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The probability of exocytosis: the dependence on Ca^{2+} sensing latency times, Ca^{2+} channel kinetic parameters and clustering.

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Abstract

The release of secrete from secretory vesicles and granules requires a transient increase of intracellular Ca^{2+} concentration in the vicinity of the fusion site. Usually there is a short delay to the onset of the actual fusion of membranes, that is described as a latency time of the Ca^{2+} sensing system of the secreting machinery. The delay time affects in an essential way the probability for a certain granule to fuse with the plasmalemma and to release its contents into the extra-cellular space. We have investigated here, theoretically and numerically, the extent of this influence and its dependence on the parameters of Ca^{2+} channels, channel clustering, the Ca^{2+} sensing system, and the length of depolarizing pulses. We used a linear probabilistic model for a random opening and closing of channels, that yields an explicit expression for the Laplace transforms of the waiting time distributions for at least one channel to be open during the latency time. This allows one in principle to calculate the probability that a vesicle will fuse with plasmalemma during the action potential. We used the theoretical results to test our numerical simulations.

Introduction

In many types of secreting cells such as neurons, pancreatic β-cells and others, secrete is released into extra-cellular space when Ca^{2+} entering through voltage-sensitive Ca^{2+} channels triggers the fusion of secrete-containing vesicles with the plasma membrane (Katz 1969; Rorsman 1997). The structural and functional organization of mechanisms involved in Ca^{2+} sensing, the fusion of vesicle and cell membranes, and the release of substances have been investigated lately in great detail, mainly in neurons (Bennett and Scheller 1994) but also in other cells that use the same vesicular way of exporting their products (Lang 1999). A set of proteins involved in these processes, for example, the plasma membrane SNARE proteins syntaxin and SNAP-25 (Sheng, Westenbroek, and Catterall 1998; Kim and Catterall 1997) and the putative Ca^{2+}-sensing molecule synaptotagmin (Wiser 1999), have been identified. It is widely believed that these Ca^{2+} sensing proteins are localized in the close vicinity of Ca^{2+} channels, since the triggering of release requires high local concentrations – 50-200 μM – of the ion. The importance of this is emphasized by the observation that exocytosis echoes Ca^{2+} channel activity and stops immediately upon repolarization (Ämmälä 1993). Additionally, the sensing molecules must be subjected to the increased levels of Ca^{2+} during some latency time for the fusion of a vesicle with the plasma membrane to occur. These latency times or the delay of fusion vary from several ms in neurons to several tens of ms in other hormone secreting cells (Yazejian, Sun, and Grinnell 2000; Barg 2001). Obviously, during the latency period certain structural changes take place inside the docking complex that finally lead to the fusion of membranes.

The existence of latency time introduces into the secretory system an additional parameter that essentially alters the exocytosis probability, i.e. the probability that a vesicle will fuse with the plasmalemma and release its contents during the action potential. If there is no delay in the sensing of increased Ca^{2+}, the exocytosis probability is determined by the mean first latency time of Ca^{2+} channels, since any opening of a channel situated close enough to the fusion site will trigger the process. The latency of fusion activation asks that at least one channel must be open during a time period equal to the latency time. This inevitably leads to the decrease of exocytosis probability. The amount of change will depend on how large is the latency time in the Ca^{2+} sensing system and on the kinetics of Ca^{2+} channels involved. In case of simple two-state channels this effect is fully defined by the mean open and shut times of the channel. Obviously, channel clustering in the vicinity of docked granules is a very effective way to increase the probability of exocytosis. In synapses, where the exocytosis speed is essential, more than 60 channels may open simultaneously per single granule. Such an arrangement is possible in places of high concentration of Ca^{2+} channels, as is the case in neuronal synapses. In other cell types, e.g. in pancreatic β-cells, the number of Ca^{2+} channels is small (450 channels per cell), latency times larger (Barg 2001), and geometrical constraints on channel and granule placing may be much harder to satisfy because of the impossibility to use many channels to secure the release of hormone from a single granule. Therefore it is important to understand in detail how the probability of exocytosis depend on Ca^{2+} channel kinetic parameters such as mean open and shut times, m_o and m_s, the latency time of the Ca^{2+} sensing system and the channel clustering around the docking sites of secretory granules or vesicles.

Here we investigate the distribution of waiting times for the event that at least one channel is open for a certain time. We also calculate the probability for a single exocytosis event in some special cases.

Theory

Throughout this paper it is assumed that all N ion channels localized in the vicinity of a secretory granule are identical and may be in two states: open or shut. Open times are assumed to be identically distributed non-negative random variables having distribution function F_o, finite mean m_o and a density function f_o. Similarly, shut times are identically distributed non-negative random variables with distribution function F_s, finite mean m_s and a density function f_s. The Ca^{2+} sensing system of the vesicle has a latency time t_l. Having these initial conditions we shall calculate the probability of a single exocytosis event assuming that the cell was subjected to the depolarizing pulse of duration t_p. As explained above a single exocytosis event will take place if the vesicle is subjected to a high local Ca^{2+} concentration during at least the latency time t_l. The necessary increase in Ca^{2+} concentration is achieved only (Ämmälä 1993) when one or several Ca^{2+} channels in the vicinity of the granule stays open during t_l. Channels stay closed in a resting state. After a depolarizing pulse of duration $t_p > t_l$ has been applied these channels start opening at random times. In this situation, the sought of probability that an elementary exocytosis event occurs equals the probability of an event that the waiting time for at least one channel to be continuously open during t_l is less than t_p.

Mathematical model

The waiting time distribution can be analyzed in a simple model, assuming each channel to obey a simple exponential decay law for its switching between the open and closed states. Consider a single channel, and let $P_0(t)$ and $P_1(t)$ be the probability that the channel is open and closed, respectively, at a certain point t in time. Then P_0 and P_1 evolve according to

$$\dot{P}_0 = -aP_0 + bP_1, \quad (1)$$
$$\dot{P}_1 = aP_0 - bP_1, \quad (2)$$

consistently with $P_0 + P_1 = 1$. Here a and b denote the switching rates, $a = 1/m_s, b = 1/m_o$

For a collection of N identical (but independent) channels, define P_k as the probability that precisely k of the N channels are open at a certain time, defining a vector $\mathbf{P} =$

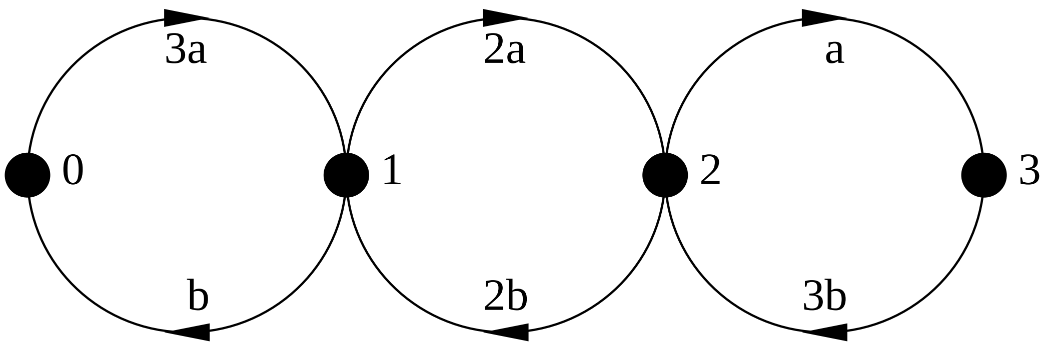

Figure 1: Transition rates between the states of a system of 3 identical channels, in terms of the opening rate $a = 1/m_s$ and the closing rate $b = 1/m_o$. The small black circles represent states with 0, 1, 2, and 3 channels open, respectively.

$(P_0, P_1, \ldots, P_N)$. Then, the evolution of $\mathbf{P}$ is governed by a linear equation,

$$\dot{\mathbf{P}} = \mathbf{M}\mathbf{P}, \tag{3}$$

in terms of a sparse matrix $\mathbf{M}$,

$$\mathbf{M} = \begin{pmatrix} -Na & b & 0 & \cdots & 0 & 0 \\ Na & -(N-1)a-b & 2b & \cdots & 0 & 0 \\ 0 & (N-1)a & -(N-2)a-2b & \cdots & 0 & 0 \\ \cdots & \cdots & \cdots & \cdots & \cdots & \cdots \\ 0 & 0 & 0 & \cdots & -a-(N-1)b & Nb \\ 0 & 0 & 0 & \cdots & a & -Nb \end{pmatrix}, \tag{4}$$

consistent with the conservation of probability, $\sum_k P_k = 1$. The diagonal element $M_{kk} = -(N-k)a - kb$ determines the distribution $f_k(t)$ of dwell times in a state with k channels open,

$$f_k(t) = ((N-k)a + kb)\,\mathrm{e}^{-((N-k)a+kb)t} \tag{5}$$

while the off-diagonal elements yield the transition rates for $k \to k \pm 1$, see Fig. 1 for an illustration. Although not symmetric in itself, $\mathbf{M}$ is equivalent to a symmetric matrix[1], implying real eigenvalues. Indeed, it is easily diagonalized, and has the eigenvalues $0, -(a+b), -2(a+b), \ldots, -N(a+b)$, with the eigenvector associated with the zero eigenvalue corresponding to a binomial equilibrium distribution,

$$P_k^* = \begin{pmatrix} N \\ k \end{pmatrix} \frac{a^k b^{N-k}}{(a+b)^N}, \tag{6}$$

[1]By means of a similarity transform

for the number k of open channels.

Dwell time distributions

We next need to compute the distribution of dwell times in the aggregate of states with at least one channel open, i.e. $k \geq 1$. Therefore, define $g_k(t)$ as the distribution of dwell times in states with at least k units open. Obviously, $g_N = f_N$.

For lower k, g_k can be obtained recursively as follows. Denote by d_k the rate for $k \to k-1$, and by u_k the rate for $k \to k+1$; they amount to

$$
\begin{aligned}
d_k &= kb, && (7)\\
u_k &= (N-k)a. && (8)
\end{aligned}
$$

First note that each time interval with at least $k > 0$ channels open must start with a transition $k-1 \to k$. Then, the next change in k is either, with probability $d_k/(d_k+u_k)$, back to $k-1$ – and that's the end of that interval –, or, with probability $u_k/(d_k+u_k)$, a jump up to $k+1$, after which the systems dwells with more than k channels open for a period of time distributed according to g_{k+1}, after which it will return to k, and we are back at square one. Thus, after the initial transition from $k-1$ to k, the number of open channels may jump back and fourth between k and more than k any number (possibly zero) of times, and finally the interval ends with a transition from k back to $k-1$.

As a result, $g_k(t)$ must satisfy the integral equation

$$
\begin{aligned}
g_k(t) \;=\; & \frac{d_k}{u_k+d_k} f_k(t) + \\
& \frac{u_k}{u_k+d_k} \int_0^t \mathrm{d}x \int_0^{t-x} \mathrm{d}y\, f_k(x)\, g_{k+1}(y)\, g_k(t-x-y). \qquad (9)
\end{aligned}
$$

This is most easily solved by means of the Laplace transform, yielding

$$
\tilde{g}_k(s) = \frac{d_k}{u_k+d_k}\tilde{f}_k(s) + \frac{u_k}{u_k+d_k}\tilde{f}_k(s)\tilde{g}_{k+1}(s)\tilde{g}_k(s), \qquad (10)
$$

in terms of the Laplace-transformed distributions,

$$
\tilde{g}_k(s) = \int_0^\infty g_k(t)e^{-st}\mathrm{d}t, \qquad (11)
$$

etc. From this, $\tilde{g}_k(s)$ can be extracted as

$$\tilde{g}_k(s) = \frac{d_k \tilde{f}_k(s)}{d_k + u_k \left(1 - \tilde{f}_k(s)\tilde{g}_{k+1}(s)\right)}, \tag{12}$$

Using that f_k has the simple Laplace transform

$$\tilde{f}_k(s) = \frac{u_k + d_k}{s + u_k + d_k} \tag{13}$$

that follows from Eq. 5, we obtain the recursive equation for $\tilde{g}_k$,

$$\tilde{g}_k(s) = \frac{d_k}{s + u_k + d_k - u_k \tilde{g}_{k+1}(s)}, \quad \text{for } 0 < k < N \tag{14}$$

which in $N-1$ steps yields $\tilde{g}_1(s)$, starting from $\tilde{g}_N(s) = \frac{u_N + d_N}{s + u_N + d_N} = \frac{Nb}{s + Nb}$. Note that this must yield $\tilde{g}_1$ as a rational function of s, corresponding to $g_1(t)$ being a linear combination of exponentials,

$$\tilde{g}_1(s) = \sum_{i=1}^{N} \frac{C_i}{s + \omega_i}, \tag{15}$$

$$g_1(t) = \sum_{i=1}^{N} C_i e^{-\omega_i t} \tag{16}$$

The coefficients C_i and the exponents ω_i can be obtained by making a partial fraction expansion of $\tilde{g}_1(s)$.

Eq. 16 can also be understood by altering the dynamics (3), replacing $\mathbf{M}$ by a modified matrix $\hat{\mathbf{M}}$ where the connections between $k = 0$ and $k = 1$ are set to zero, corresponding to cutting the two leftmost arcs in Fig. 1). As a result, probability is no longer conserved – the probability previously transferred between $k = 0$ and 1 is now lost. From an initial state with $k = 1$, the total probability will decay according to $g_1(t)$. This process can be analyzed in terms of the eigenvectors of $\hat{\mathbf{M}}$, implying that g_1 must have the form (16), with $\omega_i > 0$ the negatives of the eigenvalues of $\hat{\mathbf{M}}$.

While $f_0(t)$ describes the length distribution of time intervals with all channels closed, $g_1(t)$ plays the same role for the interlaced intervals where at least one channel is on.

The waiting time distribution

The sought distribution $w(t)$ of waiting times, for at least one channel to be open during a period of time of length at least t_l, can now be derived from f_0 and g_1; also this step is best done in terms of Laplace transforms. First, we must split g_1 in two parts, $g_<$ and $g_>$, according to

$$g_<(t) = \begin{cases} g_1(t), & t < t_l \\ 0, & t > t_l \end{cases} \quad ; \quad g_>(t) = \begin{cases} 0, & t < t_l \\ g_1(t), & t > t_l \end{cases} \quad . \tag{17}$$

Obviously, $g_< + g_> = g_1$. The corresponding Laplace transforms can be simply obtained once $\tilde{g}_1$ is written in the form (15), yielding

$$\tilde{g}_<(s) = \sum_{i=1}^{N} \frac{C_i}{s+\omega_i}\left(1 - e^{-t_l(s+\omega_i)}\right), \tag{18}$$

$$\tilde{g}_>(s) = \sum_{i=1}^{N} \frac{C_i}{s+\omega_i} e^{-t_l(s+\omega_i)}. \tag{19}$$

Then we can run the same trick again: The waiting time interval starts with $k = 0$ for a period of time distributed according to f_0, after which k stays non-zero for a period of time distributed according to g_1. If this is larger than t_l, we only count t_l, and exit; otherwise, k becomes zero again, and the whole process starts over. This implies the following integral equation for $w(t)$, in terms of the probability $p_< = \tilde{g}_>(0)$ that the dwell time with $k > 0$ is larger than t_l,

$$w(t) = p_> f_0(t - t_l)\Theta(t - t_l) + \int_0^t \mathrm{d}x \int_0^{t-x} \mathrm{d}y f_0(x) g_<(y) w(t - x - y). \tag{20}$$

For the Laplace transforms, this becomes

$$\tilde{w}(s) = \tilde{g}_>(0)\tilde{f}_0(s)e^{-t_l s} + \tilde{f}_0(s)\tilde{g}_<(s)\tilde{w}(s), \tag{21}$$

and $\tilde{w}$ can be solved for, yielding

$$\tilde{w}(s) = \frac{\tilde{g}_>(0)\tilde{f}_0(s)e^{-t_l s}}{1 - \tilde{f}_0(s)\tilde{g}_<(s)}. \tag{22}$$

Note that in the limit $t_l \to 0$ of vanishing latency time, we obtain $\tilde{w}(s) \to \tilde{f}_0(s)$, as is to be expected. The only remaining problem then is to extract the waiting time distribution $w(t)$ from its Laplace transform $\tilde{w}(s)$, a task complicated by the appearance of exponential factors in $\tilde{g}_<$ in the denominator in Eq. 22.

The average waiting time

Here we will limit ourselves to the simpler task of extracting the average waiting time; more elaborate calculations will be made in a forthcoming article. For a length distribution such as w, the average and higher moments are readily available by means of a Taylor expansion of $\tilde{w}$,

$$\tilde{w}(s) = \sum_{n=0}^{\infty} \frac{(-s)^n}{n!} \langle t_w^n \rangle \tag{23}$$

yielding $\tilde{w}(0) = 1$, reflecting the normalization of $w(t)$, and

$$\langle t_w \rangle = - \left. \frac{\mathrm{d}\tilde{w}}{\mathrm{d}s} \right|_{s=0} \tag{24}$$

etc. Keeping in mind that $g_<$ and $g_<$ are normalized not to unity, but to $p_<$ and $p_>$, respectively, differentiating Eq. 21 with respect to s, and setting $s = 0$, yields

$$\langle t_w \rangle = t_l + \frac{1}{p_>} \langle t_0 \rangle + \frac{p_<}{p_>} \langle t_< \rangle \tag{25}$$

where $\langle t_0 \rangle = \frac{1}{Na}$ is the average interval length in the distributions f_0, while

$$\langle t_< \rangle = \frac{1}{p_<} \sum_i \frac{C_i}{\omega_i^2} \left(1 - (1 + t_l \omega_i)\, e^{-t_l \omega_i} \right) \tag{26}$$

is the corresponding average in $g_<$. We also need

$$p_> = \sum_i \frac{C_i}{\omega_i} e^{-t_l \omega_i} \tag{27}$$

and $p_< = 1 - p_>$. Thus, we finally obtain the average waiting time as

$$\langle t_w \rangle = t_l + \frac{\frac{1}{Na} + \sum_{i=1}^{N} \frac{C_i}{\omega_i^2} \left\{ 1 - (1 + t_l \omega_i)\, e^{-t_l \omega_i} \right\}}{\sum_{i=1}^{N} \frac{C_i}{\omega_i} e^{-t_l \omega_i}} \tag{28}$$

Cases $N = 1$, 2 and 3.

For the case of a single unit, we have $\tilde{g}_1(s) = \tilde{f}_1(s) = \frac{b}{s+b}$, yielding $C_1 = \omega_1 = b$, and we have for $N = 1$ the result

$$\langle t_w \rangle = t_l + \frac{e^{bt_l}}{a} + \frac{e^{bt_l} - 1 - bt_l}{b} \tag{29}$$

For $N = 2$, we obtain

$$\tilde{g}_1(s) = \frac{b(s+2b)}{s^2 + (a+3b)s + 2b^2} \tag{30}$$

yielding

$$\omega_{1,2} = \frac{1}{2}\left(a + 3b \pm \sqrt{a^2 + 6ab + b^2}\right) \tag{31}$$

$$C_{1,2} = \frac{b\left(\pm(a-b) + \sqrt{a^2 + 6ab + b^2}\right)}{2\sqrt{a^2 + 6ab + b^2}} \tag{32}$$

Similarly, for $N = 3$, we obtain

$$\tilde{g}_1(s) = \frac{b\left(s^2 + (a+5b)s + 6b^2\right)}{s^3 + (3a+6b)s^2 + \left(2a^2 + 7ab + 11b^2\right)s + 6b^3} \tag{33}$$

from which $\omega_{1,2,3}$ and $C_{1,2,3}$ can be extracted, by solving a cubic equation.

Numerical simulations

To investigate numerically the dependence of exocytosis probability on ion channel clustering and to test our theoretical results, we carried out numerical simulations on a system of identical and independent two-state channels. A set of dwell times for a single channel in shut and open states was generated with the help of a source equation

$$\tau_k = -\tau \ln(\mathrm{Rnd}) \tag{34}$$

where τ is equal to m_o or m_s, τ_k $(k = 1, 2, 3, \ldots, n)$ are the consecutive dwell times in a given state, and Rnd is a random number chosen uniformly inside the interval 0 to 1. At the beginning of the simulation process all channels were assumed to be in the shut state. This corresponds to the experimental situation just before the application of a depolarizing pulse to a resting plasma membrane when all Ca^{2+} channels are shut. In case of two two-state channels dwell times were calculated in two independent 10 sec. runs and the obtained single channel records were superimposed; for three channels the same calculations were repeated three times with the subsequent superimposition of single records, and so on. The resultant superposition process for three channels ($m_o = 1.9$ ms and $m_s = 5$ ms) is seen in Fig. 2.

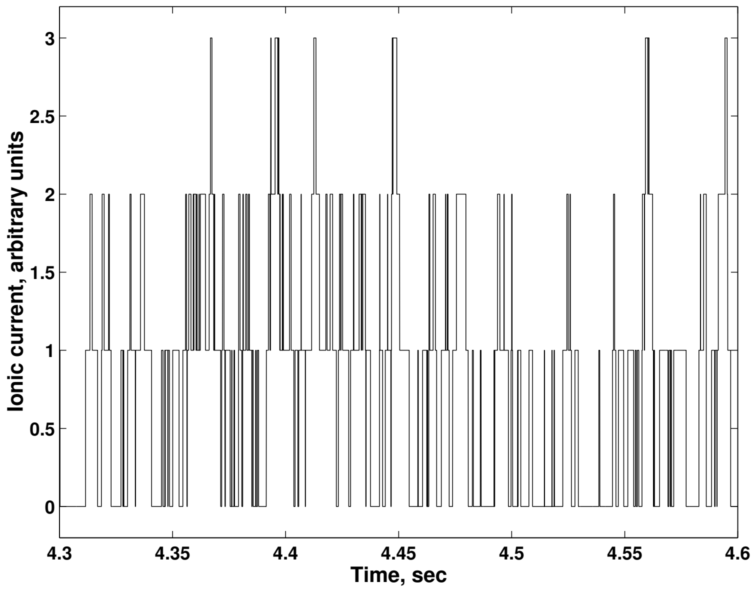

Figure 2: A continuous segment (300 ms) of the simulated superposition process for the three channel, two-state system with $m_o = 1.9$ ms and $m_s = 5$ ms.

For every individual simulation process, i.e. for every size of channel cluster, the waiting time for the event "at least one channel is open for t_l" was detected; this was repeated 400 times. Then the distribution of waiting times, $w(t)$, was estimated from binned data. An example of a histogram obtained is seen in Fig. 3 for a cluster that contains three channels, with $m_o = 1.9$ ms, $m_s = 5$ ms and $t_l = 5$ ms.

To compare simulation data with theoretical predictions, we calculated the average waiting time for the case of three channels, both from numerical simulation data shown in Fig. 4, yielding the value 0.0516, and theoretically, using Eq. 28, which yielded the value 0.0523, in good agreement with simulation data (see Fig. 3).

The simulation data were extended for other multiple channel systems up to ten channels; these data are summarized in Figs. 4–6. The importance of channel clustering in the case of large delay times is obvious from these figures. E.g., in the case of a 10 ms latency time, depolarizing pulses shorter than 20 ms practically do not induce exocytosis, even with clusters containing ten channels.

Discussion

We have shown here that a delay to the release of substances from secretory granules and vesicles, or a latency time in the Ca^{2+} sensing system, may change in an essential way the probability of the single exocytosis event. The analysis of this phenomenon presented above

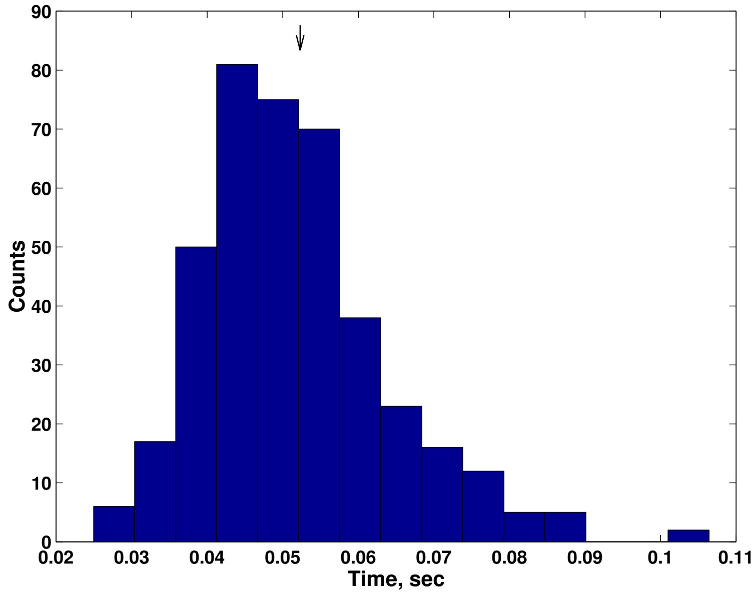

Figure 3: A histogram of the waiting times for the superposition process shown in Fig. 2. The little arrow in the upper part marks the theoretical average, 0.0523 s.

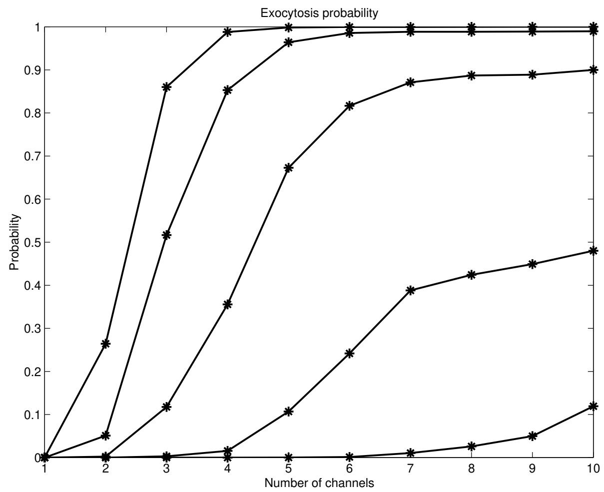

Figure 4: Exocytosis probability as it depends on the number of channels in cluster and the duration of depolarization pulse. The duration of pulse (from bottom to top): 10, 20, 30, 40 and 50 ms. t_l = 2 ms.

provides insight into how the exocytosis probability depends on the size of the delay time, on kinetic properties of Ca^{2+} channels, and on the character of the stimulus.

In a simple linear probabilistic model, we could derive expressions for the Laplace transform of the waiting time distribution, allowing us to readily calculate e.g. the average waiting time, the most important parameter from an experimental point of view. Knowledge of this and other properties of the waiting time distribution allows one to estimate the percentage of granules that will be secreted by a depolarizing pulse.

A more complete calculation of exocytosis probabilies requires more detailed knowledge about the distribution function of waiting times for the event that at least one channel is open during the latency time t_l. Work in this direction is in progress.

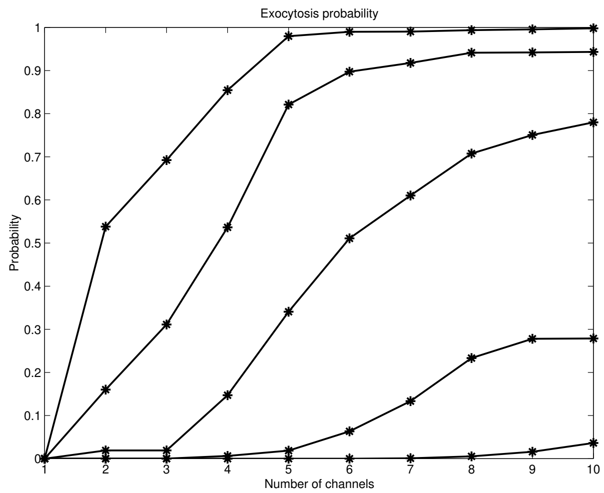

Figure 5: Exocytosis probability as it depends on the number of channels in cluster and the duration of depolarization pulse. The duration of pulse (from bottom to top): 10, 20, 30, 40 and 50 ms. t_l = 5 ms.

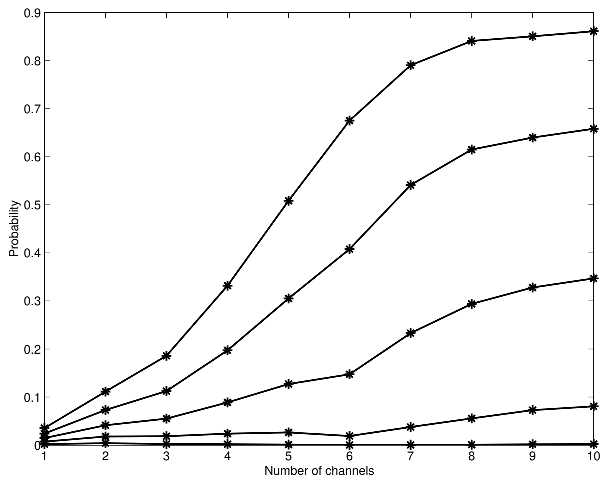

Figure 6: Exocytosis probability as it depends on the number of channels in cluster and the duration of depolarization pulse. The duration of pulse (from bottom to top): 10, 20, 30, 40 and 50 ms. t_l = 10 ms.

Besides giving the theoretical rates of exocytosis under different conditions, the results of this investigation provide also a rationale behind the structural organization of secreting machinery in various types of cells.

In synapses where the speed of exocytosis is usually most important the neutralization of the delay time in sensing the rising of Ca^{2+} concentrations is achieved by arranging a large number of Ca^{2+} channels that open per one secretory vesicle. Such an arrangement increases the probability of exocytosis practically to unity, even for short, i.e. comparable to the latency time t_l, depolarizing pulses (Wu 1999).

In other cell types where speed may be not the prime object the morphological consequences of the delay time may take different forms. For example, in insulin secreting β-cells of the pancreas, the main problem faced by the secreting machinery is the low number of Ca^{2+} channels available per one insulin granule. In case of a diffuse and random distribution of these channels over the whole surface of β-cells, the functioning of the secreting machinery will not be effective. Large amounts of energy will be needed to remove from the cytoplasm the excessive amounts of Ca^{2+} flooded into the cell in order to reach high local levels of the ion in the vicinity of releasable granules for a sufficiently long time. To counteract the effect of the latency time, which in these cells is about 10 ms, they organize Ca^{2+} channels into clusters of three. Additionally, these triplets are closely associated with docking sites of matured insulin granules (Barg 2001).

The rate of exocytosis from any type of cell is a very important factor and therefore it is subject to strict regulation. This rate can be changed through the modification of different parameters of the secreting machinery. Our investigation shows that the latency time in the Ca^{2+} sensing system and the clustering of channels may be two convenient targets for easily adjusting the rate of release for hormones or neurotransmitters from cells.

In conclusion it is to be mentioned that the kinetics of real ion channels is much more complicated than the two-state kinetics discussed in this article. Further analysis is necessary in order to assess the effect of more sophisticated kinetics on the rate of exocytosis.

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