Dynamics of Excitatory Transmitter Release: Analysis of Synaptic Responses in CA3 Hippocampal Neurons After Repetitive Stimulation of Afferent Fibers

MARCO CANEPARI AND ENRICO CHERUBINI
Biophysics Sector and Istituto Nazionale Fisica della Materia, International School for Advanced Studies, 34014 Trieste, Italy

Canepari, Marco and Enrico Cherubini. Dynamics of excitatory tracellular stimulation, believed to trigger release from a single presynaptic fiber (Voronin 1994), reflect variations in the number of quanta. When two pulses with tens of millisecond intervals are delivered to the nerve terminal, the currents evoked by the second pulse are either facilitated or depressed. The phenomenon of facilitation, well characterized at the neuromuscular junction as well as in central synapses, is thought to be due to presynaptic accumulation of residual calcium $[\text{Ca}^{2+}]_	ext{i}$ during conditioning stimulation (Katz and Miledi 1968; Magleby and Zengel 1982). The amplitude of the current evoked by the second pulse increases because the probability of release increases. Interestingly, at the neuromuscular junction, facilitation is replaced by depression in conditions of increased release probability, a phenomenon believed to be caused by depletion of synaptic vesicles (Betz 1970).

Similar results have been obtained in the hippocampus (Creager et al. 1980). As both paired-pulse facilitation and depression are considered to be generated mainly by a change in presynaptic conditions also in the central nervous system excitatory synapses, the analysis of these phenomena have been used to test the possible presynaptic site of expression of long-term potentiation (Manabe et al. 1993; McNaughton 1982; Voronin and Khunt 1990).

When nerve terminals are stimulated with more than two pulses, different presynaptic mechanisms might act together to determine the dynamics of synaptic function. In neocortical neurons, pyramid-pyramid connections are characterized by a high probability of release that most likely results in depression of postsynaptic potentials evoked by a train of presynaptic spikes (Markram and Tsodyks 1996). In contrast, in pyramid-interneuron synapses, which are apparently characterized by a lower probability of release, synaptic dynamics is dominated by facilitation (Thomson et al. 1995).

In this report, the mechanisms determining short-term changes of synaptic efficacy during short trains of presynaptic action potentials have been investigated in the CA3 region of the hippocampus. A model that takes into account the dynamics of release and the reavailability of synaptic vesicles has been developed and related to the different dynamic behaviors experimentally observed.

**METHODS**

*Slice preparation and solutions*

Slices were prepared as described in previous reports. (Sciancalepore et al. 1995). Briefly, Wistar rats, aged postnatal (P) days...
P7–P12, were decapitated under urethane anesthesia (0.5 ml ip of a 10% solution) and their brains removed. Transverse hippocampal slices (200-µm thick) were cut with a vibroslicer and placed in a bath containing (in mM) 126 NaCl, 3.5 KCl, 2 CaCl₂, 1.2 NaH₂PO₄, 1.3 MgCl₂, 14 NaHCO₃, and 11 glucose, gassed with 95% O₂-5% CO₂ at 32°C (pH 7.3). They were allowed to recover for ≥1 h before being transferred to the recording chamber in which they were superfused at 3 ml/min. The extracellular solution contained (in mM) 126 NaCl, 3.5 KCl, 2 CaCl₂, 1.2 NaH₂PO₄, 1.3 MgCl₂, 25 NaHCO₃, and 11 glucose, gassed with 95% O₂-5% CO₂ at room temperature (23°C, pH 7.3). Bicuculline (10 µM, purchased from Sigma) also was added routinely to block γ-aminobutyric acid-A (GABAₐ)–mediated synaptic responses. To block the N-methyl-d-aspartate (NMDA) component of the excitatory postsynaptic currents (EPSCs), (+)-3-([carboxyypiperazin-4-yl])–propyl-1-phosphonic acid (CPP, 20 µM) was added to the extracellular solution. In some experiments, extra- cellular calcium concentration [Ca²⁺] was varied from 0.8 to 4 mM. In additional experiments, to identify the synaptic input to the CA3 pyramidal cells, the mGluR2/mGluR3 specific agonist 2-(2,3-dicarboxyyclopropyl)glycine (DCG-IV, 1 µM, purchased from Tocris Cookson) was applied in the bathing solution.

**Patch-clamp recordings**

Recording pipettes, pulled from 2 mm borosilicate glass, had a resistance of 2–5 MΩ when filled with intracellular solution. Intracellular solution contained (in mM) 120 K-glucanote, 4 MgCl₂, 49 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES), 0.6 ethylene glycolbis (b-aminoethyl ether) N,N,N’,N’-tetraacetic acid (EGTA), 2 Na₃ATP, and 0.2 Na₂GTP, adjusted to pH 7.2 with KOH. EPSCs from CA3 hippocampal neurons were recorded at −70 mV in voltage-clamp mode, with a standard patch- clamp amplifier (EPC-7 List Medical Instruments) after optimizing capacitance and series resistance compensation. Series resistance (typically 10–15 MΩ) was checked continuously for stability during the experiment.

**Stimulation**

Patch pipettes with a tip of 10- to 30-µm diam filled with the extracellular solution were used for stimulation. The stimulating electrode was placed under visual control on the surface of the slice in the stratum lucidum-radiatum 50–200 µm above the CA3 pyramidal layer. In this way, both mossy fibers and/or associative commissural fibers could be stimulated. EPSCs were evoked by 4 or 10 pulses (frequency 10–20 Hz, each pulse 40-µs duration), repeated every 10 s. To stimulate the minimal number of fibers, the stimulus intensity was adjusted above threshold to evoke an EPSC. The mean peak EPSC amplitude plotted against stimulus intensity, showed an all-or-none behavior with an abrupt increase at a given stimulus strength corresponding to threshold and then remained constant when the stimulation intensity was kept within a given range (Jonas et al. 1993) (see also Fig. 3A). A further increase in stimulus strength gave rise to larger EPSC, indicating the involvement of more fibers. Typically the minimal intensity of stimulation required to evoke an EPSC ranged from 4 to 10 V. In some cases, discarded from the present results, unclamped antidromic spike could be detected after stimulation pulses.

**Data acquisition and analysis**

DIGIDATA 1200 (Axon Instruments) was used to acquire data and to control the stimulation. Signals were low-pass filtered at 2 kHz and acquired at 10 kHz. Data were analyzed with a Matlab 4.2C for Windows program. The program could detect the stimulation artifact and measure amplitude and rise time of EPSC. This program could fit the decay phase of the EPSC with a single exponential, to extract the decay time. A response having an amplitude smaller than the background noise (defined as 3 times the SD of the signal recorded during the 50-ms interval preceding the first stimulus artifact) was considered a failure. Statistical analysis of responses (means ± SD) included failures. Matlab 4.2C also was used to compute numerical data from the model and to compare these with experimental results.

**RESULTS**

Stimulation of the afferent pathway, in the presence of bicuculline (10 µM) and CPP (20 µM) elicited in CA3 hippocampal neurons monosynaptic α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA)–mediated EPSCs with characteristics similar to those already described (Jonas et al. 1993). The delay between the stimulation and the onset of the EPSC was usually 2–4 ms. Figure 1 shows an example of the protocol used throughout the experiments. The eight consecutive recordings (of 100) represented in Fig. 1C, demonstrate a high variability in the amplitude of EPSCs evoked by four pulses delivered at the frequency of 20 Hz. Failures in the occurrence of EPSCs after one or two of the four pulses often were observed. EPSCs fluctuated in amplitude from trial to trial, and these fluctuations are depicted in the amplitude distribution histograms represented in Fig. 1B.

**Different EPSC patterns could be evoked by four pulses of stimulation at 20 Hz**

In 57 experiments in which ≥30 recordings with four pulses of stimulation at 20 Hz have been considered, the sequences of the four mean EPSC amplitudes (EPSC patterns) could be classified in terms of increase (facilitation, F) or decrease (depression, D) in the amplitude of the nth EPSC with respect to the (n − 1)th. For example, FDD indicates facilitation of the second EPSC with respect to the first one and depression of the third with respect to the second and of the fourth with respect to the third. EPSC patterns therefore could be grouped in eight cases.

The case with three consecutive facilitations (FFF) was observed only six times (Fig. 2), whereas the case with three consecutive depressions (DDD) was more common (19 times). Cases FFD and FDD, considered as intermediate cases, were observed altogether 19 times. All the other cases in which at least one facilitation occurred after a depression were observed 13 times.

As shown in Fig. 2B, the four normalized averaged EPSCs in the sequence had a similar decay time, suggesting that AMPA receptor desensitization does not play an important role in determining different EPSC patterns (Jones and Westbrook 1996).

To test whether distinct excitatory postsynaptic potentials (EPSP) patterns could be generated in the same cell by stimulation of different fibers, in additional experiments (n = 10), the intensity of stimulation was increased gradually. In the representative example illustrated in Fig. 3A, the voltage range between 4 and 5.5 V corresponded to the minimal stimulation required to evoke an EPSC. One or more addi-
FIG. 1. Excitatory postsynaptic currents (EPSCs) evoked in CA3 hippocampal neurons by stimulation of the stratum lucidum-radiatum. A: hippocampal slice obtained from a P9 rat with a recording electrode positioned in the CA3 pyramidal region and a stimulating electrode placed in the stratum lucidum-radiatum. EPSCs \( (n = 100) \) were evoked by 4 pulses at 20 Hz. B: amplitude distribution histograms for the 1st 4th EPSCs. Number of failures (see traces 1, 2, 7, and 8 in C). These were 10 (1st pulse), 10 (2nd pulse), 8 (3rd pulse), and 8 (4th pulse). C: sampling traces showing 8 consecutive recordings (7 8). Average of 100 recordings (including 1 8) is shown in 9. Means SD of the amplitude (in pA) of the 4 EPSCs were 26.8 16.9 (1st pulse), 26.0 17.0 (2nd pulse), 28.4 16.7 (3rd pulse), and 30.9 16.8 (4th pulse). Delay between the stimulation and the occurrence of the EPSC was the same for all pulses (3.0 0.5 ms). Rise and decay times were also the same for all pulses (1.2 0.3 and 11 4 ms, respectively).

Identification of presynaptic fibers responsible for EPSCs

In the adult guinea pig hippocampus, differences in short-term plasticity between synaptic responses evoked by repetitive stimulation of mossy fibers and those elicited by associative commissural fibers have been reported (Salin et al. 1996). To see whether the high variability in EPSC patterns observed in the present experiments reflect the activation of different types of synaptic inputs, the mGluR2/mGluR3-specific agonist DCG-IV (1 mM) was used. Activation of mGluR2/mGluR3, which are localized on the mossy but not on the associative commissural fibers (Shigemoto et al. 1997), induces a reduction of neurotransmitter release and
FIG. 2. Different EPSC patterns evoked by 4 pulses. 

A: each trace is the average of 40 recordings obtained from 3 different cells (1–3). Mean amplitudes ± SD of the 4 EPSC amplitudes (in pA) in the 3 cases were 10.5 ± 6.6, 13.4 ± 7.3, 16.0 ± 9.1, and 17.8 ± 9.3 (A1); 6.7 ± 6.9, 11.2 ± 10.0, 11.7 ± 8.8, and 11.2 ± 8.7 (A2); and 22.9 ± 10.2, 18.3 ± 9.4, 16.0 ± 10.1, and 14.9 ± 9.7 (A3). A1 and 3, show the extreme cases of EPSC patterns in which there is facilitation or depression of the 2nd, 3rd, and 4th EPSC with respect to the previous 1 (FFF and DDD). A2 represents an intermediate case in which the 4th EPSC is depressed slightly in comparison with the previous 1 (FFD). All the other patterns of activity in which a facilitation occurs after a depression (DDF, DFD, DFF, or FDF) have been also observed. 

B: 4 mean currents in the 3 cases shown in A were normalized and superimposed (1–3). Notice that in each case the kinetics of the evoked currents were the same for all pulses of stimulation. 

C: summary of the results obtained in 57 experiments. Cases 1 and 3 have been observed 6 and 19 times, respectively. Intermediate cases have been observed 19 times (FFD = 9, FDD = 10) and the other cases 13 times (DDF = 6, DFD = 1, DFF = 1, FDF = 5).

therefore allows us to distinguish between the two different synaptic inputs (Kamiya et al. 1996). In three of eight cells in which DCG-IV was tested, a reduction of the mean amplitude of the first EPSC (>30%) was observed, whereas in the remaining cases, the mean EPSC amplitude was unchanged. In the presence of DCG-IV, modifications of EPSC patterns occurred as shown in the representative example of Fig. 4A, in which a DDD pattern changed to a FDD pattern. Interestingly, in the DCG-IV–sensitive cases, the mean amplitude of the first EPSC was >20 pA with a coefficient of variation (CV) >70%, whereas in the DCG-IV–insensitive responses, the first EPSCs had either a mean amplitude <20 pA or a CV <50% and almost no failures. These results suggest that EPSCs evoked by the release of a single quantum at mossy fiber synapses are generally larger in amplitude than those at associative commissural fibers as recently dem-
DYNAMICS OF SYNAPTIC RESPONSES IN CA3 NEURONS

Effects of changing $[\text{Ca}^{2+}]_o$ on EPSC patterns

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FIG. 4. Identification of presynaptic fibers responsible for EPSCs. A and B: traces representing the average of 40 recordings of 2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV)–sensitive (A) and -insensitive (B) synaptic responses in control condition and in the presence of 1 μM DCG-IV. Means ± SD of the amplitude of the 4 EPSCs (in pA) were A: 38 ± 29, 32 ± 26, 29 ± 25, and 27 ± 28 (control); 20 ± 24, 29 ± 30, 28 ± 29, and 27 ± 28 (DCG-IV); B: 10.5 ± 10.1, 9.9 ± 10.4, 8.5 ± 9.3, and 6.5 ± 8.9 (control); 10.0 ± 10.0, 9.7 ± 10.5, 9.0 ± 9.7, and 6.0 ± 8.5 (DCG-IV);...

the two action potentials; \( f(t) \) must satisfy the following requirements

\[
\begin{align*}
  f(0) &= 1 \quad \text{(no reavailability immediately after the first release)} \quad (4a) \\
  f(t \rightarrow \infty) &\rightarrow 0 \quad \text{(complete reavailability after a long time)}. \quad (4b)
\end{align*}
\]

The simplest way to express the reavailability function is to use a single exponential function \( e^{-ct} \) or a sum of several exponential functions indicating different possible time constants.

If \( j \) action potentials reach the synaptic terminal with a constant time interval \( \Delta t \), Eq. 3 can be generalized for the mean number of vesicles released during the \( j \)th action potential as

\[
\langle n_j \rangle = P(\text{[Ca}^{2+}\text{]}_0 + \Delta_j \cdot \text{[Ca}^{2+}\text{]}) \cdot \left[ N - \sum_{i=1}^{j-1} \langle n_i \rangle \cdot f((j - i) \cdot \Delta t) \right] \quad (5)
\]

where \( \Delta_j \cdot \text{[Ca}^{2+}\text{]} \) is the change of effective calcium concentration produced by the first \( j - 1 \) spikes. EPSC amplitudes can be considered approximately proportional, to the first order, to the number of released vesicles, and therefore the mean EPSC amplitude (\( A \)) can be expressed by the relation

\[
A_j = \sum_{n=1}^{i} \beta_h \cdot P_h(\text{[Ca}^{2+}\text{]}_0 + \Delta_j \cdot \text{[Ca}^{2+}\text{]}) \times \left\{ N_0 - \sum_{i=1}^{j-1} \langle n_i \rangle \cdot f((j - i) \cdot \Delta t) \right\} \quad (7)
\]

In this expression, the possible delay between the release at different terminals and/or releasing sites is neglected. By choosing particular forms of \( P(\text{[Ca}^{2+}\text{]}) \) and \( f(t) \) and by introducing a possible dependence of \( P_j \) on the \( j \)th action potential, this general model could account not only for calcium dependent exocytosis dynamics but also for other possible mechanisms of modulation of calcium entry and transmitter release during the activation of the synaptic terminal. A probability function 2 in which there is a summation of constant contributions to residual calcium \( (\Delta_j \cdot \text{[Ca}^{2+}\text{]}) = (j - 1) \cdot \Delta(\text{Ca}^{2+}) \) can account for simple mechanisms of facilitation. Each contribution depends on \( \Delta t \) as the residual calcium decays in time.

It should be stressed that Eq. 7 can also describe postsynaptic mechanisms if a dependence of \( \beta_h \) on \( j \) or on \( \langle n_j \rangle \) is introduced. As already mentioned, the kinetics of EPSCs in the sequence did not change, suggesting that receptor desensitization does not play a relevant role in these measurements. Moreover, in contrast to EPSPs recorded from neocortical interneurons (Thomson and Deuchars 1994), in the present experiments, self-facilitation at NMDA-receptor–mediated synapses did not give any contribution because EPSCs were recorded at −70 mV and in the presence of NMDA blockers. Finally, another possible postsynaptic mechanism that can contribute to different EPSC patterns is the AMPA receptor saturation, although this issue is still controversial (Clements 1996). However...
FIG. 5. EPSC patterns evoked in the presence of different [Ca\(^{2+}\)]\(_o\). A: traces representing the average of 40 recordings obtained in the presence of 0.8 (1), 2 (2), and 4 mM (3) [Ca\(^{2+}\)]\(_o\). Means ± SD of the amplitude of the 4 EPSCs (in pA) in the 3 cases were 2.1 ± 4.4, 3.6 ± 5.2, 4.1 ± 7.6, and 3.0 ± 5.9 (A1); 18.4 ± 10.7, 17.51 ± 8.3, 18.52 ± 11.1, and 12.8 ± 9.0 (A2); 30.6 ± 12.5, 16.9 ± 9.0, 12.0 ± 8.6, and 10.0 ± 9.7 (A3). Numbers of failure (not shown) were 24, 19, 18, and 18 (A1); 5, 2, 2, and 5 (A2); and 1, 1, 3, and 5 (A3). B: ratios between the 4 mean EPSC amplitudes and the 1st one in the 3 experimental conditions are shown in B1. In B2, each point represents the mean amplitude of the 1st EPSC evoked in the 3 different [Ca\(^{2+}\)]\(_o\); error bars represent SD. C: light and dark columns indicate the mean of the mean EPSC amplitudes obtained in 6 experiments with 2 and 4 mM [Ca\(^{2+}\)]\(_o\), respectively. Standard deviations are also indicated (error bars). In all cases the ratios between the 2nd, 3rd, and 4th mean EPSC amplitudes and the 1st 1 were smaller in the presence of 4 mM Ca\(^{2+}\); and in this condition DDD EPSC patterns were observed 5 times.
AMPA receptor saturation has not been tested in the present experiments.

EPSC patterns generated by different parameters

Although the synchronous release of neurotransmitter generated by a single action potential in a presynaptic terminal can be described by a simple probabilistic model based on the binomial distribution, release of neurotransmitter triggered by a train of action potentials is a much more complicated stochastic process. However, the discrete dynamic system represented in Eq. 7 can account for the mean EPSC amplitudes elicited by trains of spikes. To compare the experimental results with the predictions obtained by Eq. 7 in different conditions, EPSC amplitudes were normalized to the amplitude of the first one. In the case of one terminal, normalized EPSC amplitudes do not depend on the initial number of available vesicles (N), while in the case of more than one terminal, the number of terminals and/or releasing sites (N_k) can be included in the coefficients of the weighted sum by replacing β_k with α_k = β_k · N_k.

To reproduce different EPSC amplitude patterns, Eq. 7 has been applied to different conditions of release probability. In the simplest case of one synaptic terminal, FFF cases are generated by a low probability of release produced by calcium entry in the synaptic terminal, whereas the other extreme cases of DDD are the result of a high probability of release. Figure 6A shows three EPSC patterns calculated from Eq. 7 by using a single terminal with the probability function of Eq. 2, a summation of constant contributions to residual calcium and f(t) expressed by a single exponential function; these patterns resemble those shown in Fig. 2A and are obtained by varying only the relative calcium concentrations in the probability function.

Other cases can also be obtained with a high probability of release and a relatively fast reavailability time constant (i.e., a DDF case produced by Δt/τ = 0.25, P_{max} = 1, [Ca^{2+}]_a = 2 · K^{-1}, Δ[Ca^{2+}]_a = 1 · K^{-1}). However, the contribution of more than one releasing site and/or terminal to the EPSC (see Fig. 3) or the statistical error deriving from a small number of recordings may account for the cases in which a facilitation follows a depression. Although the probability function prevails in determining the EPSC patterns elicited by the first four pulses, reavailability dynamics plays a fundamental role in shaping EPSCs occurring toward the end of a train of more than four pulses. Figure 6B shows four EPSC patterns generated by Eq. 7 with 10 stimulation pulses in which a single exponential function with four different values for Δt/τ has been used. The EPSC amplitudes always reach a stationary value that is equal to 0 in the absence of reavailability (Δt/τ = 0), whereas it is proportional to P_{max} · N in the case of a full reavailability (Δt/τ → ∞). It is finally interesting to predict from the model possible EPSC patterns generated at different frequencies of stimulation; in this case, both the consecutive contributions of residual calcium and the timing of reavailability change. Figure 6, C and D, shows 100 EPSC patterns generated by simulating an increase in the interval between two presynaptic spikes from 50 to 500 ms in the case of a synapse with a low release probability and in the case of a synapse with a high release probability, respectively. These simulations are obtained by progressively reducing both Δ[Ca^{2+}] and Δt in Eq. 7.

Comparison of experimental data with those generated by the model

As already shown in the experimental results session, changes in the mean amplitude of EPSCs evoked by trains of stimuli were obtained by changing [Ca^{2+}], or by activating mGluR2/mGluR3 in the case of mossy fibers. In terms of parameters of Eq. 7, an increase in [Ca^{2+}] leads to an increase of both [Ca^{2+}] and Δ[Ca^{2+}]. To compare experimental data with those generated by the model, sets of parameters were chosen by using a semiautomatic procedure to reproduce experimental patterns in control conditions (2 mM [Ca^{2+}]_o). This procedure was based on the random generation of parameter sets within a given range until a good fit of the experimental patterns was obtained. Once the control condition was reproduced, the higher [Ca^{2+}] condition was mimicked by changing [Ca^{2+}] and Δ[Ca^{2+}] (Fig. 7A). A very good agreement was found between all the experimental data in which [Ca^{2+}] was changed and those generated by the model.

In the same way, to reproduce the EPSC patterns recorded in the presence of DCG-IV (and shown in Fig. 4A), the possible reduction in calcium entry after inhibition of voltage-dependent calcium channels by activation of mGluR2/mGluR3 (Takahashi et al. 1996) was simulated with a decrease of both [Ca^{2+}] and Δ[Ca^{2+}] (Fig. 7C).

Furthermore, to see how changes in the frequency of presynaptic spikes could affect EPSC patterns in our system, 10 pulses at 10–20 Hz were delivered to afferent fibers. In these experiments, EPSC patterns were reproduced by increasing Δt/τ and sometimes decreasing Δ(Κ · [Ca^{2+}]) at the lower stimulation frequency (Fig. 7B). In the case shown in Fig. 7B, f(t) was a weighted sum of a fast (τ ~ 50 ms) and a slow (τ ~ 500 ms) exponential function.

DISCUSSION

In the present experiments, it was shown that, after activation of afferent fibers with trains of stimuli, EPSC patterns are highly variable. A discrete dynamic system derived as the average of the stochastic process, in which both the release probability and the number of synaptic vesicles available for exocytosis change has been proposed. The comparison between the experimental results and this model indicates that short-term changes of presynaptic conditions can account for this variability.

Dynamic behavior of synapses reflects presynaptic changes

The correlation between the probability of release and the behavior of synaptic transmission generated by more than one action potential has been studied in the rat neocortex and hippocampus (Debanne et al. 1996; Thomson et al. 1995). Although in these reports pairing of monosynaptically coupled cells were used to analyze EPSPs or EPSCs activated by single presynaptic fibers, in the present study a minimal extracellular focal stimulation was applied in the vicinity of
FIG. 6. Different EPSC patterns calculated from Eq. 7. A and B: ratios between the nth mean EPSC amplitude and the 1st one calculated from Eq. 7. In both cases, a single negative exponential function with time constant $\tau$ was used as reavailability function, and Eq. 2, with a summation of constant contributions to residual calcium, was used as probability function. In the 3 examples shown in A, only the probability function parameters were varied. Set of parameters was $\Delta t/\tau = 0.2$, $P_{\text{max}} = 0.5$, $[\text{Ca}^{2+}] = 0.66 \cdot \text{K}^{-1}$ (1), $0.76 \cdot \text{K}^{-1}$ (2), and $1.45 \cdot \text{K}^{-1}$ (3), $\Delta [\text{Ca}^{2+}] = 0.14 \cdot \text{K}^{-1}$ (1), $0.21 \cdot \text{K}^{-1}$ (2), and $0.4 \cdot \text{K}^{-1}$ (3). In the 4 examples shown in B, only the reavailability exponential function was changed, whereas the other parameters were the ones used in A2 (values of $\Delta t/\tau$ are indicated in the figure). Notice the strong similarity between the patterns represented in A and the experimental ones shown in Fig. 2A. C and D: 100 ratios between the nth mean EPSC amplitude and the 1st one calculated from Eq. 7 by simulating a progressive change in the interval between 2 presynaptic spikes from 50 to 500 ms. In C, the set of parameters of A1 was used to produce the values at 50-ms stimulation interval; the contribution to residual calcium was linearly reduced and reached 0 at 300 ms. In D, the set of parameters at 50-ms stimulation interval was $\Delta t/\tau = 0.2$, $P_{\text{max}} = 0.5$, $[\text{Ca}^{2+}] = 2 \cdot \text{K}^{-1}$, $\Delta [\text{Ca}^{2+}] = 0.4 \cdot \text{K}^{-1}$; the contribution to residual calcium was linearly reduced and reached 0 at 500 ms. EPSC patterns represented in B–D are calculated for 10 pulses of stimulation.

the recording cell. In comparison with pair recordings, with this technique it is impossible to guarantee that only one presynaptic fiber is stimulated. Nevertheless, in voltage-clamp recordings, possible postsynaptic voltage-dependent changes of synaptic efficacy are eliminated.

In comparison with the neocortex (Thomson and Deuchars 1994; Thomson et al. 1993, 1995), synaptic responses in the hippocampus, are highly unreliable (Allen and Stevens 1994). There is also evidence that mechanisms underlying paired pulse facilitation and depression at single pyramid-pyramid connections are presynaptically mediated and dependent on the amplitude of the first EPSP or EPSC (Debanne et al. 1996; Deuchars and Thomson 1996; Dobrunz and Stevens 1997). In keeping with this, synapses with different release probabilities have been found in hippocampal pyramidal neurons in culture (Murthy et al. 1997; Rosenmund et al. 1993) and in slices (Hessler et al. 1993). In the experiments shown in the present report, the high variability of EPSC patterns, present in both mossy fibers and associative commissural fibers, could be ascribed to presynaptic mechanisms. In synapses in which the release elicited by the first action potential occurs with a low probability, the synapse will be generally potentiated by the increased probability generated by the following spikes. In contrast, in synapses in which the first action potential produces the release of a larger number of quanta, the number of vesicles available for release at the following spikes will be decreased; this depletion will compensate and eventually overcome the effect of the increased probability resulting in a depression of the synaptic efficacy (Dobrunz and Stevens 1997). This conclusion confirms the hypothesis that the balance between the effect of residual calcium accumulation and the depletion of vesicles can account for the synaptic response to a burst of action potentials in CA3 hippocampal pyramidal neurons (Miles and Wong 1986), although other mechanisms besides vesicle depletion may contribute to synaptic depression (Zucker 1989).

In the model that has been proposed, after a few stimulations of the presynaptic terminal, the probability of release will not increase any more because the accumulation of residual calcium saturates the probability function; this condition will determine an equilibrium between the
FIG. 7. Comparison between experimental and computed data. A–C: ratios between the nth mean EPSC amplitude and the 1st one obtained in some experiments (left) in comparison with patterns calculated from Eq. 7 (right). A is relative to 3 experiments in which 2 and 4 mM [Ca\(^{2+}\)] were used. In patterns computed from the model, only the parameters in the probability function (Eq. 2 with a summation of constant contributions to residual calcium) were changed to reproduce variations in [Ca\(^{2+}\)]. A single exponential function with \(\Delta t/\tau = 0.1\) (4), 0.2 (5), and 0.5 (6) was used as reavailability function, whereas the other fixed parameter \(P_{\text{max}}\) was 0.45 (4), 0.32 (5), and 0.78 (6). [Ca\(^{2+}\)] was varied from 1·K\(^{-1}\) to 2·K\(^{-1}\) (4), from 1·K\(^{-1}\) to 1.4·K\(^{-1}\) (5), and from 1·K\(^{-1}\) to 2·K\(^{-1}\) (6), whereas \(\Delta [\text{Ca}^{2+}]\) was varied from 0.2·K\(^{-1}\) to 0.25·K\(^{-1}\) (4), from 0.13·K\(^{-1}\) to 0.25·K\(^{-1}\) (5), and from 0.1·K\(^{-1}\) to 0.2·K\(^{-1}\) (6). In B, ratios obtained from data shown in Fig. 4A were compared with those obtained with the model. Experimental condition in which 1 \(\mu\)M DCG-IV was added to the external solution was simulated by decreasing both [Ca\(^{2+}\)] and \(\Delta [\text{Ca}^{2+}]\). The timing of the reavailability function was \(\Delta t/\tau = 0.8\), while the other fixed parameter \(P_{\text{max}}\) was 0.3. [Ca\(^{2+}\)] was varied from 3·K\(^{-1}\) to 1.05·K\(^{-1}\), while \(\Delta [\text{Ca}^{2+}]\) was varied from 2·K\(^{-1}\) to 0.45·K\(^{-1}\). In C, ratios obtained from 1 experiment in which EPSCs were evoked by 10 pulses of stimulation at 50 and 100 ms stimulation interval were compared with those computed with Eq. 7. Reavailability function was \(\beta_1 \exp(\Delta t/\tau_1) + \beta_2 \exp(\Delta t/\tau_2)\). \(\Delta t\) was changed from 50 to 100 ms to reproduce the modifications in the frequency of stimulation. Fixed parameters were \(P_{\text{max}} = 0.35\), [Ca\(^{2+}\)] = 2·K\(^{-1}\), \(\Delta [\text{Ca}^{2+}] = 0.5\cdot K^{-1}\), \(\tau_1 = 500\) ms, \(\tau_2 = 50\) ms, \(\beta_1 = 0.45\), \(\beta_2 = 0.55\). Patterns calculated from the model qualitatively reproduce experimental data.
mechanisms of exocytosis and reavailability of synaptic vesicles that eventually will result in a steady-state value of the mean number of available vesicles. Therefore the stationary EPSC value depends on the frequency of stimulation (exocytosis time course) and on the time course of reavailability of synaptic vesicles. In the comparison between experimental data and the model, the reavailability function used to mimic EPSC patterns obtained experimentally was the sum of two exponential functions, one with a time constant of the order of tens of milliseconds and the other with a time constant of the order of hundreds of milliseconds. This suggests that reavailability mechanisms of synaptic vesicles with different time scales can be present in these synapses. Particularly, mechanism of flickering of fusion pores responsible for a ‘‘kiss and run’’ type of secretion (Alvarez de Toledo et al. 1993; Breckenridge and Almers 1987; Fesce et al. 1994) may be a candidate for the fastest dynamics of release.

The consequences of different dynamic behaviors of synapses in terms of synaptic function and neural code have been discussed in the neocortex (Tsodyks and Markram 1997). In this system, as excitatory connections to pyramidal cells and to interneurons exhibit different features, it has been proposed that modulations of synaptic properties generated by synaptic plasticity can regulate the temporal codes expressed by neocortical circuits. The high variability of EPSC patterns after stimulation of the mossy or the associative commissural pathway suggests that, in the hippocampus, activity-dependent regulation of synaptic efficacy can markedly change the dynamics of intercellular communication.

How the analysis of transmitter release dynamics can be used to investigate changes in synaptic function

The analysis of postsynaptic response fluctuations evoked by stimulation of presynaptic fibers has been used to study the mechanisms of expression of long-term plasticity (Bekkers and Stevens 1990; Manabe et al. 1993; Oliet et al. 1994; Stevens and Wang 1994). This method is called quantal analysis (reviewed by Voronin 1994), and one of its particularly efficient variants is the coefficient of variation method (Faber and Korn 1991; Kullmann 1994).

Up to now, the quantal analysis has been applied mainly to analyze currents or potentials evoked by a single or at most two presynaptic spikes. A more detailed analysis of the stochastic process of release induced by several action potentials can be an alternative and possibly more powerful way to investigate changes in synaptic function. In the model proposed in this paper, only the behavior of the mean EPSCs evoked by a train of presynaptic action potentials (Eq. 7) has been discussed. The comparison between experimental data and the model shows that this kind of analysis is able to indicate the possible underlying changes in the probability of release and in the time course of reavailability of synaptic vesicles by using a relatively small number (30–40) of recordings.

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