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Transmitter Metabolism as a Mechanism of Synaptic Plasticity: A Modeling Study

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Axmacher, Nikolai, Martin Stemmler, Dominique Engel, Andreas Draguhn, and Raphael Ritz. Transmitter metabolism as a mechanism of synaptic plasticity: a modeling study. *J Neurophysiol* 91: 25–39, 2004.. First published September 17, 2003; First published September 17, 2003; 10.1152/jn.00797.2003. The nervous system adapts to experience by changes in synaptic strength. The mechanisms of synaptic plasticity include changes in the probability of transmitter release and in postsynaptic responsiveness. Experimental and neuropharmacological evidence points toward a third variable in synaptic efficacy: changes in presynaptic transmitter concentration. Several groups, including our own, have reported changes in the amplitude and frequency of postsynaptic (miniature) events indicating that alterations in transmitter content cause alterations in vesicular transmitter content and vesicle dynamics. It is, however, not a priori clear how transmitter metabolism will affect vesicular transmitter content and how this in turn will affect pre- and postsynaptic functions. We therefore have constructed a model of the presynaptic terminal incorporating vesicular transmitter loading and the presynaptic vesicle cycle. We hypothesize that the experimentally observed synaptic plasticity after changes in transmitter metabolism puts predictable restrictions on vesicle loading, cytoplasmic–vesicular transmitter concentration gradient, and on vesicular cycling or release. The results of our model depend on the specific mechanism linking presynaptic transmitter concentration to vesicular dynamics, that is, alteration of vesicle maturation or alteration of release. It also makes a difference whether differentially filled vesicles are detected and differentially processed within the terminal or whether vesicle filling acts back onto the terminal by presynaptic autoreceptors. Therefore, the model allows one to decide, at a given synapse, how transmitter metabolism is linked to presynaptic function and efficacy.

INTRODUCTION

Chemical synapses are the key structures for plastic adaptations of the central nervous system (CNS) and hence for learning and memory. Synapses are complex computational devices that respond to different temporal patterns of activity with a variety of short- and long-term changes involving both transmitter release and postsynaptic responsiveness. Several lines of evidence indicate that—besides these traditional mechanisms—the concentration of transmitter in the presynaptic bouton can be varied in a functionally relevant manner (for a review, see Sulzer and Pothos 2000). In fact, the transmitter content of aminergic synapses is an important target of neuro- or psychoactive drugs, most prominently in the treatment of Parkinson's disease with L-DOPA. Similarly, drugs that increase the concentration of the inhibitory transmitter GABA (γ-aminobutyric acid) at central inhibitory synapses yield anticonvulsant effects (Engel et al. 2000; Gram et al. 1988; Löscher et al. 1989; Taylor et al. 1992). Although these examples are based on therapeutic interventions, several recent studies indicate that changes in transmitter metabolism represent a genuine plasticity mechanism in the CNS. Synaptic inhibition seems to be regulated by changes in transmitter metabolism in a way supporting homeostasis of overall network activity: after epileptic seizures, inhibitory interneurons in the rat hippocampus increase the expression of glutamate decarboxylase (GAD), the key enzyme for the synthesis of GABA (Esclapez and Houser 1999; Feldblum et al. 1990). Conversely, GABA production is downregulated after deafferentation of cortical areas (Garraghty et al. 1991; Gierdalski et al. 1999; Hendry and Carder 1992). A direct role for GABA metabolism in synaptic plasticity is indicated by genetically modified mice that are devoid of GAD65, the most strongly regulated isoform of the GABA-producing enzyme. These animals show specific changes in the age-dependent forms of plasticity of ocular dominance columns in the visual cortex (Fagiolini and Hensch 2000; Hensch et al. 1998).

At the microphysiological level, experimental alterations of transmitter content have caused changes in quantal size and—more surprisingly—also in release rates at many different synapses. Incubation of midbrain dopaminergic neurons with the dopamine precursor L-dihydroxyphenylalanine (L-DOPA) increases the number of released dopamine molecules per vesicle (Pothos et al. 1998a). Conversely, suppression of the dopamine-synthetizing molecule tyrosine hydroxylase by activation of D_2-autoreceptors reduces quantal size (Pothos et al. 1998b). At the same time, the frequency of quantal release was lowered and this effect could be reversed by application of L-DOPA, indicating that the filling state of vesicles is paralleled by changes in the readily releasable pool or in release probability. At the *Xenopus* neuromuscular junction, overexpression of the vesicular transporter for acetylcholine increases the quantal size as well as the frequency of miniature postsynaptic events, again pointing toward a relationship between variations in vesicle filling and vesicle dynamics (Song et al. 1997). Several acute biochemical manipulations of acetylcholine content or loading at frog neuromuscular junctions result in altered size of postsynaptic quantal events (Van der Kloot et al. 2000, 2002). However, these manipulations seem to affect

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neither the size of the readily releasable pool nor the size of individual vesicles. At GABAergic synapses, quantal size can be increased by blocking GABA degradation (Engel et al. 2001) or can be decreased by suppressing GABA synthesis (Golan and Grossman 1996; Murphy et al. 1998). Again, reduced GABA synthesis results in a decreased miniature inhibitory postsynaptic current (mIPSC) frequency (Murphy et al. 1998) or probability of release (Golan and Grossman 1996), whereas elevated presynaptic GABA levels are paralleled by an increased frequency of mIPSCs (Engel et al. 2001). The latter result contrasts, however, with recent observations by Overstreet and Westbrook (2001), who found a decrease in quantal size and in mIPSC frequency upon acute incubation of hippocampal slices with vigabatrin, an inhibitor of the GABA-degrading enzyme GABA-transaminase. Complex changes in synaptic efficacy have been observed after genetic ablation of the GABA-synthetizing enzyme GAD65: although basal synaptic function appears to be unchanged, sustained massive activation of presynaptic terminals results in a diminished GABA release, again indicating a relation between presynaptic transmitter concentration and supply of vesicles (Tian et al. 1999). Thus functional changes upon altered transmitter metabolism are diverse and may be confounded by additional effects of the experimental manipulations [e.g., increased tonic inhibition through nonvesicular release of GABA (Overstreet and Westbrook 2001; Wu et al. 2003; Yee et al. 1998)]. Nevertheless, multiple experimental findings indicate that vesicle filling and presynaptic vesicle dynamics can both be altered by changes in transmitter metabolism.

If transmitter metabolism is a genuine mechanism of synaptic plasticity, as we propose, there must be functional links between cytosolic transmitter content, vesicular loading, and the transition of vesicles between the different presynaptic compartments, including release. We have constructed a reduced compartmental model of the presynaptic terminal (Südhof 1995) and have analyzed the possibilities and constraints for such links. Using parsimonious assumptions, we find that changing the presynaptic cytosolic transmitter content will indeed profoundly alter the filling state of vesicles. The vesicle cycle could, in principle, be influenced by transmitter content at different stages and by different mechanisms. Our simulations show that different stages and mechanisms of links between transmitter metabolism and vesicle dynamics have unique, experimentally testable functional consequences. Such effects include states of sustained enhanced vesicular transmitter release upon elevation of presynaptic transmitter concentration, as experimentally observed.

METHODS

Presynaptic vesicle cycle

We describe the distribution of vesicles in the presynaptic terminal with respect to their different transmitter content ν and, in the dynamic case, with respect to time. Presynaptic vesicles undergo a complex cycle between release, recovery, and maturation (Südhof 1995) that for the present purpose has been reduced to transitions between 3 functionally distinct vesicle pools: *1*) the reserve pool (n, *Eqs. a*), *2*) the readily releasable pool (n_{RRP}, *Eqs. b*), and *3*) the pool of empty, fused vesicles (n_f, *Eqs. c*). The faster "kiss-and-run" pathway will be modeled as a case with infinitely small reserve pool [see *Effects of transmitter concentration on directly recycling vesicles (shortcut pathway)*]. In the full model, the vesicle cycle is given by transition from the reserve pool into the RRP (rate β), subsequent synaptic release (rate r), and, finally, recovery of fused vesicles into the reserve pool (rate γ; see Fig. 1 for illustration). Transitions between pools are described by the following set of ordinary differential equations

$$\frac{\mathrm{d}}{\mathrm{d}t} n(t) = \gamma n_f(t) - \beta n(t) \tag{1a}$$

$$\frac{\mathrm{d}}{\mathrm{d}t} n_{\mathrm{RRP}}(t) = \beta n(t) - r n_{\mathrm{RRP}}(t) \tag{1b}$$

$$\frac{\mathrm{d}}{\mathrm{d}t} n_f(t) = r n_{\mathrm{RRP}}(t) - \gamma n_f(t) \tag{1c}$$

with the normalization $n(t) + n_{\mathrm{RRP}}(t) + n_f(t) = \text{constant}$, assuming that the total amount of vesicles stays constant. In the steady state, the mean number of vesicles in each of the 3 compartments can easily be solved analytically giving

$$\gamma \bar{n}_f = \beta \bar{n} \tag{2a}$$

$$\beta \bar{n} = r \bar{n}_{\mathrm{RRP}} \tag{2b}$$

$$r \bar{n}_{\mathrm{RRP}} = \gamma \bar{n}_f \tag{2c}$$

or

$$\bar{n} = [1 + \beta(\gamma^{-1} + r^{-1})]^{-1} \tag{3a}$$

$$\bar{n}_{\mathrm{RRP}} = [1 + r(\gamma^{-1} + \beta^{-1})]^{-1} \tag{3b}$$

$$\bar{n}_f = [1 + \gamma(\beta^{-1} + r^{-1})]^{-1} \tag{3c}$$

In these equations, it is assumed that the total number of vesicles equals 1 (i.e., $\bar{n}_a$ denotes the fraction of vesicles in each pool).

In the initial part of the RESULTS section, we model the filling of vesicles and their resulting distribution with respect to transmitter content. For the sake of clarity, in this section the reserve pool and the readily releasable pool were collapsed into a single pool of vesicles that reduced the mathematical model to only 2 differential equations.

Filling of vesicles

Neurotransmitters are transported into synaptic vesicles in exchange with protons that are previously accumulated by H^+-ATPases (Masson et al. 1999). We assume that this process saturates with

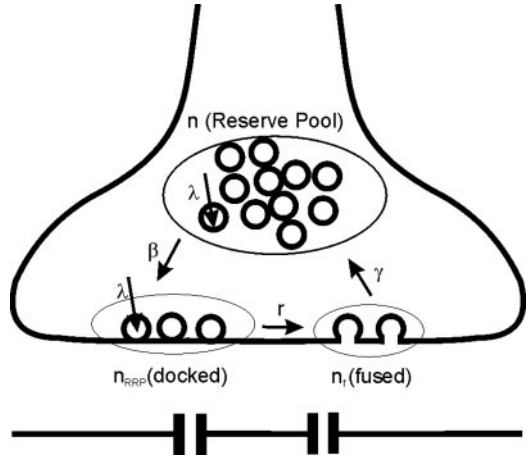

FIG. 1. Simplified model of the synaptic vesicle cycle. Synaptic vesicles are contained in 3 different compartments (n, reserve vesicles; n_{RRP}, readily releasable vesicles; n_f, fused, empty vesicles). Vesicles in the reserve pool and in the RRP are being filled with a rate λ. Transitions between the 3 pools are described by rate constants γ, β, and r, respectively. For details see main text.

increasing vesicular transmitter concentration. This assumption is justified by 2 main reasons. First, transport energy increases with an increasing concentration gradient between axonal cytoplasm and vesicle interior. Thus the accumulation of transmitter molecules in vesicles is a self-limiting process. Second, nonsaturating vesicle loading should result in bigger quanta at low release rates and smaller quanta at high release rates. Such a release-dependent reduction of quantal size indeed occurs after massive repetitive stimulation of frog motor endplates (Naves and Van der Kloot 2001) and may contribute to the frequency-dependent fading of inhibitory and excitatory postsynaptic currents at central synapses (Galarreta and Hestrin 1998). At low to moderate release rates, however, many central synapses show quantal sizes for evoked or spontaneous (miniature) release that are independent from the frequency of release (e.g., Edwards et al. 1990; Kraszewski and Grantyn 1992; Ropert et al. 1990; Sahara and Takahashi 2001; Van der Kloot 1996) or that even increase upon increasing frequency (Behrends and ten Bruggencate 1998). Thus vesicular loading seems to be saturating and can limit vesicular transmitter content only at very fast release rates (see Fig. 2).

Subsequently, we model saturating vesicle loading in the most parsimonious way (i.e., as a bidirectional flux of transmitter molecules that depends on transmitter concentration on either side). With flux rates into (λ^+) and out of (λ^-) the vesicles, a cytosolic transmitter concentration c, and resulting vesicular transmitter concentration ν we get a net flux λ of

$$\lambda(c, \nu) = \lambda^+ c - \lambda^- \nu \tag{4}$$

for which filling saturates at $\nu_{max} = (\lambda^+/\lambda^-)c$.

In this equation, loading depends on the cytosolic transmitter concentration and leakage depends on intravesicular transmitter concentration (see Fig. 2*A*), which does not alter our qualitative results. Vesicles are still being filled until an equilibrium of influx and efflux is reached. Experimental observations show that the presynaptic cytosolic transmitter concentration can affect quantal size (Engel et al. 2001; Murphy et al. 1998; Pothos et al. 1998a). Our model reproduces this effect: an increase in c will increase the resulting amount of transmitter ν in the vesicle until equilibrium is reached (see RESULTS and Fig. 4). This qualitative result persists under the alternative assumption that leakage is independent of ν (Wang and Floor 1994).

Combining cycling and filling

To reproduce the experimentally observed dependency of synaptic function on transmitter metabolism, we then introduce the vesicular transmitter concentration ν as an additional variable into the description of vesicle distribution between the 3 pools. The total number of vesicles in the reserve pool and in the RRP, respectively, is now given by the integral of their distribution with respect to ν; that is: $n(t) = \int_0^\infty n(\nu, t)\rho(\nu)\mathrm{d}\nu$ and $n_{RRP}(t) = \int_0^\infty n_{RRP}(\nu, t)\rho(\nu)\mathrm{d}\nu$, where $\rho(\nu)$ is the integral of ν needed here to get the dimensions right (the pool of empty, fused vesicles n_f is independent from ν). Our model should account for experimental data, which suggest that presynaptic transmitter content affects vesicle dynamics. Therefore we will assume that the transition rates between different pools can, in principle, depend on ν [i.e., $\beta(\nu)$, $r(\nu)$]. In principle, the rates could be modeled to depend on cytosolic transmitter concentration c rather than on ν. In this case, however, increases in cytosolic transmitter concentration would exert effects on incompletely filled vesicles and therefore mean quantal size would be reduced. Below, we will systematically examine how the dependency of rate constants on vesicular transmitter content influences the distribution of vesicles between the 3 compartments. In general terms, the presynaptic vesicle dynamics is now determined by the following set of partial differential equations [the recovery of empty vesicles (γ) can, of course, not depend on the filling state]

$$\frac{\partial n(\nu, t)}{\partial t} = -\frac{\partial}{\partial \nu}[\lambda(\nu, c)n(\nu, t)] - \beta(\nu)n(\nu, t) \tag{5a}$$

$$\frac{\partial n_{RRP}(\nu, t)}{\partial t} = \beta(\nu)n(\nu, t) - \frac{\partial}{\partial \nu}[\lambda(\nu, c)n_{RRP}(\nu, t)] - r(\nu)n_{RRP}(\nu, t) \tag{5b}$$

$$\frac{\partial n_f(t)}{\partial t} = \int_0^\infty r(\nu)n_{RRP}(\nu, t)\rho(\nu)\mathrm{d}\nu - \gamma n_f(t) \tag{5c}$$

In this system, the supply of the reserve pool with vesicles from the pool of fused vesicles $n_f(t)$ is given by the boundary equilibrium condition

$$\frac{\partial}{\partial \nu}[\lambda(\nu)n(\nu, t)]_{\nu=0} = \gamma n_f(t) \tag{6}$$

To specify the dependency of transition rates on vesicular transmitter concentration, we introduce the following equations for transition into the RRP (β) and release (r)

$$\beta(\nu) = \beta_0[1 - \exp(-\nu/\nu_0)] \tag{7}$$

and

$$r(\nu) = r_0[1 - \exp(-\nu/\nu_0)] \quad \text{with } \nu_0 = 1 \text{ mM} \tag{8}$$

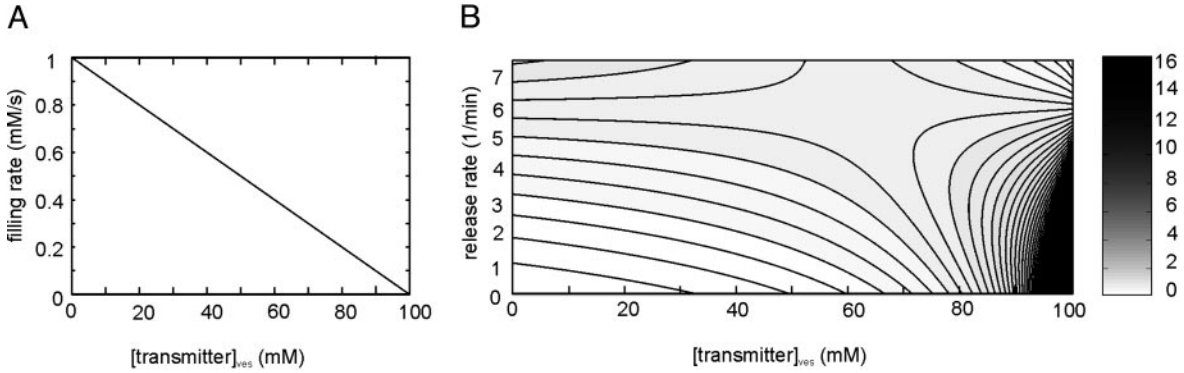

FIG. 2. Filling of vesicles and equilibrium distribution of differentially filled vesicles for different release rates. *A*: effective filling rate (λ) is assumed to decrease linearly with increasing vesicular transmitter concentration, reaching equilibrium ($\lambda = 0$) at 100 mM. *B*: distribution of vesicles with different intravesicular transmitter concentrations (gray values coding the number of vesicles as indicated in the scale bar). Note that most vesicles are (almost) maximally filled. Distribution increases monotoneously with transmitter concentration. At higher release rates, the distribution broadens toward lower filling states. If the release rate increases further, the distribution flattens before it becomes monotonously decreasing, indicating that for most vesicles the cycling time is insufficient for a complete filling.

These relationships establish a monotonous function, starting at minimal rates for 0 transmitter concentration and saturating for highly filled vesicles. It should be noted, however, that the equations are purely illustrative because we do not know of any experimental results supporting either this or an alternative function.

Biological interpretation of variables and choice of parameters

We have modeled spontaneous release as a random process with a low probability in each time step. Fused vesicles (n_f) are recycled with a constant rate γ. The number of recycled empty vesicles sets the boundary condition $n(0, t)$ for the vesicular filling process in the reserve pool $n(\nu, t)$.

The system of *Eqs. 1a–c* contains 3 variables (the number of vesicles in each compartment n, n_{RRP}, and n_f) and 3 parameters (the transition rates β, r, and γ). The numerical values of parameters will differ between different types of synapses and situations but it should be noted that this will not influence the qualitative results (i.e., changes in synaptic function after changes in presynaptic transmitter concentration). As a typical case, we will consider 10 vesicles in the release-ready pool (Borges et al. 1995; Kirischuk and Grantyn 2000; Kraushaar and Jonas 2000; Murthy and Stevens 1999; Stevens and Tsujimoto 1995). The number of fused vesicles $\bar{n}_f$ will also be set to 10 and the vesicle content of the reserve pool $\bar{n}$ is set to 80, based on data by Liu and Tsien (1995). Under steady-state conditions, the influx into each compartment equals the efflux into the next compartment. The spontaneous (action potential-independent) release rate of individual vesicles (r) in hippocampal slices is unknown (but see Murthy and Stevens 1999 for cultured hippocampal cells). We will assume a value of $r = 0.01$/s, translating into one vesicle per 10 s with 10 vesicles in the readily releasable pool. This is is below the rate of synaptic depression at inhibitory synapses (Galarreta and Hestrin 1998) and yields realistic values for the frequency of miniature postsynaptic currents (e.g., 5/s for 50 presynaptic terminals). With these assumptions, the parameters for the steady state in *Eqs. 3* are: $\beta = 1.25 \times 10^{-3}$/s; $r = 1 \times 10^{-2}$/s; $\gamma = 1 \times 10^{-2}$/s. Different assumptions for the absolute numbers of these parameters will not alter the qualitative conclusions in the RESULTS section (e.g., distribution of vesicles with respect to their transmitter content).

There is increasing evidence that vesicular recycling does not only occur on the classical timescale of 10s of seconds, but also on a faster timescale ("kiss-and-run"; Aravanis et al. 2003; Gandhi and Stevens 2003; Murthy and Stevens 1998; Sara et al. 2002; Stevens and Williams 2000; Valtorta et al. 2001). Fast cycling vesicles do not go through a reserve pool but seem to enter directly into a release-ready state after recovery from fusion. In the framework of our model, this faster pathway may have 2 important consequences: *1*) cycling might become faster than the filling of vesicles, leading to the release of incompletely filled vesicles (Naves and Van der Kloot 2001; see Figs. 2*B* and 3*B* for illustration within our 3-compartment model); *2*) the RRP cannot be refilled from a large reserve pool after losing vesicles (i.e., our presynaptic model is effectively reduced to 2 compartments). Within the present model, this direct pathway can be considered a limiting case for increased values of β. Under these conditions, the number of vesicles in the reserve pool approaches zero and β loses its rate-limiting function. The consequences of such an increase in β are analyzed in detail below [see *Effects of transmitter concentration on directly recycling vesicles (shortcut pathway)*] and in Fig. 6. Alternatively, we also simulated the direct pathway in a 2-compartment model (containing only RRP and fused vesicles), which yielded similar results (data not shown).

Vesicular filling was modeled as a bidirectional flux of transmitter, with influx depending on cytosolic transmitter concentration c and efflux depending on vesicular transmitter concentration ν. Experimental data on cytosolic transmitter concentrations in the presynaptic terminal are surprisingly scarce; recent evidence, however, suggests that it is in the order of 1–10 mmol (Ishikawa et al. 2002; Yamashita et al. 2003). Therefore, we will assume transmitter concentrations in the order of magnitude of the vesicular transporter affinity constant K_m (~5 mmol for GABA; see Kish et al. 1989; McIntire et al. 1997). The cytosolic transmitter concentration c cannot be chosen too low as compared to K_m because net transport of transmitter into vesicles must be fast enough to guarantee filling within the presynaptic cycling time of vesicles (~20 s). Otherwise, vesicular transmitter concentration would strongly depend on release rate. As mentioned above, this seems to be the case only during very high cycling rates (Naves and Van der Kloot 2001). On the other hand, if c was much higher than K_m, vesicular transmitter transporters would be permanently saturated and changes in cytosolic transmitter concentration would not translate into changes in vesicular transmitter content, in contrast to experimental observations (Engel et al. 2001; Pothos et al. 1998a).

What is the maximal vesicular transmitter concentration that can be reached? Experimental and modeling studies have suggested that vesicular transmitter concentration can reach values of at least 100 mmol (Burger et al. 1989; Busch and Sakmann 1990). It is generally assumed that vesicular transmitter transporters do not build up very steep gradients between the inner and outer vesicular compartment

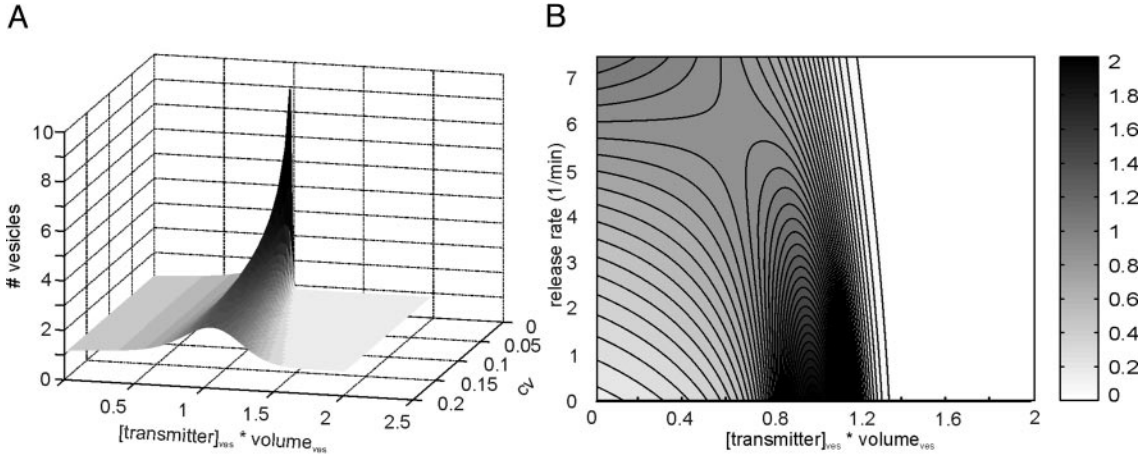

FIG. 3. Vesicular transmitter content assuming variation in vesicle volume. *A*: number of vesicles with different transmitter content ([transmitter]$_{ves}$ × volume$_{ves}$) for different coefficients of variation (CV) in the distribution of vesicular diameters. Note that the distribution broadens with increasing CV. *B*: distribution of differentially filled vesicles for different release rates (CV = 0.12). With the assumed variability of vesicular volume, the resulting distribution is not monotonous and depends only weakly on release rate (as long as the release rate is low or modest). Scale bar indicates number of vesicles.

(see, e.g., Fonnum et al. 1998), consistent with recent data from the calyx of Held, which suggest a cytosolic transmitter concentration around 1 mM (Yamashita et al. 2003). We tested various sets of parameters for vesicle loading until vesicular filling was saturating within about 20 s, and filling was dependent on c. Parameters used are: $\lambda^+ = 10$/s, $\lambda^- = 0.1$/s, c from 1 to 10 mM, resulting in ν_{max} ranging from 100 to 1000 mM. Using *Eq. 4*, these assumptions yield a mean value of $\lambda = 5$ mmol/s for $c = 1$ mmol.

The system of partial differential equations (*Eqs. 5*) was implemented by Monte Carlo simulations using Matlab (The Mathworks, Natick, MA) and was executed on Intel Pentium II–powered computers running under the Linux operating system.

RESULTS

To gain insight into the functional effects of altered presynaptic transmitter concentration, we first model vesicular filling and the resulting distribution of vesicles for different presynaptic transmitter concentrations. We then examine the influence of time on vesicle filling by looking at different release rates. Finally, we assume that one of the transition rates for vesicles inside the terminal depends on vesicular transmitter content [$\beta(\nu)$, or $r(\nu)$, respectively]. Under these conditions, the filling state of released vesicles depends on the cytosolic transmitter supply. We first compute the steady-state situation and afterward dynamic changes in presynaptic transmitter content. Together, the results show how synaptic efficacy changes with presynaptic transmitter metabolism.

Distribution of vesicular transmitter content

As a first step, we model a single, homogeneous population of vesicles

$$\frac{\partial n(\nu, t)}{\partial t} = -\frac{\partial}{\partial \nu}[\lambda(\nu) n(\nu, t)] - r n(\nu, t)$$

that is loaded according to *Eq. 4*: $\lambda(c, \nu) = \lambda^+ c - \lambda^- \nu$ (see Fig. 2*A*). For the steady-state situation, this equation can be solved analytically and results in a distribution of vesicles that peaks at ν_{max}, such that most vesicles are almost maximally full (Fig. 2*B*). The depicted number of vesicles in each filling state is proportional to the probability of being in this filling state. (Note that even if no vesicles are released, i.e., in the situation of $r = 0$, several vesicles are incompletely filled, reflecting the equilibrium of influx and efflux.) Increasing the release rate up to 5/min (corresponding to a cycling time of 12 s) results in reduced transmitter content of released vesicles but leaves the distribution qualitatively unchanged. At central synapses the shortest possible cycling time of single vesicles has been reported to be about 15 s (Klingauf et al. 1998; Liu and Tsien 1995; Ryan and Smith 1995; Ryan et al. 1993) except in special situations that are likely to involve kiss-and-run release (Aravanis et al. 2003; Burgoyne et al. 2001; Gandhi and Stevens 2003; Graham et al. 2002; Machado et al. 2000, 2001; Palfrey and Artalejo 1998; Sara et al. 2002). Thus for normal release processes vesicular transmitter content is high and relatively stable. If the release rate increases further, however, it reaches the filling time of vesicles that has been set to 6 s; at this point, the distribution flattens (Fig. 2*B*). An even higher release rate results in a monotonically decreasing distribution of vesicles because an increasing number of vesicles is incompletely filled (cf. Naves and Van der Kloot 2001).

For modest release rates, the distribution of presynaptic vesicles in Fig. 2*B* shows a very sharp peak at maximal values of ν (Fig. 2*B*). Miniature postsynaptic currents, however, usually show a skewed distribution with a large coefficient of variance of up to about 0.5 (e.g., Frerking et al. 1995; Sahara and Takahashi 2001). There is good indication that this variance is at least partially attributed to the release of differentially filled vesicles (Frerking et al. 1995), although different postsynaptic receptor numbers at different synaptic sites may contribute to the variance (Nusser et al. 1997). Recent evidence suggests that at some synapses variance of vesicular volume V (rather than of vesicular transmitter concentration) may underlie the variable transmitter content (Bruns et al. 2000; Colliver et al. 2000), although such a correlation has not been found at the neuromuscular junction (van der Kloot et al. 2002).

In our model, we introduced variance by convoluting the distribution of concentrations (Fig. 2*B*) with the distribution of vesicular volumes in presynaptic endings. The latter was based on analyses of vesicle diameters in cerebellar and hippocampal neurons (Bekkers et al. 1990; Palay and Chan-Palay 1974), yielding a coefficient of variance of about 0.12. This Gaussian distribution of diameters was transformed into the 3rd-order Gaussian describing vesicular volume (Frerking et al. 1995). From hereon, the distribution of vesicles in the readily releasable pool (RRP) and in the reserve pool will be plotted as $n(\nu \times V)$ (i.e., with respect to transmitter content), rather than concentration. Figure 3*A* illustrates the influence of vesicular size variance on the distribution of differentially filled vesicles for a case of low release rate ($r = 1$/min). Obviously, at zero variance the distribution peaks at maximal transmitter content (yielding a distribution similar to Fig. 2*B* for $r = 1$/min). If such a distribution of vesicular transmitter content would underlie the experimentally observed amplitude distribution of miniature postsynaptic currents, variance would almost exclusively be attributed to postsynaptic factors, contrary to experimental evidence (e.g., Frerking et al. 1995; Sahara and Takahashi 2001). At higher values of CV the distribution becomes smoother, consistent with a role for differentially filled vesicles. Finally, we modeled the distribution of vesicles $n(\nu \times V)$ at different release rates (diameter variance was set to 0.12 for this simulation; Fig. 3*B*). For modest release rates, this distribution is less sensitive to release rate than the data shown in Fig. 2*B*, and it yields a clear peak for highly, but not maximally, filled vesicles. Consequently, postsynaptic current amplitudes are largely independent of release rate within some range, consistent with experiments (Edwards et al. 1990; Kraszewski and Grantyn 1992; Ropert et al. 1990; Sahara and Takahashi 2001; Van der Kloot 1996). Only at high sustained release rates, filling becomes incomplete (Naves and Van der Kloot 2001; see Fig. 3*B* for release rates above ~5/s).

Dependency of release rate r on transmitter content

There is experimental evidence that an increase in presynaptic transmitter concentration can increase the frequency or probability of vesicle release (Engel et al. 2001; Golan and Grossman 1996; Murphy et al. 1998; Pothos et al. 1998b; Song et al. 1997). To establish mechanisms for these observations within our model, we will now consider transition rates that depend on transmitter concentration. In contrast to the previous section, we will now use the full presynaptic model as intro-

duced in *Eqs. 5*; that is, vesicles are distributed between a reserve pool (80% of vesicles in equilibrium), RRP (10% of vesicles), and fused vesicles waiting for recovery from the presynaptic membrane (10% of vesicles; numbers chosen to illustrate a typical case; see METHODS). Our model allows for concentration-dependent modulation of release rate r as well as of the transition rate β (flow into the readily releasable pool). We start with the case where the release rate r depends on vesicular transmitter concentration: $r = r(\nu) = r_0[1 - \exp(-\nu/\nu_0)]$. Afterward we will consider the alternative scenario where the supply of vesicles from the reserve pool to the RRP depends on ν [i.e., $\beta = \beta(\nu)$].

For $r(\nu) = r_0[1 - \exp(-\nu/\nu_0)]$, the distribution of vesicles in the RRP becomes smoother and is shifted toward larger values when the cytosolic transmitter concentration is raised (Fig. 4*B*). Because in this scenario vesicles with higher transmitter content are released at higher rates than those with low transmitter content, the distribution of released vesicles maintains a relatively sharp peak at high transmitter content (Fig. 4*C*). Whereas Fig. 4 focuses on distributions of vesicles with respect to transmitter content, Fig. 5 shows the number of vesicles in the RRP and the number of vesicles per time undergoing exocytosis. An increase in cytosolic transmitter concentration c results in a drastic reduction of the number of vesicles in the RRP (Fig. 5*A*). The number of released vesicles per time remains relatively constant, however, because the reduced number of releasable vesicles is compensated by the increased release rate of these (fuller) vesicles (Fig. 5*B*). Thus in a model with distinct pools, a filling-dependent release rate cannot reproduce a strong influence of cytosolic transmitter concentration on the frequency of vesicular release.

The results depicted in Fig. 5 can be derived from numerical simulations of the system of differential *Eqs. 5a–c*, but can also be approximated with analytical methods for the equilibrium situation (see continuous lines in Fig. 5): in the steady state, the flux from each compartment into the next one is equal. Given that most of the vesicles are in the reserve pool, the rate β for the transition of vesicles from the reserve pool into the RRP is much smaller than r and γ. Therefore the total flux of vesicles in our scenario depends mainly on the slowest transition rate β. The number of vesicles in each pool, on the other hand, changes reciprocally with changes in the transition rates out of the respective pool. For example, an x-fold increase in the release rate r will lead to an x-fold decrease in the

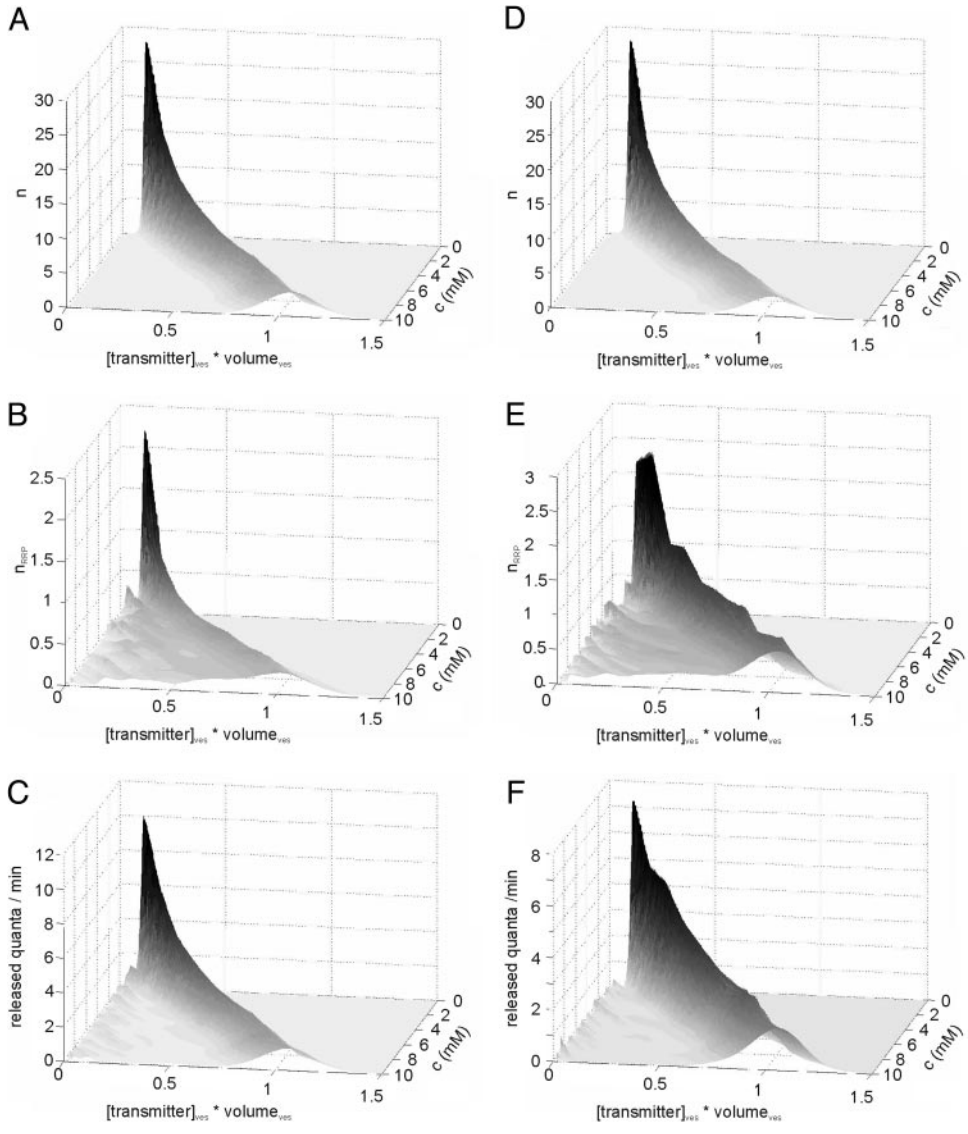

FIG. 4. Distribution of vesicles in different pools for transition rates that depend on vesicular filling state. *Left row:* release rate r increases with transmitter concentration ν as in *Eq. 8*. *Right row:* supply to the readily releasable pool β increases with ν as in *Eq. 7*. *A*, *B*, and *C* show the resulting distribution of transmitter content for the reserve pool (*A*), for the RRP (*B*), and for released vesicles (*C*). *Right panels:* respective distributions for the reserve pool (*D*), RRP (*E*), and released vesicles (*F*) for $\beta = \beta(\nu)$. Note the larger number of vesicles in the RRP at high values of c when supply depends on transmitter content as compared to $r = r(\nu)$ for a presynaptic transmitter concentration $c = 10$ mM. Total amount of released vesicles is about 9/min in *C* and about 15/min in *F* if the presynaptic transmitter concentration c is increased to $c = 10$ mM.

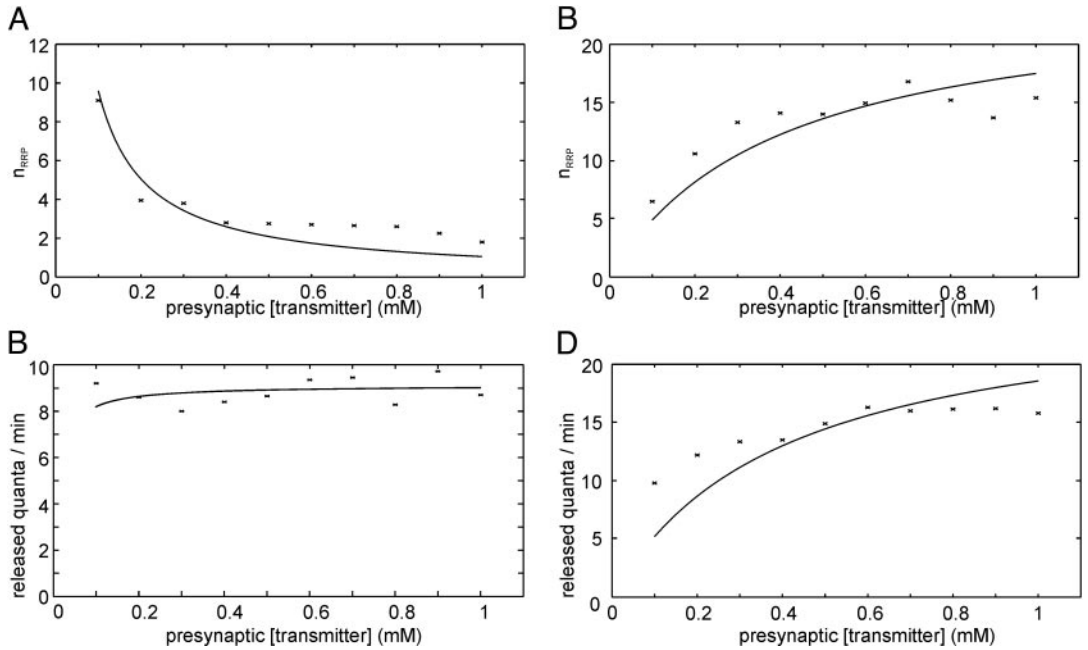

FIG. 5. Total number of vesicles in the readily releasable pool and number of released vesicles depend on presynaptic transmitter concentration. *A*, *B*: results for $r = r(\nu)$ as in *Eq. 8*, *C*, *D*: results for $\beta = \beta(\nu)$ as in *Eq. 7*. Note that for concentration-dependent release (*A*, *B*) the number of vesicles in the RRP declines with increasing concentration, whereas the number of released vesicles stays almost constant. If, however, the supply to the RRP depends on transmitter concentration, both the number of vesicles in the RRP (*C*) and the number of released vesicles (*D*) increase with *c*. Lines represent analytic solutions of *Eq. 2a–c*; dots show numerical results of *Eqs. 5a–c*.

number of vesicles in the RRP. The analytic approximations match the numerical simulations quite well. The small mismatches between both approaches reside in the fact that the analytical solution uses direct changes in rates, whereas the numerical simulations are based on alterations in *c*, which are first translated into vesicular transmitter content and subsequently processed by *Eqs. 5*.

Dependency of vesicle supply β on transmitter content

We will now consider the alternative scenario $\beta = \beta(\nu)$, where the transmitter content of a vesicle determines the rate of transition from the reserve pool into the RRP. In a broad sense, this scenario can be understood as "vesicle maturation": filling is a precondition for efficient translocation into the RRP. The distribution of vesicles in the reserve pool and in the RRP are shown in Fig. 4, *D* and *E*, respectively. Increasing cytosolic transmitter concentration results in a broadening of the distribution of differentially filled vesicles in the RRP. In contrast to Fig. 4*B* [$r = r(\nu)$], the total number of vesicles in the RRP increases with higher values of *c*. The distribution of released vesicles is largely similar to the distribution resulting from $r(\nu)$ but is slightly broader and reaches a larger integral when cytosolic transmitter concentration is increased (Fig. 4*E*). Figure 5*B* shows how many vesicles reside in the RRP and Fig. 5*D* illustrates how many vesicles are being released per unit time when the supply of vesicles from the reserve pool depends on their filling state: the size of the RRP will now increase with increasing cytosolic transmitter concentration and the release rate also sharply increases, in parallel to the size of the RRP. Similar to the numerical data, the analytical approximation yields a drastic increase in release number/time with increasing *c*, as indicated by the continuous line superimposed on the numerical data in Fig. 5*D*.

The above simulations show that changes in vesicular filling state as well as changes in vesicle dynamics can be caused by changes in presynaptic transmitter concentration. We found that effects of *c* on the frequency of vesicular release can be best explained if the supply of vesicles into the RRP depends on vesicular filling, rather than the release rate itself (see also Brager et al. 2002).

Effects of transmitter concentration on directly recycling vesicles (shortcut pathway)

As mentioned in METHODS, there is increasing evidence that vesicles are recycled not only on the classical path involving the reserve pool, but also directly ("kiss-and-run"). We introduced such an alternative pathway into our model by increasing the rate β_0, thereby diminishing the size of the reserve pool. As β_0 approaches infinity, the reserve pool is effectively eliminated. The lifetime of an individual vesicle on this shortcut pathway may be as short as 1 s (Gandhi and Stevens 2003). Although the transmitter content of fast cycling vesicles has not directly been measured, it is possible that filling equilibrium cannot be reached in such vesicles (Naves and Van der Kloot 2001). In our model, this situation corresponds to very fast release rates in Figs. 2*B* and 3*B*, where the sharp peak in vesicle distribution broadens. Figure 6*A* shows the number of vesicles in each compartment as a function of increasing values of β_0 according to *Eqs. 3*. The size of the reserve pool is reciprocally proportional to the velocity of "maturation" and the pool vanishes at high values of β_0. Conversely, the pools of releasable and of fused vesicles, respectively, increase. What happens now in this system if the presynaptic transmitter

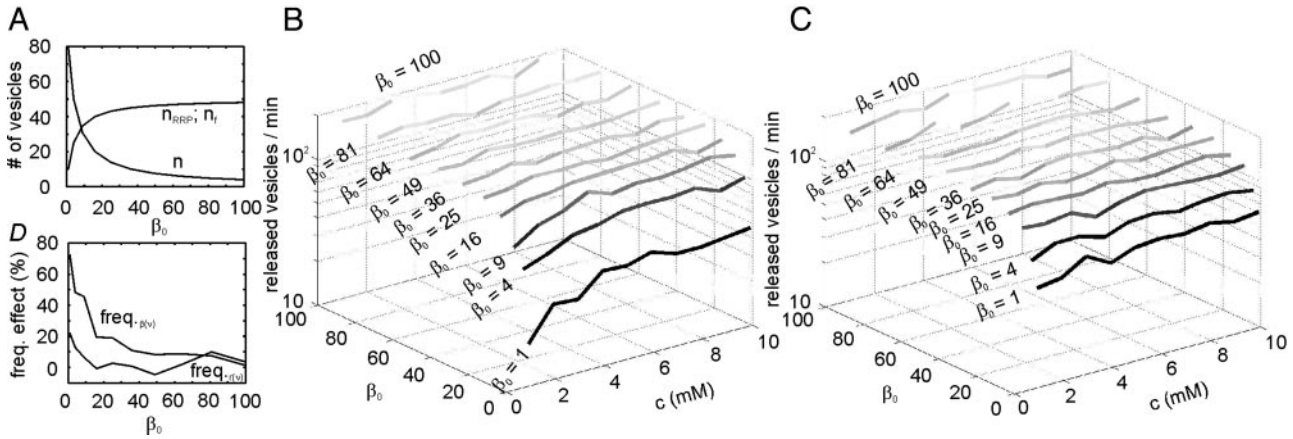

FIG. 6. Dependency of the frequency of vesicle release on presynaptic transmitter concentration decreases in a fast vesicle cycle. *A*: dependency of the number of vesicles in the reserve pool (n), the RRP (n_{RRP}), and of fused vesicles (n_f) on an increased vesicle supply into the RRP (increase in β_0). *B*: scenario where the vesicle supply into the RRP depends on the vesicular transmitter concentration, $\beta = \beta(\nu)$. Only if β is slow ($\beta_0 = 1$), the number of released quanta depends on the presynaptic transmitter concentration, c. *C*: scenario where the release rate r depends on the vesicular transmitter concentration, $r = r(\nu)$. There is no dependency of the number of released quanta on the presynaptic transmitter concentration. *D*: overview of the "frequency effect" (increase in the number of released quanta upon a 10-fold increase of c) as a function of β_0 for both $\beta(\nu)$ and $r(\nu)$.

concentration c is increased? Again, we must distinguish between effects of c on vesicle maturation β (Fig. 6*B*) and effects on release rate r (Fig. 3*C*). When β_0 is increased, the steep correspondence between transmitter concentration and release (see Fig. 5*D* and case $\beta_0 = 1$ in Fig. 6*B*) is lost, and the number of released vesicles per time becomes largely independent from c. This is also illustrated in Fig. 6*D*. At low values of β ($\beta_0 = 1$), vesicular release is strongly increased after a 10-fold increase in c. Increasing β_0 reduces the rate-limiting role of β and thereby abolishes any effects of transmitter concentration on the frequency of vesicle release. At high values of β_0, one might assume that effects of c on the release rate r [implemented as $r(\nu)$; see above] become more pronounced. However, Fig. 6*C* shows that the weak effect of c on release is lost when β_0 is increased. This is caused by a reciprocal compensation of 2 effects: at increased values of c, vesicles are being filled more rapidly and are released with higher probability, if r increases with ν. On the other hand, this will reduce the number of vesicles available in the RRP. Therefore, the product rn_{RRP} is roughly constant.

In summary, the simulations within our parameter regime show that any effects of transmitter concentration on presynaptic vesicular dynamics requires the existence of a reserve pool. Simulations within a 2-compartment model (consisting of only the RRP and a pool of fused vesicles) yielded equivalent results, that is, that the synaptic release is always independent of transmitter concentration in the absence of the reserve pool.

Dynamic alterations of vesicle cycling

To further demonstrate the differences between alterations of vesicular release rate $r(\nu)$ and vesicle recruitment $\beta(\nu)$, we subsequently computed dynamic changes of vesicular release for a stepwise increase in c. Although such a sudden increase in cytosolic transmitter content will not happen in natural neurons, the data can still be interpreted in a biologically realistic manner. For the scenario with $r = r(\nu)$, a stepwise increase in c corresponds to a stepwise change in the rate of release (e.g., by a high-frequency stimulus train). This experimental paradigm is being used by many authors to induce processes of synaptic plasticity (for a review, see Zucker and Regehr 2002) or to probe the size of the RRP (e.g., Kirischuk and Grantyn 2000; Rosenmund and Stevens 1996). For the alternative scenario [$\beta = \beta(\nu)$], the change in c translates into a situation of increased flow of vesicles into the RRP. Experimental data suggest that the supply of vesicles can indeed be varied by different mechanisms, including increased presynaptic Ca^{2+} influx and activation of protein kinase C (PKC) (Gillis et al. 1996; Smith et al. 1998; Stevens and Sullivan 1998; Stevens and Wesseling 1998; Wang and Kaczmarek 1998).

Figure 7 shows the results of the stepwise increase in c for the 2 different scenarios: in the case of an isolated increase in r, the release rate will briefly increase and then decrease to reach a new plateau of release that is only about 10% above the prestimulus level (Fig. 7*A*). This small increase in release rate in equilibrium has already been demonstrated in Figs. 4 and 5. After returning to normal transmitter content c, the terminal shows a decreased release of vesicles until the RRP is filled again. Such a transient decrease in vesicular release is regularly observed upon depletion of the RRP by high-frequency stimuli (short-term depression; Brager et al. 2002; Dobrunz and Stevens 1997). In the other case, where $\beta = \beta(\nu)$, an increase in c will be followed, with some delay, by a proportional and sustained increase in vesicular release because of the increasing number of vesicles flowing into the RRP (Fig. 7*B*). This situation would allow for a stable increase in synaptic transmission without fatigue.

Predictions derived from different implementations of $r = r(\nu)$ or $\beta = \beta(\nu)$

What could be the underlying causes for the dependency of vesicle processing on vesicular transmitter concentration? How can we experimentally distinguish between these possibilities? We can imagine two principally different links between vesicle transitions and vesicular transmitter content: first, an intrinsic detection mechanism that selects highly filled vesicles for further processing. This would correspond to "vesicle matura-

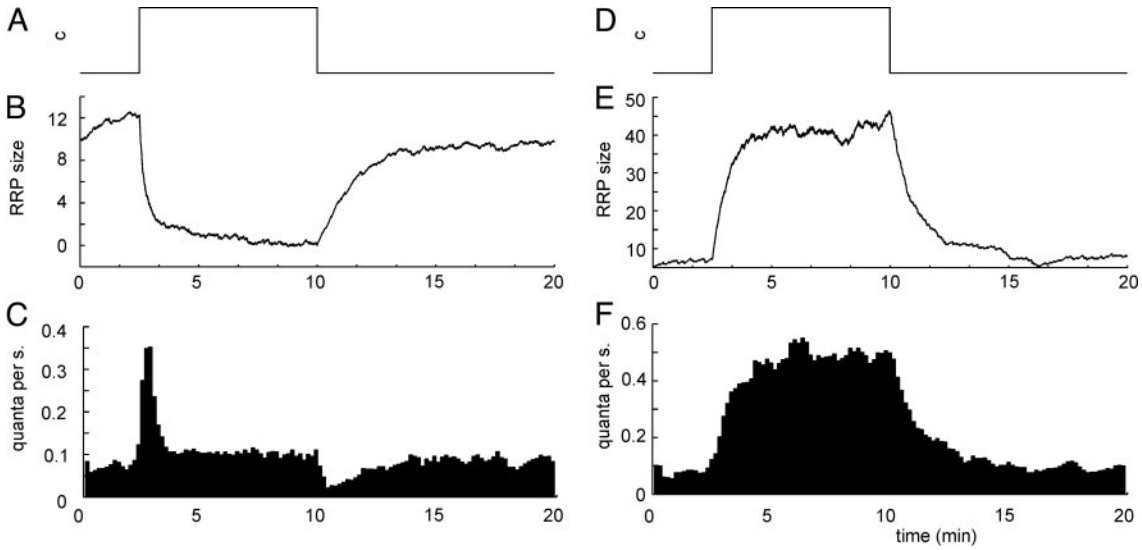

FIG. 7. Dynamic effects of changes in presynaptic transmitter concentration as in *A* and *D*. *B*, *C*: results for $r = r(\nu)$ as in *Eq. 8*. *E*, *F*: results for $\beta = \beta(\nu)$ as in *Eq. 7*. Note that for effects of vesicular filling on release rate r there is only a minor persistent change in released quanta (*C*). In contrast, if β increases with ν, the number of released vesicles per unit time shows a sustained increase for increases in c (see *F*).

tion" as a precondition for transition into the RRP or for release. Second, the released transmitter may exert feedback effects on r or β by presynaptic autoreceptors. Each vesicle could then, by virtue of its released transmitter content, influence the fate of subsequent vesicles, but not its own dynamics. In the DISCUSSION we will give examples for such positive feedback mechanisms and contrast them to the better-known negative presynaptic feedback mechanisms. In total, these considerations allow for four different scenarios: $\beta(\nu)$ or $r(\nu)$; both either mediated by a detection mechanism or by autoreceptors. The following experiments may help to distinguish between the scenarios.

1) If r was increased by transmitter released from previous vesicles (feedback), then vesicular release would tend to occur in bursts. Results of a simulation of this mechanism are plotted in Fig. 8. Notably, release of vesicles in brief bursts, similar to the results from our simulation, has been observed at hippocampal GABAergic synapses with increased transmitter content (Engel et al. 2001). This observation is thus compatible with presynaptic GABAergic autoreceptors that are positively coupled to vesicular release.

2) Another experimentally testable prediction is pointed out in Fig. 7. Sustained high-frequency stimulation of presynaptic fibres leads to depletion of the RRP and short-term depression (Dobrunz and Stevens 1997; Liu and Tsien 1995; for modeling, see Brager et al. 2002; Matveev and Wang 2000; and our Fig. 7*B*). If presynaptic transmitter content affects the transition of vesicles into the RRP [$\beta(\nu)$], increasing c will increase the

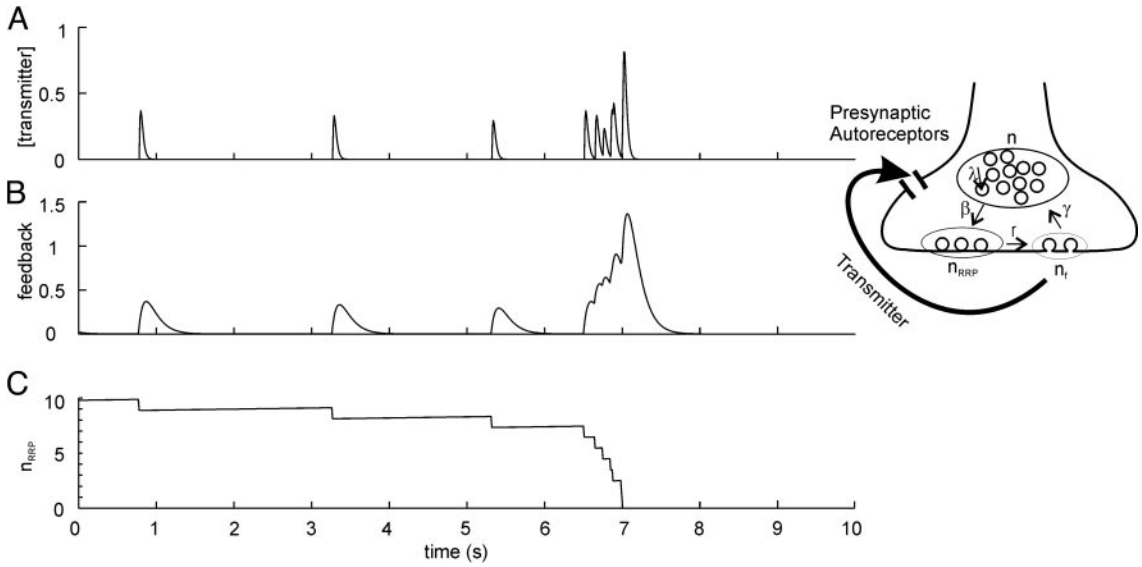

FIG. 8. Effects of positive feedback of released transmitter on subsequent release. *Right scheme:* schematic drawing of the suggested mechanism (presynaptic autoreceptors). *A*: time course of transmitter in the synaptic cleft (arbitrary units). Note the cluster of released quanta, resulting in a burstlike event at about t = 7 s. *B*: time course of the positive feedback parameter on subsequent release (arbitrary units). *C*: number of vesicles in the RRP.

TABLE 1. *Experimental predictions from the model if the presynaptic transmitter concentration c is increased*

	$r(\nu)$	$\beta(\nu)$
Intrinsic feedback	Frequency of events ($\uparrow$)	Frequency of events $\uparrow$
	Depletion $\uparrow$	Depletion $\downarrow$
	Recovery $\downarrow$	Recovery $\uparrow$
	Event correlation $\leftrightarrow$	Event correlation $\leftrightarrow$
Autoreceptors	Frequency of events ($\uparrow$)	Frequency of events $\uparrow$
	Depletion $\uparrow$	Depletion $\downarrow$
	Recovery $\leftrightarrow$	Recovery $\leftrightarrow$
	Event correlation $\uparrow$	Event correlation $\leftrightarrow$

Either the release rate r or the supply of vesicles β are assumed to increase with the presynaptic transmitter concentration ν. All effects are opposite if r or β decrease with ν (negative feedback). ($\uparrow$), slight increase; $\uparrow$, increase; $\leftrightarrow$, no effect; $\downarrow$, decrease.

number of vesicles in the RRP and the synapse should become more resistant toward fatigue. Conversely, if $r = r(\nu)$, the time constant for depletion should become faster when c is increased. A recent experimental and theoretical study on the modulation of short-term synaptic plasticity by PKC has revealed a very similar distinction between changes in vesicle supply versus changes in release probability (Brager et al. 2002). The effects of transmitter concentration on vesicle supply and depletion do, of course, reverse when either r or β decreases with ν (i.e., in the case of a negative presynaptic feedback mechanism).

3) Refilling of the RRP after high-frequency stimulation is a process that depends essentially on β and has time constants in the range of seconds to minutes (Pyott and Rosenmund 2002; Stevens and Tsujimoto 1995). After depletion, the rate of release of vesicles from the RRP is very small; therefore, any feedback mechanism acting by presynaptic autoreceptors is very ineffective in this situation. Thus if an increased presynaptic transmitter concentration leads to a faster recovery from depletion, it is likely that the rate of transition into the RRP β depends directly on vesicular transmitter concentration (detection and faster processing of full vesicles). The different scenarios are summarized in Table 1.

DISCUSSION

The present study was prompted by our and others' experimental observations suggesting complex relationships between transmitter metabolism and synaptic function. We tested different scenarios in which presynaptic transmitter concentration can be linked to vesicle filling and vesicular release. Our model revealed several experimentally testable results: *1*) the variability of vesicular transmitter content is—at least partially—based on an endogenous variance of vesicles (e.g., their size), rather than on the variance of transmitter loading; *2*) changes in presynaptic transmitter concentration can affect vesicular transmitter content as well as the frequency of release; *3*) these effects differ strongly depending on the step within the vesicle cycle that is regulated by transmitter concentration; *4*) dynamic changes of release are also different depending on the mechanisms linking transmitter content and behaviour of vesicles ("maturation" or presynaptic feedback). The model allows for the definition of experiments that can help to elucidate the causal relation between transmitter metabolism and synaptic function.

Filling of vesicles and transmitter content

At present, we lack information on many parameters of vesicular loading, the most important of which are the number of transmitter transport molecules per vesicle and the local cytosolic transmitter concentration. We therefore used the most parsimonious model, which takes into account the following experimental findings: *1*) an increased cytosolic transmitter concentration enhances vesicular transmitter content (Engel et al. 2001; Pothos et al. 1998a); *2*) transmitter can flow in and out of the vesicles; *3*) changes in transport rate change the resulting vesicular transmitter content (Colliver et al. 2000; Song et al. 1997; Van der Kloot et al. 2000); and *4*) transmitter content is equal at low and modest release rates (Behrends and ten Bruggencate 1998; Edwards et al. 1990; Kraszewski and Grantyn 1992; Ropert et al. 1990; Sahara and Takahashi, 2001; Van der Kloot 1996). We chose an equilibrium model that reaches a balance between inflow and outflow at a time defined by the relative weight of the rate constants, λ^+ and λ^-. In this model, filling of vesicles depends on presynaptic transmitter concentration and there is no fixed value for maximal transmitter content (see Williams 1997). Although equilibrium models have been challenged by observations at the neuromuscular junction (Naves and Van der Kloot 1996; Van der Kloot et al. 2000), our model does account for the main observations at central synapses with varying transmitter concentration.

Any equilibrium model requires some minimal time until equilibrium is reached. After fusion and endocytosis, vesicles at central synapses need at least 20 s to reenter the readily releasable pool (Ryan and Smith 1995; Ryan et al. 1993; Stevens and Tsujimoto 1995; von Gersdorff and Matthews 1997). An alternative, very fast recycling track for vesicles (Sara et al. 2002) seems to follow partial release and therefore does not require complete refilling (Graham et al. 2002; Machado et al. 2000, 2001). Thus, 20 s is sufficient to guarantee complete filling of recycled vesicles at central synapses (Dobrunz and Stevens 1997). Consistent with experimental observations, our model yields stable vesicular filling states over a wide range of release frequencies (Edwards et al. 1990; Kraszewski and Grantyn 1992; Ropert et al. 1990; Sahara and Takahashi, 2001; Van der Kloot 1996). At higher rates, quantal size may decrease, as has been observed upon continuous stimulation of the neuromuscular junction (Naves and Van der Kloot 2001).

Our model produced a surprisingly uniform population of equally and almost completely filled vesicles. To reproduce the observed variance of postsynaptic responses we introduced some variability of vesicle size, consistent with experimental and theoretical work on the variance of mIPSCs (Bekkers et al. 1990; Frerking et al. 1995; Palay and Chan-Palay 1974). Recently variations in vesicular dopamine content of pheochromocytoma cells have been shown to cause parallel changes in the volume of large dense core vesicles (Colliver et al. 2000). It should be noted, however, that vesicles at the neuromuscular junction do not change their size with changing acetylcholine content (Van der Kloot et al. 2002). Variance between vesicles can certainly result from alternative mechanisms. For example, the rate constants λ^+ and λ^- may differ between vesicles, possibly attributable to variable numbers of H^+-ATPase or VGAT molecules (see Song et al. 1997). In any case, the introduction of an intrinsic variability of vesicles led to a

distribution of vesicular transmitter content consistent with the experimentally observed variability of postsynaptic miniature currents.

Relationship between vesicular transmitter content, pool sizes, and vesicular release

Effects of vesicular filling state on synaptic function were modeled by assuming that one of the rate constants of the presynaptic vesicle cycle depends on transmitter content. The presynaptic vesicle cycle consists of multiple steps (Südhof 1995, 2000) that, for the present purpose, have been condensed to transitions between 3 major groups of vesicles: the readily releasable pool (RRP), the reserve pool, and empty vesicles after fusion. The RRP (Rosenmund and Stevens 1996) at central synapses is generally considered to contain 5–10 vesicles. Recent evidence indicates that the size of the RRP can be reduced after extensive activation of the synapse, possibly because of the disruption of release sites by fused vesicles or because of the depletion of certain molecule(s) needed for fusion (Stevens and Wesseling 1999; see *capacity restrictions*). This mechanism would tend to limit the capacity for increased vesicular release and thus is not likely to account for the observed increase in frequency of miniature postsynaptic current frequency upon increased transmitter loading of vesicles (Engel et al. 2001; Song et al. 1997). Our "reserve pool" contains all vesicles inside the terminal that might become available for release after going through additional steps of activation. At central synapses this pool is far greater than the RRP (Südhof 2000) and constitutes 80% of all vesicles in our model. The transition of these vesicles into the RRP has been condensed into one rate constant β, which also includes the equilibrium between forward and backward reactions (e.g., the undocking of vesicles) (Murthy and Stevens 1999; Oheim et al. 1999). In reality, multiple different transitions may occur between various subpools, including more remote reserve pools (Wang and Zucker 1998), an alternative route through the endosome (Südhof 2000), or a fast track for individual vesicles (Murthy and Stevens 1998; Sara et al. 2002; Stevens and Williams 2000; Valtorta et al. 2001). However, our 3-pool model is a parsimonious approach to distinguish between effects of vesicular transmitter content at 2 principally different stages: *1*) direct effects on the probability of release, modeled as $r(\nu)$, or *2*) effects on the rate of recruitment into the RRP, modeled as $\beta(\nu)$ (see below for a discussion of fast recycling).

Figure 5 illustrates the main difference between these two possibilities. If the probability of release is directly affected by the filling state of vesicles [$r(\nu)$], the effects of transmitter content on release rate will be rather mild and may escape detection. If, on the other hand, the supply of vesicles into the RRP is affected by their transmitter content [$\beta(\nu)$], changes in vesicular filling will massively alter the size of the RRP and thus the release rate. This latter possibility is favored by experimental data showing that increased loading of vesicles increases the frequency of miniature postsynaptic currents (Engel et al. 2001; Song et al. 1997; see also Pothos et al. 1998b). Conversely, hippocampal synapses that were depleted of the transmitter GABA exhibit a reduced frequency of mIPSCs (Murphy et al. 1998). It should be noted, however, that other experimental approaches have revealed no (Van der Kloot et al. 2000; Zhou et al. 2000) or even opposite effects of transmitter content on the rate of miniature postsynaptic currents (Overstreet and Westbrook 2001). The reasons for these differences remain to be elucidated but may hint toward some variability in presynaptic mechanisms at different synapses, besides more technical experimental differences. At GABAergic synapses, receptor desensitization, increased tonic inhibitory activity, and reversed function of GABA uptake may be confounding factors (Overstreet and Westbrook 2001; Wu et al. 2003). In summary, experimental evidence from some, but not all, systems is compatible with the idea that increasing presynaptic/vesicular transmitter concentration enhances the supply of vesicles into the RRP.

Changes in β as a mechanism of synaptic plasticity

There is good evidence that the transition of vesicles into the RRP can be modulated by various physiological tools. Increased calcium levels within the presynaptic terminal increase the rate of replenishing of the RRP (Stevens and Wesseling 1998; Wang and Kaczmarek 1998). In addition, and independently from this mechanism, activation of PKC increases the size of the RRP and speeds up its refilling (Gillis et al. 1996; Stevens and Sullivan 1998). Interestingly, Stevens and Sullivan (1998) also report an increase in the frequency of miniature postsynaptic currents after activation of PKC. This effect is partially explained by the larger size of the RRP, but its overproportional size indicates additional, more direct effects of PKC on release probability (Brager et al. 2002). In chromaffine cells, RRP size is regulated by calcium through at least 2 mechanisms, a PKC-dependent one and a PKC-independent one (Smith et al. 1998). It thus appears that increases in the transition rate β (or one time-limiting step, which we have included in this common rate constant) are a mechanism of synaptic plasticity and that an increased size of the RRP leads to a concomitant increase in the frequency of miniature postsynaptic currents.

Mechanisms of implementation

How may the filling state of vesicles affect their dynamics within the presynaptic terminal? In our model, we have compared 2 principally different sites of action: a feedback mechanism through presynaptic transmitter receptors and a detection mechanism inside the terminal that leads to different processing of differentially filled vesicles ("maturation" of vesicles by filling). Presynaptic autoreceptors mediating feedback effects on transmitter release are well known from many different synapses. In the case of GABA, most GABAergic neurons are equipped with $GABA_B$ receptors at the axon terminal that decrease the probability of release by various mechanisms (Misgeld et al. 1995). There is increasing evidence, however, that presynaptic axon terminals also carry ionotropic autoreceptors that, in some cases, can positively modulate the probability of release (for glutamatergic kainate autoreceptors see Rodriguez-Moreno et al. 1997; Schmitz et al. 2000). Ionotropic GABA receptors ($GABA_A$ and $GABA_C$ receptors) have been identified at various synapses including retinal horizontal cells (Kamermans and Werblin 1992; Matthews et al. 1994), cerebellar granule cells (Pouzat and Marty 1999), and the glutamatergic Schaffer collaterals in the rodent hippocampus (Stasheff et al. 1993). The latter exert a depolarizing effect

(probably because of a low Cl^- gradient in the axon terminals) that can trigger antidromically conducted action potentials. Similarly, GABA release from retinal horizontal cells is facilitated by previously released GABA (Kamermans and Werblin 1992). Depolarizing actions of GABA at GABAergic terminals would tend to increase the influx of calcium and thereby increase the release rate. Calcium might also trigger the PKC-dependent and PKC-independent facilitation of RRP loading described above. In the case of a positive feedback of GABA on subsequent vesicular release, the temporal sequence of mIPSCs should be influenced by the history of the terminal: release of large vesicles would then be especially prone to trigger further release of vesicles, resulting in bursts of mIPSCs, as indicated in our Fig. 8 and as observed in CA3 neurons from cultured hippocampal slices after treatment with the GABA-enhancing agent γ-vinyl-GABA (Engel et al. 2001).

If, on the other hand, vesicles with larger transmitter content would be transferred more easily into the readily releasable pool, there would be no such temporal pattern of release. This mechanism would require a detection of the filling state within the terminal; that is, filling would be a necessary step in vesicle maturation. A recent study has revealed that in dopaminergic neurons vesicle diameter increases with vesicular transmitter content (Colliver et al. 2000). This will enlarge the surface of the vesicle and may facilitate the interaction of vesicular membrane proteins with the molecular transport machinery within the presynaptic terminal, thereby speeding up the translocation of vesicles into the RRP. Small synaptic vesicles (SSV) at central nervous synapses may also vary in size according to their transmitter content (Frerking et al. 1995). At peripheral synapses, however, differentially filled vesicles did not reveal any alteration in size (Van der Kloot et al. 2002). In principle, alternative mechanisms for the detection of vesicular filling states are feasible (e.g., molecular conformation changes induced by the dissipation of the pH or voltage gradient upon filling), although we are not aware of experimental evidence for this.

Negative modulation of release probability and vesicle supply

We have focused on modeling a positive effect of vesicular transmitter concentration on release rate or vesicle supply, although the model can also account for negative presynaptic feedback effects as exerted by presynaptic $GABA_B$ receptors (Hammond 2001; Isaacson and Hille 1997; Rohrbacher et al. 1997). Recently, Overstreet and Westbrook (2001) reported a $GABA_B$ receptor-independent downregulation of mIPSC frequency in acutely prepared slices with enhanced GABA content. If this effect is indeed attributed to an enhanced presynaptic GABA concentration, the observation is opposite to our previous result from longer incubations of cultured hippocampal slices with γ-vinyl-GABA (Engel et al. 2001). Both examples may, however, be mediated by presynaptic $GABA_A$ autoreceptors: it is feasible that GABA can increase or decrease transmitter release in different preparations, dependent on the presynaptic chloride gradient.

Similar to the positive feedback described above, our model allows for experimentally testable distinctions between different implementations of negative feedback mechanisms: if released GABA decreases the release rate r, increased vesicular content will yield an increased size of the RRP. The release frequency will be only slightly affected because the higher number of vesicles in the RRP will partially compensate for the reduced probability of release. If the supply of vesicles (rate β) was reduced by vesicular transmitter content, the size of the RRP would decrease with increasing cytosolic transmitter concentration. Accordingly, the frequency of miniature postsynaptic currents will decrease. Thus, effects of transmitter concentration on the frequency of release are more pronounced if the supply of vesicles $\beta(\nu)$ is modulated as compared to direct effects on $r(\nu)$, similar to the positive modulation described above. It should be noted, however, that the difference between effects on r or β, respectively, is less pronounced in the case of downmodulation, given that a decrease in r will reduce the difference between r and the slowest transition constant β.

Classical and fast recycling

It is probable that vesicular cycling is a combination of the "classical" pathway by a resting pool and rapid recycling (i.e., a direct transition of fused vesicles into the RRP). Besides, more remote pools might also play a role (Wang and Zucker 1998). Furthermore, recent evidence suggests that the relative weight of different vesicle pathways may depend on release rate: the percentage of rapidly recycling vesicles might increase (Sara et al. 2002) or decrease (Gandhi and Stevens 2003) during high-frequency release or might become more important upon induction of LTD (Zakharenko et al. 2002). Even without explicitly modeling all these possibilities, we can posit the following: to reproduce a clear dependency of vesicular release on presynaptic transmitter concentration within our regime of parameters, one has to assume that the rate-liming factor is both transmitter concentration dependent and *upstream* of the RRP. This is not the case in a "rapid" vesicle cycle lacking a reserve pool. In a combination of fast and slow cycling, the effect of presynaptic transmitter concentration on vesicle release increases with the contribution of the classical pathway. This prediction might shed a new light on controversial results concerning the impact of vesicular filling on vesicular cycling (Engel et al. 2001; Zhou et al. 2000): it is conceivable that such differences are at least partly attributable to different cycling regimes that are indeed variable and depend on release rate (Gandhi and Stevens 2003; Sara et al. 2002).

Capacity restrictions

Our model belongs to the general class of rate-limited models. This implies that the slowest transition rate, in our case β, has the strongest influence on the overall behavior of the system. New experimental findings suggest, however, that vesicular cycling is restricted not only by rates, but also by the "capacity" of pools, especially of the RRP (Stevens and Wesseling 1998, 1999). In our model, this could be described as follows

$$\frac{\partial n(\nu, t)}{\partial t} = -\frac{\partial}{\partial \nu}[\lambda(\nu, c)n(\nu, t)] - \beta(\nu)n(\nu, t)\left[C_{nRRP} - \int_0^\infty n_{RRP}(\nu, t)\rho(\nu)d\nu\right] \quad (9a)$$

with C_{nRRP} being the "capacity" of the RRP.

$$\frac{\partial n_{\mathrm{RRP}}(\nu, t)}{\partial t} = \beta(\nu) n(\nu, t) \left[C_{\mathrm{nRRP}} - \int_0^\infty n_{\mathrm{RRP}}(\nu, t) \rho(\nu) \mathrm{d}\nu \right] - r(\nu) n_{\mathrm{RRP}}(\nu, t) - \frac{\partial}{\partial \nu} [\lambda(\nu, c) n_{\mathrm{RRP}}(\nu, t)] \quad (9b)$$

$$\frac{\partial n_f(t)}{\partial t} = \int_0^\infty r(\nu) n_{\mathrm{RRP}}(\nu, t) \rho(\nu) \mathrm{d}\nu - \gamma n_f(t) \quad (9c)$$

The boundary equilibrium condition is again

$$\frac{\partial}{\partial \nu} [\lambda(\nu) n(\nu, t)]_{\nu=0} = \gamma n_f(t) \quad (9d)$$

How would a capacity limitation affect the main findings of our study, that is, the dependency of transmitter release on presynaptic transmitter concentration? One of the main differences toward an unlimited RRP is the following: a pronounced effect of the presynaptic transmitter concentration c on transmitter release is observed even if the only rate that depends on vesicular filling is r. If r is increased because of faster vesicular filling, the release of vesicles from the RRP is enhanced and the increased flux from the RRP decreases the number of vesicles in the RRP, so that new "empty" slots in the capacity-limited RRP become available, and thus the refilling of vesicles into the RRP increases. This example shows that a capacity restriction of RRP refilling is equivalent to a refilling that depends on vesicular release. In *Eqs. 9*, this is immediately evident because the flux into the RRP now depends on the number of vesicles in the RRP, which in turn depends on the vesicular release r. There are even data suggesting a biochemical basis for this coupling of influx into and efflux from the RRP by their common dependency on Ca^{2+} (Stevens and Wesseling 1998). More generally, a coupling between vesicular release and maturation might also provide a mechanism to ensure a relatively stable RRP during periods of rest and release at higher frequency.

In conclusion, there is increasing evidence that presynaptic transmitter content provides an independent mechanism for synaptic plasticity in normal and pathological situations. Our model reveals different ways in which transmitter metabolism may be linked to vesicular filling and dynamics. The molecular mechanisms that govern the variance of vesicular size and filling as well as the regulation of vesicular cycling inside presynaptic terminals remain to be elucidated.

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