis window was then shifted by 2 s such that the responses contained in the first 2 s of the previous analysis window were dropped and two seconds of new responses were added at the end of the new analysis window. A new set of numbers for the same four parameters was calculated by fitting the responses contained in the new analysis window and plotted in Fig. 6, B–E. Subsequently, this window was shifted again by 2 s, and the procedure was repeated until the analysis window reached the end of the 2-min conditioning period. This procedure of shifting an analysis window of a 10-s width in increments of 2 s was adopted to compromise between the need to fit the model to a sufficiently large number of synaptic responses to obtain accurate values for the computed parameters and the need for a high temporal resolution in this analysis. In some cases, the small number of points per analysis window leads to fluctuations in the parameters as for example in Fig. 6B. The periodic appearance of these slow changes can be attributed to the high overlap of adjacent analysis windows (8 s).

Figure 6, B–E, left, shows changes of the correlation coefficient and the three model parameters during the 120-s conditioning period that were obtained with the procedure described in the preceding text. Figure 6B indicates that correlation coefficients improved significantly during the 2-min conditioning period in the sample cell presented here as well as in almost all of the 11 neurons tested with this protocol. This result suggests that our simple vesicle release model performs well in capturing the dynamics of synaptic transmission in synapses that have been active for prolonged periods of time but performs significantly less well in rested synapses or synapses that have been driven with a only a small number of stimuli. Changes in pool size, release probability, and recovery time constant over the 2-min conditioning period are shown in Fig. 6, C–E, indicating that pool size decreased, release probability increased, and recovery time constant decreased consistently and significantly over the 2-min conditioning period.

**FIG. 6.** Model performance during the transition from rested to active state. Model parameters were calculated for overlapping 10-s segments of the 2-min conditioning period. A: responses during a 10-s window of the conditioning period containing on average 200 Poisson-distributed stimuli were used to calculate 1 set of values for the model parameters. Subsequently the 10-s window was advanced by 2 s and a 2nd set of values was calculated, and the procedure repeated until the analysis window reached the end of the conditioning period. Responses to the 1st second of the conditioning period (i.e., the 1st 20 stimuli) were eliminated from this analysis as these values are dominated by the initial steep buildup of synaptic depression. B–E: values for the model parameters calculated with this method. Left: the continuous changes in parameters over the 120-s conditioning period for 1 sample neuron. Middle: the linear fits associated with the data traces of all 11 neurons tested with this procedure, and right: the average value of the first analysis window with the average value obtained with the last analysis window. Note that data from one neuron was omitted from the center graph of E to allow for better scaling. This neuron had an initial time constant of 560 ms, which decreased to 350 ms with conditioning.
These results suggest some functional differences between rested and active synapses. In summary, the data shown in Fig. 6 suggest that key parameters of synaptic transmission change during the transition from rested to active state and that the dynamics of rested synapses are not as well captured by the simple release model as the dynamics of chronically active synapses are.

DISCUSSION

The main question this study addresses is how well the response of the calyx of Held synapse to complex trains of continuous activity can be described using vesicle release models. We found that a very basic model including only a constant release probability and a single-exponential time course for vesicle replenishment can predict EPSC amplitudes already with very high accuracy. Two key features of the present study are the use of very long-lasting and statistically complex stimuli as well as the embedding of these into chronic “spontaneous” background activity.

Spontaneous activity is one of the hallmarks of auditory brain stem neurons in vivo but for methodological reasons is lost in slice preparations, such that auditory brain stem neurons in vitro are typically tested against a background of silence. Previous vesicle release models describing synaptic transmission at the calyx of Held (Graham et al. 2001, 2004; Weis et al. 1999) were based on electrophysiological data recorded from silent in vitro preparations (i.e., without the naturally present spontaneous activity), and thus only capture the dynamics of synaptic transmission under these conditions. However, previously we found that synaptic transmission at the calyx of Held in such silent slice preparations differs from transmission under natural activity levels in a number of key parameters such as synaptic amplitude, latency, synaptic depression during trains, recovery from depression, and fidelity of transmission (Hermann et al. 2007).

Therefore the central goals of this study were to model the transmission dynamics of chronically active calyces, compare results obtained with several extensions of the model, and test the performance of the model during complex trains of activity, including activity resulting from sound stimulation with natural and biologically relevant sound clips.

The surprising result is that already the most basic model including only a constant release probability and a single-exponential time course for vesicle replenishment performs exceptionally well in predicting EPSC amplitudes. Extending the model by increasing the number of parameters to account for additional biophysical effects such as receptor desensitization, two vesicle pools, or synaptic facilitation on a separate time course does not increase the prediction accuracy. Why does the most basic model perform so well although it disregards a number of physiological mechanisms that have clearly been shown to be present in the calyx of Held, albeit the rested calyx of Held in silent brain slices? It is unlikely that the stimulus trains used in the present study were too simplistic to capture the fine details of transmission dynamics. We note that the Poisson distributed activity, which was used to compute the model parameters, inherently includes a high degree of statistical complexity. Varying the mean frequency of the Poisson distribution over almost two levels of magnitude introduces additional complexity and ensures that the entire range of activity a MNTB neuron may encounter in the intact brain is covered by the stimulus train. We also tested trains with regular spaced inter spike intervals and stimulus trains derived from sound stimulation with natural sound clips, which increase the amount of statistical complexity even further. Therefore we assume that any model able to predict responses to the stimuli used in this study correctly should be able to predict responses to any naturally occurring sound situation equally well.

Species, age, and temperature differences may account for some of our findings. Most previous in vitro work on the calyx of Held has been performed in preparations from rat or mouse not gerbil. However, we previously found that synaptic transmission in the calyx of Held of the gerbil is very similar to that of other rodents in basic parameters. Receptor desensitization, while being very prominent in preparations from younger animals, plays a much smaller role in preparations from older animals such as the ones used in this study (Joshi and Wang 2002; Renden et al. 2005; Taschenberger et al. 2005). This finding may help explain why the model variation with added receptor desensitization did not yield higher correlation coefficients. EPSC amplitudes, or vesicle pools, are known to recover with double-exponential time courses, not single-exponential ones as used by our basic model (Sakaba and Neher 2002; Wang and Kaczmarek 1998). However, of the two associated time constants, one is very slow and on the order of seconds. Therefore interspike intervals on the order of seconds would be required to measure, or compute, this time constant properly. Due to spontaneous activity in the auditory brain stem, such long periods of silence are uncommon in vivo. Because the goal of our study was to capture the dynamics of synaptic transmission under conditions as closely as possible to those in the intact brain, our stimulus trains did not contain any interspike intervals long enough to properly measure the slow time constant. This might explain why the model variant with the double exponential did not yield higher correlation coefficients than the basic version of the model. It might also explain why the slow time constants were virtually eliminated from the computations during the parameter fitting process, either by assuming infinite values for the time constant or values that were virtually identical to those of the fast time constant.

We are uncertain why the model variant with added facilitation did not seem to yield improved predictions over the basic variant. Synaptic facilitation, i.e., an accumulation of calcium in the presynaptic terminal, must certainly be present during chronic stimulation at relatively high frequencies. It is possible that the introduction of chronic background activity into the slice preparations changes the dynamics of synaptic transmission such that the role of the various mechanisms of short-term plasticity are altered in comparison to the state of a rested synapse. Long-term stimulation of a slice preparation with thousands of stimuli might put calyces of Held into a functional state that is much less well understood than the functional state of rested calyces. Thus it cannot be ruled out that facilitation with an independent time scale, possibly also double-exponential recovery, might just not have a significant influence over EPSC dynamics during chronic long-term activity. It is also possible that the time courses of these are too similar to the basic features of the model such that the parameter optimization cannot extract them as separate parameters. In this case, the parameters computed by our model would represent a combination of physiological effects and the number of phys-
ologically relevant parameters is actually higher than estimated by the modeling process. On the other hand, the values for release probability and the fast recovery time constant as determined by our model are similar to values previously reported in the literature, suggesting that they correspond at least to a high degree to these physiological parameters rather than an abstract combination of several physiological effects. Nevertheless, the possibility that our model underestimates the number of physiological parameters relevant for transmission in active synapses cannot be ruled out completely.

An alternative explanation that can resolve the discrepancies between this study and older studies performed in rested synapses is that active synapses are in a different physiological state that can be described in mathematically simpler terms. This explanation implies that mechanisms of synaptic transmission known to influence transmission of rested synapses play a much smaller role in active synapses. Not much is known about the chronically active calyx of Held, and therefore more data are needed to verify or falsify this interpretation. Our own analysis of the transition between rested and active state revealed that model parameters change during the transition between rested and active states, suggesting that at least some biophysical parameters of synaptic transmission are affected by the chronic activity. More importantly, the same experiment revealed that our model performs significantly less well with data from rested synapses, suggesting that it is not an appropriate mathematical description of the rested state, supporting the interpretation of functional differences between rested and active states of the calyx of Held.

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