Transmitter metabolism as a mechanism of synaptic plasticity:

A modeling study

Nikolai Axmacher\textsuperscript{1,2}, Martin Stemmler\textsuperscript{2}, Dominique Engel\textsuperscript{3}, Andreas Draguhn\textsuperscript{4}, and Raphael Ritz\textsuperscript{2}

(1) Johannes-Müller-Institut für Physiologie
   Humboldt Universität Berlin
   Tucholskystr. 2, 10117 Berlin, Germany

(2) Institut für Theoretische Biologie
   Humboldt Universität Berlin
   Invalidenstr. 43, 10115 Berlin, Germany

(3) Physiologisches Institut der Universität Freiburg
   Hermann-Herder-Str. 7, 79104 Freiburg, Germany

(4) Institut für Physiologie und Pathophysiologie
   Ruprecht-Karls-Universität Heidelberg
   Im Neuenheimer Feld 326, 69120 Heidelberg, Germany

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Correspondance should be addressed to:
Raphael Ritz
Institut für Theoretische Biologie
Humboldt-Universität Berlin
Invalidenstr. 43
10115 Berlin
Germany
tel +49 30 2093 9120
fax +49 30 2093 8801
email r.ritz@biologie.hu-berlin.de
Abstract

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 neuro-pharmacological evidence points towards a third variable in synaptic efficacy, namely 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 following 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, i.e. alteration of vesicle maturation or alteration of release. It does also make a difference whether differentially filled vesicles are detected and differentially processed within the terminal or whether vesicle filling acts back onto the terminal via presynaptic autoreceptors. Therefore, the model allows one to decide, at a given synapse, how transmitter metabolism is linked to presynaptic function and – efficacy.
1. Introduction

Chemical synapses are the key structures for plastic adaptations of the central nervous system 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 which increase the concentration of the inhibitory transmitter GABA (γ-aminobutyric acid) at central inhibitory synapses yield anticonvulsant effects (Gram et al. 1988; Löscher et al. 1989; Engel et al. 2000; Taylor et al. 1992). While these examples are based on therapeutic interventions, several recent studies indicate that changes in transmitter metabolism represent a genuine plasticity mechanism in the central nervous system. Synaptic inhibition seems to be regulated by changes in transmitter metabolism in a way supporting homeostasis of overall network activity: following epileptic seizures, inhibitory interneurons in the rat hippocampus increase the expression of glutamate decarboxylase (GAD), the key enzyme for the synthesis of GABA (Feldblum et al. 1990; Esclapez and Houser 1999). Conversely, GABA production is down-regulated after deafferentiation of cortical areas (Garraghty et al. 1991; Hendry and Carder 1992; Gierdalski et al. 1999). A direct role for GABA-metabolism in synaptic plasticity is indicated by genetically modified mice which
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 (Hensch et al. 1998; Fagiolini and Hensch 2000).

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-synthesizing molecule tyrosine hydroxylase by activation of D2-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 quantal size as well as the frequency of miniature postsynaptic events, again pointing towards 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; Van der Kloot et al. 2002). However, these manipulations seem to affect 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 mIPSC frequency (Murphy et al. 1998) or probability of release (Golan and Grossman 1996), while elevated presynaptic GABA levels are paralleled by an increased frequency of miniature inhibitory postsynaptic currents (Engel et al. 2001). The latter result contrasts, however, to 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 following genetic ablation of the GABA-synthetizing enzyme GAD65: While 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 non-vesicular release of GABA (Yee et al. 1998; Overstreet and Westbrook 2001; Wu et al. 2003). 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 analysed 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.

2. Glossary

- $c$: presynaptic transmitter concentration (mM)
- $n$: number of vesicles in the reserve pool
- $n_{RRP}$: number of vesicles in the readily releasable pool
- $n_f$: number of fused vesicles
- $r$: release rate (1/s)
- $\beta$: transition rate into the readily releasable pool (1/s)
- $\gamma$: rate of recovery from the pool of fused vesicles (1/s)
- $\lambda^+$: rate of influx into vesicles (1/s)
- $\lambda^-$: rate of efflux from vesicles (1/s)
- $\dot{\lambda}$: net vesicular filling (mM/s)
- $\nu$: vesicular transmitter concentration (mM)
- $\nu_{max}$: maximal vesicular transmitter concentration (mM)
3. Materials and Methods

3.1. Presynaptic vesicle cycle

We describe the distribution of vesicles in the presynaptic terminal with respect to their different transmitter content \( \nu \) and - in the dynamic case - with respect to time. Presynaptic vesicles undergo a complex cycle between release, recovery and maturation (Südhof 1995) which, for the present purpose, has been reduced to transitions between three functionally distinct vesicle pools: (1) the reserve pool \( (n, \text{equations a}) \), (2) the readily releasable pool \( (n_{RRP}, \text{equations b}) \) and (3) the pool of empty, fused vesicles \( (n_f, \text{equations c}) \). The faster “kiss-and-run” pathway will be modelled as a case with infinitely small reserve pool in section 4.4. In the full model, the vesicle cycle is given by transition from the reserve pool into the RRP (rate \( \beta \)), subsequent synaptic release (rate \( r \)) and, finally, recovery of fused vesicles into the reserve pool (rate \( \gamma \), see Fig. 1 for illustration). Transitions between pools are described by the following set of ordinary differential equations:

\[
(1a) \quad \frac{d}{dt} n(t) = \gamma n_f(t) - \beta n(t)
\]

\[
(1b) \quad \frac{d}{dt} n_{RRP}(t) = \beta n(t) - r n_{RRP}(t)
\]

\[
(1c) \quad \frac{d}{dt} n_f(t) = r n_{RRP}(t) - \gamma n_f(t)
\]
with the normalization \( n(t) + n_{RRP}(t) + n_f(t) = \text{const.} \), assuming that the total amount of vesicles stays constant. In the steady state, the mean number of vesicles in each of the three compartments can easily be solved analytically giving

\begin{align}
(2a) \quad \gamma \bar{n}_f &= \beta \bar{n} \\
(2b) \quad \beta \bar{n} &= r \bar{n}_{RRP} \\
(2c) \quad \bar{n}_{RRP} &= \gamma \bar{n}_f
\end{align}

or

\begin{align}
(3a) \quad \bar{n} &= [1 + \beta (\gamma^{-1} + r^{-1})]^{-1} \\
(3b) \quad \bar{n}_{RRP} &= [1 + r(\gamma^{-1} + \beta^{-1})]^{-1} \\
(3c) \quad \bar{n}_f &= [1 + \gamma (\beta^{-1} + r^{-1})]^{-1}.
\end{align}

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

In the initial part of the results section (4.1), we model the filling of vesicles and their resulting distribution with respect to transmitter content. For the sake of clarity, in this chapter the reserve pool and the readily releasable pool were collapsed into a single pool of vesicles which reduced the mathematical model to only two differential equations.

3.2. Filling of vesicles

Neurotransmitters are transported into synaptic vesicles in exchange with protons which are previously accumulated by H⁺-ATPases (Masson et al. 1999). We assume that this process saturates
with increasing vesicular transmitter concentration. This assumption is justified by two 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, non-saturating 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 does indeed occur 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 which are independent from the frequency of release (e.g., Edwards et al. 1990; Ropert et al. 1990; Kraszewski and Grantyn 1992; Van der Kloot 1996; Sahara and Takahashi 2001) or which even increase upon increasing frequency (Behrends and ten Bruggencate 1998). Thus, vesicular loading seems to be saturating and can only limit vesicular transmitter content at very fast release rates (see Fig. 2).

Subsequently, we model saturating vesicle loading in the most parsimonious way, i.e. as a bi-directional flux of transmitter molecules which depends on transmitter concentration on either side. With flux rates into \( \dot{\lambda}^+ \) and out of \( \dot{\lambda}^- \) the vesicles, a cytosolic transmitter concentration \( c \) and resulting vesicular transmitter concentration \( v \) we get a net flux \( \lambda \) of:

\[
\lambda(c, v) = \dot{\lambda}^+ c - \dot{\lambda}^- v.
\]
Filling saturates at $v_{\text{max}} = \frac{\lambda^+}{\lambda^-} c$.

In this equation, loading depends on the cytosolic transmitter concentration and leakage depends on intravesicular transmitter concentration (see Fig. 2A). This 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 (Murphy et al. 1998; Pothos et al. 1998a; Engel et al. 2001). Our model reproduces this effect: an increase in $c$ will increase the resulting amount of transmitter $v$ 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 $v$ (Wang and Floor 1994).

### 3.3 Combining cycling and filling

In order to reproduce the experimentally observed dependence of synaptic function on transmitter metabolism, we then introduce the vesicular transmitter concentration $v$ as an additional variable into the description of vesicle distribution between the three 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 $v$, i.e. $n(t) = \int_0^\infty n(v,t) \rho(v) dv$ and $n_{\text{RRP}}(t) = \int_0^\infty n_{\text{RRP}}(v,t) \rho(v) dv$ where $\rho(v)$ is the integral of $v$ needed here to get the dimensions right (the pool of empty, fused vesicles $n_f$ is independent from $v$). 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 \( v \) (i.e. \( \beta(v) \), \( r(v) \)). In principle, the rates could be modelled to depend on cytosolic transmitter concentration \( c \), rather than on \( v \). 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 dependence of rate constants on vesicular transmitter content influences the distribution of vesicles between the three compartments. In general terms, the presynaptic vesicle dynamics is now determined by the following set of partial differential equations (the recovery of empty vesicles (\( \gamma \) can, of course, not depend on the filling state):

\[
\frac{\partial n(v,t)}{\partial t} = - \frac{\partial}{\partial v} \left[ \lambda(v,c)n(v,t) \right] - \beta(v)n(v,t)
\]

\[
\frac{\partial n_{\text{RRP}}(v,t)}{\partial t} = \beta(v)n(v,t) - \frac{\partial}{\partial v} \left[ \lambda(v,c)n_{\text{RRP}}(v,t) \right] - r(v)n_{\text{RRP}}(v,t)
\]

\[
\frac{\partial n_f(t)}{\partial t} = \int_0^\infty r(v)n_{\text{RRP}}(v,t)\rho(v)dv - \gamma n_f(t).
\]

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:

\[
\frac{\partial}{\partial v} \left[ \lambda(v,n(v,t)) \right] \Bigg|_{v=0} = \gamma n_f(t).
\]

In order to specify the dependence of transition rates on vesicular transmitter concentration, we introduce the following equations for transition into the RRP (\( \beta \)) and release (\( r \)):

\[
\beta(v) = \beta_0 [1 - \exp(-v/v_0)]
\]

and
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 as we do not know of any experimental results supporting either this or an alternative function.

3.4. Biological interpretation of variables and choice of parameters

We have modelled spontaneous release as a random process with a low probability in each timestep. Fused vesicles \( f_n \) are recycled with a constant rate \( \gamma \). The number of recycled empty vesicles sets the boundary condition \( n(0,t) \) for the vesicular filling process in the reserve pool \( n(v,t) \).

The system of equations (1a) – (1c) contains three variables (the number of vesicles in each compartment \( n, n_{RRP} \) and \( n_f \)) and three parameters (the transition rates \( \beta, r \) and \( \gamma \)). 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 following changes in presynaptic transmitter concentration. As typical case, we will consider 10 vesicles in the release-ready pool (Borges et al. 1995; Stevens and Tsujimoto 1995; Murthy and Stevens 1999; Kraushaar and Jonas 2000; Kirischuk and Grantyn 2000). The number of fused vesicles \( f_j \) 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 compartment.
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 seconds 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 equations 3 are: \( \beta = 1.25 \cdot 10^{-3}/s \); \( r = 1 \cdot 10^{-2}/s \); \( \gamma = 1 \cdot 10^{-2}/s \). 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 tens of seconds, but also on a faster timescale (“kiss-and-run”, Murthy and Stevens 1998; Stevens and Williams 2000; Valtorta et al. 2001; Sara et al. 2002; Aravanis et al. 2003; Gandhi and Stevens 2003). 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 two 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. 2B, 3B for illustration within our three-compartment model); (2) the RRP can not be re-filled from a large reserve pool after losing vesicles, i.e., our presynaptic model is effectively reduced to two compartments. Within the present model, this direct pathway can be considered a limiting case for increased values of \( \beta \). Under these conditions, the number of vesicles in the reserve pool approaches
zero and $\beta$ loses its rate-limiting function. The consequences of such an increase in $\beta$ are analysed in detail in section 4.4 and Fig. 6. Alternatively, we also simulated the direct pathway in a two-compartment model (only containing RRP and fused vesicles), which yielded similar results (data not shown).

Vesicular filling was modelled as a bi-directional flux of transmitter, with influx depending on cytosolic transmitter concentration $c$ and efflux depending on vesicular transmitter concentration $v$. 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 $K_m$ ($\sim$5 mmol for GABA, see Kish et al. 1989, McIntire et al. 1997). The cytosolic transmitter concentration $c$ can not 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 ($\sim$ 20s). Otherwise, vesicular transmitter concentration would strongly depend on release rate. As mentioned above, this seems only to be the case 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 (Pothos et al. 1998a; Engel et al. 2001).
What is the maximal vesicular transmitter concentration that can be reached? Experimental and modelling 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 (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 $\nu_{\text{max}}$ ranging from 100 to 1000 mM. Using equation (4), these assumptions yield a mean value of $\lambda = 5$ mmol/s for $c = 1$ mmol.

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

4. Results

In order 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 afterwards dynamic changes in presynaptic transmitter content. Together, the results show how synaptic efficacy changes following changes in presynaptic transmitter metabolism.

4.1 Distribution of vesicular transmitter content

As a first step, we model a single, homogeneous population of vesicles that is loaded according to equation 4: \[ \lambda(c,\nu) = \lambda^c - \lambda \nu \]
(see Fig. 2A). For the steady-state situation, this equation can be solved analytically and results in a distribution of vesicles that peaks at \( \nu_{\text{max}} \), such that most vesicles are almost maximally full (Fig. 2B). 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 (Ryan et al. 1993; Ryan and Smith 1995; Liu and Tsien 1995; Klingauf et al. 1998) except in special situations which are likely to involve kiss-and-run release (Sara et al. 2002; Palfrey and Artalejo 1998; Burgoyne et al. 2001; Machado et al. 2000, 2001; Graham et al. 2002; Aravanis et al. 2003; Gandhi and Stevens 2003). 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
which has been set to 6 sec.; at this point, the distribution flattens (Fig. 2B). 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. 2B shows a very sharp peak at maximal values of $v$ (Fig. 2B). Miniature postsynaptic currents show, however, usually a skewed distribution with a large coefficient of variance of up to ~0.5 (e.g., Frerking et al. 1995; Sahara and Takahashi 2001). There is good indication that this variance is at least partially due 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. 2B) with the distribution of vesicular volumes in presynaptic endings. The latter was based on analyses of vesicle diameters in cerebellar and hippocampal neurons (Palay and Chan-Palay 1974; Bekkers et al. 1990), yielding a coefficient of variance of ~0.12. This gaussian distribution of diameters was transformed into the third-order gaussian describing vesicular volume (Frerking et al. 1995). From hereon, the distribution of vesicles in the readily releasable pool and in the reserve pool will be plotted as $n(v \times V)$, i.e. with respect to transmitter content, rather than concentration. Fig. 3A illustrates the influence of vesicular size variance on the distribution of differentially filled vesicles for a case of low release rate ($r = 1/\text{min}$). Obviously, at zero variance the distribution peaks at
maximal transmitter content (yielding a distribution similar to Fig. 2B for \( r = 1/\text{min} \)). If such a distribution of vesicular transmitter content would underlie the experimentally observed amplitude distribution of miniature postsynaptic currents, variance would almost exclusively be due 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 modelled the distribution of vesicles \( n(\nu \times V) \) at different release rates (diameter variance was set to 0.12 for this simulation; Fig. 3B). For modest release rates, this distribution is less sensitive to release rate than the data shown in Fig. 2B, 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; Ropert et al. 1990; Kraszewski and Grantyn 1992; Van der Kloot 1996; Sahara and Takahashi 2001). Only at high sustained release rates, filling becomes incomplete (Naves and Van der Kloot 2001; see Fig. 3B for release rates above \( \sim 5/\text{sec} \)).

4.2 Dependence 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 (Golan and Grossman 1996; Song et al. 1997; Murphy et al. 1998; Pothos et al. 1998b; Engel et al. 2001). In order to establish mechanisms for these observations within our model, we will now consider transition rates which depend on transmitter concentration. In contrast to the previous section, we will now use the full presynaptic model as introduced in equations 5, i.e. vesicles are distributed between a reserve pool (80% of
vesicles in equilibrium), a readily releasable pool (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 \( \beta \) (flow into the readily releasable pool). We start with the case where the release rate \( r \) depends on vesicular transmitter concentration: 
\[
r = r(\nu) = r_0[1 - \exp(-\nu/\nu_0)].
\]
Afterwards, we will consider the alternative scenario where the supply of vesicles from the reserve pool to the readily releasable pool depends on \( \nu \), 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 towards larger values when cytosolic transmitter concentration is raised (Fig. 4B). Since 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. 4C). While Fig. 4 focusses 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. 5A). The number of released vesicles per time remains relatively constant, however, as the the reduced number of releasable vesicles is compensated by the increased release rate of these (fuller) vesicles (Fig. 5B). Thus, in a model with distinct pools, a filling-dependent release rate can not 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 equations 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. Due to the fact that most of the vesicles are in the reserve pool, the rate $\beta$ for the transition of vesicles from the reserve pool into the RRP is much smaller than $r$ and $\gamma$. Therefore, the total flux of vesicles in our scenario depends mainly on the slowest transition rate $\beta$. 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 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 equations 5.

4.3 Dependence of vesicle supply $\beta$ 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. 4D 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. 4B ($r = r(\nu)$), 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. 4E). Fig. 5B shows how many vesicles reside in the RRP and Fig. 5D 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 does also sharply increase, 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. 5D.

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).

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

As mentioned in Methods, there is increasing evidence that vesicles are not only recycled 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 \( \beta_0 \), thereby diminishing the size of the reserve pool. As \( \beta_0 \) 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. 2B and 3B, where the sharp peak in vesicle distribution broadens. Fig. 6A shows the number of vesicles in each compartment as a function of increasing values of $\beta_0$ according to the equations (3). The size of the reserve pool is reciprocally proportional to the velocity of “maturation” and the pool vanishes at high values of $\beta_0$. Conversely, the pools of releasable and of fused vesicles, respectively, increase.

What happens now in this system if the presynaptic transmitter concentration $c$ is increased? Again, we must distinguish between effects of $c$ on vesicle maturation $\beta$ (Fig. 6B) and effects on release rate $r$ (Fig. 3C). When $\beta_0$ is increased, the steep correspondence between transmitter concentration and release (see Fig. 5D and case $\beta_0 = 1$ in Fig. 6B) is lost, and the number of released vesicles per time becomes largely independent from $c$. This is also illustrated in Fig. 6D. At low values of $\beta$ ($\beta_0 = 1$), vesicular release is strongly increased following a 10-fold increase in $c$. Increasing $\beta_0$ reduces the rate-limiting role of $\beta$ and thereby abolishes any effects of transmitter concentration on the frequency of vesicle release. At high values of $\beta_0$, one might assume that effects of $c$ on the release rate $r$ (implemented as $r(\nu)$, see above) become more pronounced. However, Fig. 6C shows that the weak effect of $c$ on release is lost when $\beta_0$ is increased. This is caused by a reciprocal compensation of two effects: At increased values of $c$, vesicles are being filled more rapidly and are released with higher probability, if $r$ increases with $\nu$. 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 two-compartment model (only comprising the RRP and a pool of fused vesicles) yielded equivalent results, namely that the synaptic release is always independent of transmitter concentration in the absence of the reserve pool.

4.5 Dynamic alterations of vesicle cycling

In order to further demonstrate the differences between alterations of vesicular release rate $r(v)$ and vesicle recruitment $\beta(v)$, we subsequently computed dynamic changes of vesicular release for a stepwise increase in $c$. While 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 $r = r(v)$, 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., Rosenmund and Stevens 1996; Kirischuk and Grantyn 2000). For the alternative scenario ($\beta = \beta(v)$), 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 $\text{Ca}^{2+}$ influx and activation of PKC (Gillis et al. 1996; Stevens and Sullivan 1998; Smith et al. 1998; Wang and Kaczmarek 1998; Stevens and Wesseling 1998).
Fig. 7 shows the results of the stepwise increase in $c$ for the two 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 which is only $\sim 10\%$ above the pre-stimulus level (Fig. 7A). 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; Dobrunz and Stevens 1997; Brager et al. 2002). In the other case, where $\beta = \beta(v)$, an increase in $c$ will be followed, with some delay, by a proportional and sustained increase in vesicular release, due to the increasing number of vesicles flowing into the RRP (Fig. 7B). This situation would allow for a stable increase in synaptic transmission without fatigue.

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

What could be the underlying causes for the dependence 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 which selects highly filled vesicles for further processing. This would correspond to "vesicle maturation" as a precondition for transition into the RRP or for release. Second, released transmitter may exert feedback effects on $r$ or $\beta$ via 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: $\beta(v)$ or $r(v)$; 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 which 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 (Liu and Tsien 1995; Dobrunz and Stevens 1997; for modeling, see Matveev and Wang 2000; Brager et al. 2002; and our Fig. 7A). If presynaptic transmitter content affects the transition of vesicles into the RRP ($\beta(v)$), increasing $c$ will increase the number of vesicles in the RRP and the synapse should become more resistant towards fatigue. Conversely, if $r = r(v)$, 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 protein kinase C has revealed a very similar distinction between changes in vesicle supply versus changes in release probability (Brager et al. 2002).
2002). The effects of transmitter concentration on vesicle supply and depletion do, of course, reverse when $r$ or $\beta$ decrease with $\nu$ (i.e. in case of a negative presynaptic feedback mechanism).

3) Re-filling of the RRP after high-frequency stimulation is a process which depends essentially on $\beta$ and has time constants in the range of seconds to minutes (Stevens and Tsujimoto 1995; Pyott and Rosenmund 2002) After depletion, the rate of release of vesicles from the RRP is very small; therefore, any feedback mechanism acting via 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 $\beta$ depends directly on vesicular transmitter concentration (detection and faster processing of full vesicles). The different scenarios are summarized in Table 1.
5. Discussion

The present study was prompted by our and other’s 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 which 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 does allow for the definition of experiments which can help to elucidate the causal relation between transmitter metabolism and synaptic function.

5.1 Filling of vesicles and transmitter content

At present, we are lacking information on many parameters of vesicular loading, most importantly 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: i) an increased cytosolic transmitter concentration enhances vesicular transmitter content (Pothos et al. 1998a; Engel at al. 2001); ii) transmitter can flow out of the vesicles; iii) changes in transport rate change the resulting vesicular transmitter content (Song et
al. 1997; Van der Kloot et al. 2000; Colliver et al. 2000); iv) transmitter content is equal at low and modest release rates (Edwards et al. 1990; Ropert et al. 1990; Kraszewski and Grantyn 1992; Van der Kloot 1996; Behrends and ten Bruggencate 1998; Sahara and Takahashi, 2001). We chose an equilibrium model which reaches a balance between inflow and outflow at a time defined by the relative weight of the rate constants, $\lambda^+$ and $\lambda^-$. 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 re-enter the readily releasable pool (Ryan et al. 1993; Ryan and Smith 1995; 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 re-filling (Machado et al. 2000, 2001; Graham et al. 2002). Thus, 20 s suffice 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; Ropert et al. 1990; Kraszewski and Grantyn 1992; Van der Kloot 1996; Sahara and Takahashi, 2001). 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 (almost maximally) filled vesicles. In order 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 (Palay and Chan-Palay 1974; Bekkers et al. 1990; Frerking et al. 1995). 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 $\lambda^+$ and $\lambda^-$ may differ between vesicles, possibly due 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.

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

Effects of vesicular filling state on synaptic function were modelled 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) which, for the present purpose, have been condensed to transitions between three major groups of vesicles: the readily releasable pool (RRP),
the reserve pool and empty vesicles after fusion. The readily releasable pool (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 due to disruption of release sites by fused vesicles or due to the depletion of certain molecule(s) needed for fusion (Stevens and Wesseling 1999; see section 5.7.). This mechanism would tend to limit the capacity for increased vesicular release and, therefore, is not likely to account for the observed increase in frequency of miniature postsynaptic current frequency upon increased transmitter loading of vesicles (Song et al. 1997; Engel et al. 2001). Our “reserve pool” contains all vesicles inside the terminal which 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 comprised 80% of all vesicles in our model. The transition of these vesicles into the RRP has been condensed into one rate constant $\beta$, which does also include 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 sub-pools, including more remote reserve pools (Wang and Zucker 1998), an alternative route through the endosom (Südhof 2000) or a fast track for individual vesicles (Murthy and Stevens 1998; Stevens and Williams 2000; Valtorta et al. 2001, Sara et al. 2002). However, our three-pool model is a parsimonious approach to distinguish between effects of vesicular transmitter content at two principally different stages: i) direct effects on the probability of release, modelled as $r(\nu)$ or ii) effects on the rate of recruitment into the RRP, modelled as $\beta(\nu)$ (see below for a discussion of fast recycling).
Fig. 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, 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 release rate. This latter possibility is favoured by experimental data showing that increased loading of vesicles increases the frequency of miniature postsynaptic currents (Song et al. 1997; Engel et al. 2001; see also Pothos et al. 1998b). Conversely, hippocampal synapses which 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 towards 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.

5.3 Changes in \( \beta \) 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 (Wang and Kaczmarek 1998; Stevens and Wesseling 1998). In addition, and independently from this mechanism, activation of PKC increases the size of the RRP and speeds up its re-filling (Gillis et al. 1996; Stevens and Sullivan 1998). Interestingly, Stevens and Sullivan (1998) do 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 over-proportional 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 two mechanisms, a PKC-dependent one and a PKC-independent one (Smith et al. 1998). It appears thus that increases in the transition rate $\beta$ (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 does lead to a concomitant increase in the frequency of miniature postsynaptic currents.

5.4 Mechanisms of implementation

How may the filling state of vesicles affect their dynamics within the presynaptic terminal? In our model, we have compared two principally different sites of action: a feedback mechanism through presynaptic transmitter receptors and a detection mechanism inside the terminal which 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 which decrease the probability of release by various mechanisms (Misgeld et al. 1995). There is increasing evidence, however, that presynaptic axon terminals do also carry ionotropic autoreceptors which, 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 (Pouza et al. and Marty 1999) and the glutamatergic Schaffer collaterals in the rodent hippocampus (Stasheff et al. 1993). The latter exert a depolarizing effect (probably due to a low Cl^- gradient in the axon terminals) which 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 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 \( \gamma \)-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, i.e. 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. We are not aware, however, of experimental evidence for this.

5.5 Negative modulation of release probability and vesicle supply

We have focussed on modelling a positive effect of vesicular transmitter concentration on release rate or vesicle supply. The model can, however, also account for negative presynaptic feedback effects as exerted by presynaptic GABA_b receptors (Isaacson and Hille 1997; Rohrbacher et al. 1997; Hammond 2001). Recently, Overstreet and Westbrook (2001) reported a GABA_b receptor-independent down-regulation of mIPSC frequency in acutely prepared slices with enhanced GABA content. If this effect is indeed due 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 frequency release will only be slightly affected as the higher number of vesicles in the RRP will partially compensate for the reduced probability of release. If the supply of vesicles (rate $\beta$) 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 $\beta(\upsilon)$ is modulated as compared to direct effects on $r(\upsilon)$, similar to the positive modulation described above. It should be noted, however, that the difference between effects on $r$ or $\beta$, respectively, is less pronounced in the case of down-modulation, as a decrease in $r$ will reduce the difference between $r$ and the slowest transition constant $\beta$.

5.6 Classical and fast recycling

It is probable that vesicular cycling is a combination of the “classical” pathway via 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: in order to reproduce a clear dependence of vesicular release on presynaptic transmitter concentration within our regime of parameters, one has to assume that the rate-limiting factor is both transmitter concentration-dependent and \textit{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 (Zhou et al. 2000; Engel et al. 2001): it is conceivable that such differences are at least partly due to different cycling regimes which are indeed variable and depend on release rate (Sara et al. 2002; Gandhi and Stevens 2003).

5.7 Capacity restrictions

Our model belongs to the general class of rate-limited models. This implies that the slowest transition rate, in our case $\beta$, has the strongest influence on the overall behavior of the system. New experimental findings suggest, however, that vesicular cycling is not only restricted 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:

$$\frac{\partial n(v,t)}{\partial t} = -\frac{\partial}{\partial v} \left[ \lambda(v,c)n(v,t) \right] - \beta(v)n(v,t)[C_{n\text{RRP}} - \int_0^\infty n_{\text{RRP}}(v,t)\rho(v)dv]$$

(with $C_{n\text{RRP}}$ being the “capacity” of the RRP)
The boundary equilibrium condition is again:

\[ \frac{\partial n_{\text{RRP}}(v,t)}{\partial t} = \beta(v)n(v,t)[C_{n_{\text{RRP}}} - \int_0^\infty n_{\text{RRP}}(v,t)\rho(v)dv] - r(v)n_{\text{RRP}}(v,t) - \frac{\partial}{\partial V}[\lambda(v,c)n_{\text{RRP}}(v,t)] \]

\[ \frac{\partial n_f(t)}{\partial t} = \int_0^\infty r(v)n_{\text{RRP}}(v,t)\rho(v)dv - \gamma n_f(t). \]

How would a capacity limitation affect the main findings of our study, namely the dependence of transmitter release on presynaptic transmitter concentration? One of the main differences towards 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 due to 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 the above equations (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 dependence on \( \text{Ca}^{2+} \) (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.
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 which 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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Fig. 1: Simplified model of the synaptic vesicle cycle. Synaptic vesicles are contained in three 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 \( \lambda \). The transitions between the three pools are described by rate constants \( \gamma \), \( \beta \), and \( r \), respectively. For details see main text.
Fig. 2: Filling of vesicles and equilibrium distribution of differentially filled vesicles for different release rates. A: The effective filling rate ($\lambda$) is assumed to decrease linearly with increasing vesicular transmitter concentration, reaching equilibrium ($\lambda = 0$) at 10 mM. B: Distribution of vesicles with different intravesicular transmitter concentrations (grey values coding the number of vesicles as indicated in the scale bar). Note that most vesicles are (almost) maximally filled. The distribution increases monotonously with transmitter concentration. At higher release rates, the distribution broadens towards 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.
Fig. 3: Vesicular transmitter content assuming variation in vesicle volume. A: Number of vesicles with different transmitter content ([transmitter]_{ves} \times \text{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). The scale bar indicates number of vesicles.
Fig. 4: Distribution of vesicles in different pools for transition rates which depend on vesicular filling state. Left row: release rate $r$ increases with transmitter concentration $\nu$ as in equation (8); right row: supply to the readily releasable pool $\beta$ increases with $\nu$ as in equation (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 show the 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. The total amount of released vesicles is about 9 per minute in (C) and about 15 per minute 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 $r = r(v)$ as in equation (8). C, D: Results for $\beta = \beta(v)$ as in equation (7). Note that for concentration-dependent release (A, B) the number of vesicles in the RRP declines with increasing concentration while 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 equations (2a-c), dots show numerical results of equation (5a-c).
Fig. 6: The dependence of the frequency of vesicle release on presynaptic transmitter concentration decreases in a fast vesicle cycle. A: Dependence 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 $\beta_0$). B: Scenario where the vesicle supply into the RRP depends on the vesicular transmitter concentration, $\beta = \beta(\nu)$. Only if $\beta$ 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 dependence 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 $\beta_0$ for both $\beta(\nu)$ and $r(\nu)$. 
Fig. 7: Dynamic effects of changes in presynaptic transmitter concentration as in (A, D). B, C: Results for $r = r(v)$ as in equation (8). E, F: Results for $\beta = \beta(v)$ as in equation (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 $\beta$ increases with $v$, the number of released vesicles per unit time shows a sustained increase for increases in $c$ (see panel F).
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 burst-like 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.
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<th>$r(\nu)$</th>
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Table 1: Experimental predictions from the model if the presynaptic transmitter concentration $c$ is increased. Either the release rate $r$ or the supply of vesicles $\beta$ are assumed to increase with the presynaptic transmitter concentration $\nu$. All effects are opposite if $r$ or $\beta$ decrease with $\nu$ (negative feedback). (↑): slight increase, ↑: increase, ↔: no effect, ↓: decrease.