Synaptic Vesicle Dynamics: A Simple Model of Phasic Release

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Abstract. We present a simple model of phasic neurotransmitter release which reproduces the salient features of chemical neurotransmission. The synaptic vesicle cycle has been modelled as a set of biochemical reactions represented by a system of coupled differential equations. These equations have been solved analytically to obtain the time dependent behaviour of the system on perturbation from the steady state. The scheme of the synaptic vesicle network has been emphasized and its role in determining some of the major experimentally observed properties of synaptic transmission has been discussed, which includes the biphasic decay of the rate neurotransmitter release even under sustained stimulation. Another interesting outcome of this theoretical exercise is the saturation of total release with the calcium dependent rate constant. The theoretically calculated values of total release fit very well into a sigmoidal saturating function with a fourth order cooperativity exponent similar to the empirical Dodge–Rahamimoff equation. It appears that the synaptic vesicle network itself is responsible for some of the major properties associated with chemical neurotransmission.

Key words: Synaptic, Vesicle, Dynamics, Model

1. Introduction

Recently a study of the mechanics of exocytosis of synaptic vesicles (SV) during chemical neurotransmission has been initiated [1] by applying the structural metabolic control theory [2]. It has been shown that a model of SV exocytosis in which Ca^{2+} binding to *synaptotagmin* (an integral membrane protein belonging to the synaptic vesicle membrane) and subsequent changes is assumed to remove some barrier to membrane fusion, is more consistent with certain experimental observations than other alternative models. The model (Table 1) describes the *evolution* of synaptic vesicles through different *states* ultimately leading to fusion with the presynaptic membrane and consequently, release of neurotransmitter into the synaptic cleft. In a recent review [3] the synaptic vesicle cycle has been divided into nine steps out of which the first three steps (i.e., docking, priming and fusion) have been considered explicitly in the present model (Table 1). In the present work, we show that with a simple model of the synaptic vesicle cycle one can simulate the observed pattern of biphasic decay [4] of the rate of release of neurotransmitter in the Ca^{2+} -triggered fusion of synaptic vesicles. Since this property is observed to be

Table 1. Minimal model of synaptic vesicle exocytosis

$FUV(x_1) + NT(In) \xrightarrow{k_1} FRV(x_2)$	...Step 1 (Recycling of fused vesicles)
$FRV(x_2) \xrightleftharpoons[k_3]{k_2} DV(x_3)$	...Step 2 (Docking of 'free vesicles' at release sites)
$DV(x_3) \xrightleftharpoons[k_5]{k_4} CV(x_4)$	...Step 3 (Formation of 'release ready' vesicles)
$CV(x_4) \xrightleftharpoons[k_7]{k_6} PFC(x_5)$	...Step 4 (Formation of the 'pre-fusion complex')
$PFC(x_5) \xrightarrow{k_8} FUV(x_1) + NT(Out)$	...Step 5 (Vesicle fusion and release of neurotransmitter)

CV=clamped vesicle; DV=docked vesicle; FRV=free vesicle; FUV=fused vesicle; NT=neurotransmitter; PFC=pre-fusion complex.

conserved from neuromuscular junctions to central synapses, the present exercise enhances the confidence in the generality of the model.

Another important feature of neurotransmitter release concerns the total release, which is defined as the total amount of neurotransmitter released in response to a single presynaptic stimulation. It has been shown that total release saturates with the extracellular Ca^{2+} concentration, which is described as an empirical relation by Dodge and Rahamimoff [5]. This typical form of saturation is also reproduced with our model, although, in our model external calcium concentration has been represented by a particular rate parameter.

2. Experimental Observations

Some synapses (e.g., squid giant synapse [6], retinal amacrine cells [7] etc.) are *incapable* of maintaining a high level of release for protracted periods in response to sustained depolarization. These are called *phasic* synapses in contrast to other synapses that are known to be *tonic* in function. In order to investigate the origins of this phasic behaviour, i.e., the rapid decay of rate of release of neurotransmitter in spite of a high internal Ca^{2+} concentration, we have used a simple model of synaptic vesicle dynamics. Since most of the required parameters for simulating this behaviour has been recorded for hippocampal synapses, we have chosen to focus our attention on a recent work on this particular system [4].

Goda and Stevens (1994) monitored [4] the average synaptic transmission between pairs of hippocampal pyramidal neurons in primary culture and observed that with the arrival of a presynaptic nerve impulse, the rate of neurotransmitter release (Q) after the initial rapid rise apparently decayed biphasically satisfying the empirical relation

$$Q(t) = Me^{-mt} + Ne^{-nt} + L. \quad (1)$$

Similar biphasic decay has been observed in neuromuscular junctions by Barrett and Stevens [8]. In another experiment on rat neurohypophysial nerve terminals [9]

the intracellular Ca^{2+} concentration was monitored and the rate of neurohormone release was recorded simultaneously, which showed that in spite of a high level of internal Ca^{2+} concentration, the rate of release came down sharply. This property is further exemplified by a recent experiment [7] carried out in retinal amacrine cells, where a 100 ms presynaptic step depolarization was applied and it was observed that after the initial rapid rise, within ≈ 10 ms the rate of release dropped to a low level (20–25 percent of the peak release) and thereafter decayed slowly to attain the spontaneous release level. Since the sustained depolarization is accompanied with sustained Ca^{2+} current [6], it indirectly shows that Ca^{2+} removal by diffusion is not the cause of decay of evoked release.

It is further observed that the total release of neurotransmitter has a saturating dependence on extracellular Ca^{2+} concentration which satisfies an empirical relation as given by Dodge and Rahamimoff [5]

$$\text{Total release} = \left(\frac{U[\text{Ca}^{2+}]}{V + W[\text{Ca}^{2+}]} \right)^4. \quad (2)$$

This type of saturating dependence was also reported by Goda and Stevens [4]. We have analyzed the present model in the light of these two characteristics of neurotransmitter release.

3. Formulation of the Model

The model of synaptic vesicle dynamics is described in Table 1. Here, *step 1* concerns the production of free vesicles (FRV) from fused vesicles (FUV) by an endocytotic process followed by endosome fusion, budding and neurotransmitter uptake [3] which is practically unidirectional. Supply of neurotransmitter, i.e., neurotransmitter in the cytosol which is to be packed into FRV, is assumed to come from an infinite pool so that its concentration need not be considered explicitly in the rate equations.

In *step 2*, the free vesicles are docked (DV) at the plasma membrane as a result of interaction between plasma membrane associated proteins and vesicle membrane associated proteins. Concentrations of these proteins need not be shown explicitly in the reaction scheme for the following reasons. Firstly, the effective concentration of vesicle-membrane associated proteins is limited by the concentration of the host vesicle (assumption of binary collision). This becomes obvious when one considers the almost spherical shape of synaptic vesicles which enable only those protein molecules which face the plasma membrane to take an active role in the fusion related reactions. On the other hand, the plasma-membrane associated proteins are not explicitly considered because of the fact that they are more numerous than the vesicles and therefore, the reaction is practically a pseudo-first order one.

In *step 3*, the cytoplasmic domain of synaptotagmin is assumed to ‘clamp’ the docked vesicle near the fusion site to form clamped vesicles (CV) which then

becomes part of the release-ready pool of vesicles. The step 2 and step 3 together is known as ‘priming’ [3].

Step 4 involves the formation of the pre-fusion complex (PFC) wherein synaptotagmin ‘unclamps’ the vesicles with a Ca^{2+} -dependent rate constant k_6 and allows these to form PFCs. The value of k_6 is quite low at the resting condition of the cell but increases rapidly with the rise of local Ca^{2+} concentration. If the Ca^{2+} concentration within the pre-synaptic terminal is increased (iontophoretically, by depolarization or by any other mechanism) the value of k_6 increases several-fold to cause large amount of neurotransmitter release within a short period of time, i.e., the so-called evoked release.

Step 5 indicates the completion of the vesicle fusion and release of neurotransmitter outside the cell, giving rise to fused vesicle (FUV) and NT (Out), i.e., neurotransmitter released into the synaptic cleft.

We have represented these essential steps in the evolution of synaptic vesicles through various *states* by a system of coupled differential equations. According to the above model, the vesicles can exist in 5 different ‘states’ with concentrations $x_i(t)$, where i runs from 1 to 5. Since the major supply of the vesicles have been considered to come through the endocytotic route, a constraint arises in this model, namely, the conservation of total concentration (C) of all forms of vesicles, i.e.

$$C = x_1(t) + x_2(t) + x_3(t) + x_4(t) + x_5(t) = \text{Constant.} \quad (3)$$

This leaves only four independent metabolite concentrations. The kinetic scheme for Table 1, is, therefore, given by the matrix equation

$$\frac{d\mathbf{x}}{dt} = A\mathbf{x} - F, \quad (4)$$

where $\mathbf{x}$ is a concentration vector with elements x_1, x_2, x_3 and x_4 ,

$$A = \begin{pmatrix} -(k_1 + k_8) & -k_8 & -k_8 & -k_8 \\ k_1 & -k_2 & k_3 & 0 \\ 0 & k_2 & -(k_3 + k_4) & k_5 \\ -k_7 & -k_7 & -(k_7 - k_4) & -(k_5 + k_6 + k_7) \end{pmatrix},$$

$$F = \begin{pmatrix} -k_8 C \\ 0 \\ 0 \\ -k_7 C \end{pmatrix}.$$

4. Results and Discussion

Kinetics of evoked release have been simulated by perturbing the steady state of the above dynamical system and then looking at its evolution in time. Since k_6 is the Ca^{2+} -dependent rate constant, the effect of Ca^{2+} signal (stimulation) is achieved by increasing this rate constant to a level which exhibits the experimentally observed time scale of vesicle release. Rate of neurotransmitter release (i.e., the rate of production of NT (Out), neurotransmitter outside the presynaptic terminal) is given by $k_8 x_5$.

The total release, defined as the total amount of neurotransmitter released in response to a single presynaptic stimulation, can be calculated by finding the area under the curve representing the release rate as a function of time, i.e., by integrating the function $k_8 x_5$ over time. The Equations 3 and 4 were solved analytically to obtain x_5 as a function of time. Thus the formula for total release is as follows

$$\begin{aligned} \int_0^\tau \frac{d}{dt} \text{NT (Out)} dt \\ = \int_0^\tau k_8 x_5 dt = k_8 [(C - s_1 - s_2 - s_3 - s_4)\tau + p_1 + p_2 + p_3 + p_4], \end{aligned} \quad (5)$$

where τ is a sufficiently large time after which the rate of release practically becomes equal to the basal level. The p_i s are given by the equation

$$p_i = \frac{c_i(z_{i1} + z_{i2} + z_{i3} + z_{i4})}{r_i}(1 - e^{r_i\tau}). \quad (6)$$

Here c_i s are constants of integration determined from the knowledge of boundary conditions, r_i s are the eigenvalues of the coefficient matrix A and

$$\begin{pmatrix} z_{i1} \\ z_{i2} \\ z_{i3} \\ z_{i4} \end{pmatrix}$$

are the corresponding eigenvectors. s_i s are elements of the column matrix $A^{-1}F$. However, the above relation will hold only when all the eigenvalues of the matrix A are real.

For computing with numbers we have confined our attention on the synaptic terminals associated with hippocampal neurons for which most data are available (Table 2). To reproduce the release kinetics, analytical solutions of the coupled differential equations were obtained at particular sets of *allowed* values of the parameters. On stimulation, the rate of release at first rapidly increases to reach a maximum and then decays in spite of the raised value of k_6 (Figure 1). This

Table 2. Values of the known parameters and steady state metabolite concentrations for hippocampal neurons

Parameter	Value	Reason for the choice
C	$3.66 \mu\text{M}$	Total number of vesicles per terminal $\sim 200\text{--}400$
k_1	0.01 s^{-1}	Synaptic vesicle recycling time $\sim 100 \text{ sec}$
k_2	0.1 s^{-1}	Time constant of replenishment of the readily releasable vesicles (priming) $\sim 10 \text{ sec}$
k_8	5000 s^{-1}	Synaptic delay between membrane depolarization and neurotransmitter release $\sim 0.2 \text{ ms}$
x_4^0	$0.2 \text{ to } 0.4 \mu\text{M}$	Number of readily releasable vesicles/terminal $\sim 12\text{--}24$
$k_8 x_5^0$	$2.7 \times 10^{-4} \text{ to } 5.4 \times 10^{-4} \mu\text{M/s}$	The rate of neurotransmitter release at the steady state $\sim 1\text{--}2 \text{ vesicles/minute/terminal}$
x_5^0	$5.4 \times 10^{-8} \text{ to } 1 \times 10^{-7} \mu\text{M}$	Using value of k_8

All the values are taken from Südhof (1995) (The presynaptic terminal volume = $0.1 \mu\text{m}^3$) Ranges of the *allowed* values of the other parameters are evaluated from the fixed point solution of Equation (4) with the following constraints: (a) none of the steady state concentrations can be negative; (b) all the eigenvalues of the Jacobian of Equation (4) should have negative real parts; (c) x_4^0 and x_5^0 are known with a close range; (d) there exist some intuitive ideas about the relative magnitudes of the rate parameters. The chosen values of the other rate parameters in the present computation are: $k_3 = 1.0$; $k_4 = 10.0$; $k_5 = 10.0$; $k_6 = 0.01$ (basal value); $k_7 = 0.00001$ (All values are per sec.). The values of steady state vesicle concentrations are (in μM): $x_1^0 = 0.279$; $x_2^0 = 2.82$; $x_3^0 = 0.279434$; $x_4^0 = 0.279$.

decay of neurotransmitter release rate has been found to be *biphasic* with a gradual lowering of maximal release rate with decreasing perturbation of the parameter k_6 (Figures 2a and 2b). The total release also saturates rapidly (Figure 3) with increasing values of k_6 with a cooperativity parameter of value 4. Thus, the general features of neurotransmitter release are satisfied by this model.

It is interesting to note that the expression for the rate of release has four exponential decay terms, which implies that the decay of rate of release may have four components. However, how many of them ultimately show up in the realistic physical situation depend entirely on the values of the rate parameters. We emphasize that although we have used the parameter values for hippocampal synapses to reproduce the observations of a particular experiment [4], our main goal is to show that the present model has the property that even a sustained high Ca^{2+} concentration cannot stop the rapid decay of rate of release of neurotransmitter.

Though the primary motive of the aforesaid exercise is to show the qualitative agreement of our model with the general features of the neurotransmitter release, a discussion on the basis of the actual values of the parameters used in the model may help to have a better understanding of the internal dynamics of the model and its consistency. The eigenvalues of the Jacobian of the system play a most crucial role

Figure 1. Kinetics of neurotransmitter release on the basis of the model (Table 1), using the parameter values given in Table 2. The Ca^{2+} -dependent rate constant k_6 is increased from its basal value to simulate inflow of Ca^{2+} ions in the presynaptic terminal. It may be noted that with low values of k_6 , the instantaneous rise in the release rate is low but it decays more slowly, maintaining a higher value of release rate for a considerable length of time. Goda and Stevens [4] observed the same feature while replacing Ca^{2+} by Sr^{2+} . In this theoretical exercise it appears to be a common feature at low values of k_6 , which may be assumed to represent the experimental condition obtained by replacing Ca^{2+} ions with Sr^{2+} ions in the bathing solution.

in determining the number of observable exponential decay terms. For example, in the particular range of parameters used here (Table 2), two out of four eigenvalues have absolute magnitudes ranging between a few tens to a few hundreds. These two contribute to the ultimate expression of release-rate to give rise to two exponential decay (since they are negative) terms showing time constants of the order of 0.001 to 0.1 seconds. The other two eigenvalues are found to have magnitudes beyond the aforesaid range, that is, well above thousand or well below one. Again, the term with large eigenvalue comes with a negative weight factor. Therefore it contributes to the very fast rate of rise of the release-rate curve (time constant of the order of 0.001 s or below). The other eigenvalue with extremely low magnitude gives rise to a decay term which is not in the observed time scale (i.e., the time constant is above 1 s). The weight factors, contributed by the eigenvectors of the Jacobian, also determine some features, for example, particularly the peak release rate. It is interesting to note that the large value of the rate constant k_8 compared to the other rate constants is responsible for the very large magnitude of one of the eigenvalues (several thousands) of the Jacobian. If, for example, one lowers the value of k_8 to 10 s^{-1} , the rapid decay of release rate is no longer observed (Figure 4). Some of the weight factors also become negative, and multiplied by diminishing functions

Figure 2. Biphasic nature in the decay of neurotransmitter release at two elevated values of k_6 . The dotted curves show the values calculated with the help of the model given in Table 1 using the parameter values as given in Table 2. The solid lines show the best-fitted curve using Equation (1). Fitted parameters are given as insets. Since the second phase of decay is extremely weak compared to the first phase, we have plotted these curves on a semi logarithmic scale for a better display of both phases of decay, which are otherwise not clearly distinguishable. These curves can be compared with those given in Figure 1 of [4]. Our emphasis, however, is only on the nice qualitative agreement.

of time, they contribute to a release-rate profile with a smaller rate of rise and a much slower decay. Therefore, it appears that a fast fusion process which removes the pre-fusion complexes (PFC) as soon as they are formed is necessary for the rapid decay of release. In absence of such efficient removal of PFCs, the typical synchronous rise in the release-rate followed by a rapid decay is not observed according to our model. Moreover, the increase in the rate of release in response to the stimulus (raised value of k_6) is almost an order of magnitude less than that observed when k_8 is very large which becomes evident as one compares the peak releases associated with the Figures 1 and 4.

The saturation of total release with k_6 (Figure 3) can also be rationalized from the point of view of synaptic vesicle dynamics. For k_6 values up to 1 s^{-1} (after perturbation) the total release increases very slowly. Beyond this range of k_6 total release increases steeply to reach a saturation level of $0.6 \mu\text{M}$ (approximately). A closer look at the synaptic vesicle network (Table 1) reveals that before the actual fusion process, the vesicles are available in four states (free, docked, clamped/primed, pre-fusion complex) which maintain a state of equilibrium among themselves.

Figure 3. Dependence of total release to Ca^{2+} concentration. The solid points are the values of the total release at various values of k_6 , as calculated with the help of Equation (5). The cut-off parameter $\tau = 0.5$ sec. The solid line is the curve fitted with Dodge–Rahamimoff Equation (2) in which Ca^{2+} concentration is replaced by k_6 . Values of the fitted parameters are shown as inset.

Figure 4. Dependence of the rate of neurotransmitter release with time calculated using the model (Table 1) for a lower value of k_8 (10 s^{-1}). Other parameter values are the same as in Figure 1. k_6 was perturbed to a value of 200 s^{-1} .

Conversion of FRV to DV is very slow ($k_2 = 0.1 \text{ s}^{-1}$) which is almost comparable to the rate of conversion of CV to PFC at the resting state (basal value of $k_6 = 0.01 \text{ s}^{-1}$). When the magnitude of k_6 increases with the arrival of calcium

signal, the conversion of CV to PFC becomes very fast whereas the conversion of FRV to DV remains slow as before. Therefore, with a gradual increase of k_6 , more and more DV, CV and PFCs are converted to fused vesicle which are unable to recycle back to FRV rapidly owing to the very slow time constant of that process ($k_1 = 0.01 \text{ s}^{-1}$, Table 2). When k_6 is perturbed to 100 s^{-1} or above, almost all the available store of vesicles in the form of DV, CV and PFC are exhausted, which explains why the perturbation of k_6 above 100 s^{-1} cannot bring about the release of a larger amount of vesicles. This becomes obvious when one sums up the amounts of DV, CV and PFC in the resting state (which gives a total of $0.558 \mu\text{M}$) and compares it with the level of saturation of total release ($\approx 0.6 \mu\text{M}$). The small difference between these two can be attributed to the conversion of some FRV to DV during the release process which follows the usual route to fusion.

5. Conclusion

We have earlier been able to select a proper model of synaptic vesicle dynamics [1] on the basis of experimental data and algebraic consistency. We have now used that selected model to reproduce the observed biphasic decay of neurotransmitter release in hippocampal neurons. Moreover, the model also agrees with the empirical law suggested by Dodge and Rahamimoff [5] for total release of neurotransmitter. The very structure of the synaptic vesicle cycle (along with the rate parameters) seems to hold the key to these major synaptic properties as we did not make any assumption about the role played by Ca^{2+} ions or the effect the spatio-temporal distribution of intracellular Ca^{2+} concentration on the release kinetics. In a recent work [10], synaptic vesicle exocytosis has been modeled successfully by assuming a four stage binding of Ca^{2+} -ions to the release machinery, but our model does not require any such assumption regarding the specific role of Ca^{2+} ions in the fusion process.

In case of sustained depolarization, the calcium concentration near the vesicle-fusion machinery cannot come down as rapidly as the decay of the neurotransmitter release-rate occurs. Llinás and his coworkers [6] have shown that the calcium current remains high as long as depolarization is maintained. Therefore, the rapid decay of release in such cases cannot be ascribed to the depletion of Ca^{2+} ions in the active (fusion) zone. Our model indicates that the very dynamics of the synaptic vesicle cycle is responsible for this decay. Moreover, the present model has been found to corroborate other salient features of evoked neurotransmitter release, including invariance of time course during changes in the depolarization level, external Ca^{2+} concentration or the application of conditioning prepulses [11]. Although, at this stage it may not be possible to say anything conclusive about the detailed mechanism of synaptic vesicle exocytosis entirely from a theoretical standpoint, such models certainly help in understanding the course of events in a better way.

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