The Amplitude Distribution of Release Events through a Fusion Pore

Stephen W. Jones* and David D. Friel†
*Department of Physiology and Biophysics; and †Department of Neurosciences, Case Western Reserve University, Cleveland, Ohio 44106

ABSTRACT Neurotransmitters, hormones, or dyes may be released from vesicles via a fusion pore, rather than by full fusion of the vesicle with the plasma membrane. If the lifetime of the fusion pore is comparable to the time required for the substance to exit the vesicle, only a fraction of the total vesicle content may be released during a single pore opening. Assuming 1), fusion pore lifetimes are exponentially distributed (τ_P), as expected for simple single channel openings, and 2), vesicle contents are lost through the fusion pore with an exponential time course (τ_D), we derive an analytical expression for the probability density function of the fraction of vesicle content released (F): $dP/dF = A\,(1 - F)^{(A-1)}$, where $A = \tau_D/\tau_P$. If $A > 1$, the maximum of the distribution is at $F = 0$; if $A < 1$, the maximum is at $F = 1$; if $A = 1$, the distribution is perfectly flat. Thus, the distribution never has a peak in the middle ($0 < F < 1$). This should be considered when interpreting the distribution of miniature synaptic currents, or the fraction of FM dye molecules lost during a single fusion pore opening event.

Received for publication 30 November 2005 and in final form 3 January 2006.

Address reprint requests and inquiries to Stephen W. Jones, Tel.: 216-368-5527; E-mail: swj@case.edu.

The contents of secretory vesicles may be released via a transient fusion pore, without the vesicle completely merging with the plasma membrane (1,2). Fusion pore closure could affect rapid release of small transmitter molecules (3–5), slow release of peptides or proteins (6), and loss of exogenous dyes such as FM1-43 (7,8).

DERIVATION

We begin with two assumptions: 1), fusion pore open times are exponentially distributed, and 2), vesicle contents are lost with an exponential time course as long as the pore is open.

From assumption No. 1, the probability density function for the fusion pore lifetimes (P) is:

$$dP/dt = (1/\tau_P)e^{-t/\tau_P}, \tag{1}$$

where τ_P is the mean open time of the fusion pore.

Assumption No. 2 gives the fraction of the vesicle content lost (F) during an opening of duration t:

$$F = 1 - e^{-t/\tau_D}, \tag{2}$$

where τ_D is the time constant for exit of the substance through the pore. We wish to obtain the distribution of event amplitudes: that is, the probability of observing each value of F from 0 to 1. Because F is a single-valued function of t, the desired probability density function (dP/dF) can be obtained from dP/dt by the chain rule, as follows:

$$dP/dt = dP[F(t)]/dt = (dP/dF)(dF/dt)$$
$$dP/dF = (dP/dt)/(dF/dt). \tag{3}$$

Taking the derivative of Eq. 2,

$$dF/dt = (1/\tau_D)e^{-t/\tau_D}. \tag{4}$$

Substituting Eqs. 1 and 4 into Eq. 3 gives

$$dP/dF = (\tau_D/\tau_P)e^{-t(1/\tau_P - 1/\tau_D)}. \tag{5}$$

Solving for t from Eq. 2,

$$t = -\tau_D \ln(1 - F), \tag{6}$$

and substituting Eq. 6 into Eq. 5,

$$dP/dF = (\tau_D/\tau_P)e^{[(1/\tau_P - 1/\tau_D)\tau_D \ln(1-F)]}$$
$$dP/dF = (\tau_D/\tau_P)e^{[\ln(1-F)](\tau_D/\tau_P - 1)}$$
$$dP/dF = (\tau_D/\tau_P)(1 - F)^{(\tau_D/\tau_P - 1)}.$$

Defining $A = \tau_D/\tau_P$,

$$dP/dF = A(1 - F)^{(A-1)}. \tag{7}$$

Note that this distribution depends on a single parameter, A. That is, only the ratio of τ_D/τ_P is relevant, not the absolute time constants.

In even simpler mathematical form, Eq. 7 is:

$$y = Ax^{A-1}, \tag{8}$$

where $y = dP/dF$ and $x = 1 - F$. Note that a graph of y versus x is linear on a log-log scale with slope $= A - 1$. Eq.

doi: 10.1529/biophysj.105.078881

8 might also apply to other physical situations involving two coupled exponential processes.

Fig. 1 shows the calculated distribution of fractional release of vesicle contents, for different values of A. These distributions have several interesting features. First, if $A > 1$, most fusion pore openings are too brief to release a large fraction of the vesicle contents. In fact, the maximum of the distribution is at $F = 0$. One interesting case is $A = 2$, where Eq. 7 reduces to $dP/dF = 2\ (1 - F)$. The probability decreases linearly with F, approaching zero at $F = 1$. Surprisingly, where $A = 1$, the distribution is flat, since Eq. 7 reduces to $dP/dF = 1$. That is, all possible values of F (0–1) are equally likely. For $A < 1$, a large fraction of the vesicle content is usually released, and the maximum of the distribution is at $F = 1$.

MONTE CARLO SIMULATION

Another way to calculate the distribution of F is to simulate a large number of events. Each fusion pore open time (t) was obtained by randomly sampling an exponential distribution with mean τ_P, and F was calculated from Eq. 2. A large number (10^8) of such events was binned, according to the value of F. The results were superimposible upon the analytic solutions in Fig. 1 (not shown).

The calculations above assume that the loss of vesicle contents is determined by the open time of the pore. That may not be correct if the number of molecules per vesicle is small. To address this, simulations were run where not only pore open time, but also loss of vesicle content, was assumed to be random, with 500 molecules per vesicle. Specifically, for each randomly chosen open time, the time to loss was calculated for each molecule by randomly sampling an exponential distribution with mean τ_D. If the time to loss was less than the pore open time, that molecule was assumed to be lost. The results of this simulation are shown in Fig. 1 (5×10^6 events for each τ_D/τ_P ratio). The analytical and simulated results superimpose. Although trial-to-trial variation in the number of molecules lost will affect the amplitude of any given event, the distribution of event amplitudes is unaffected.

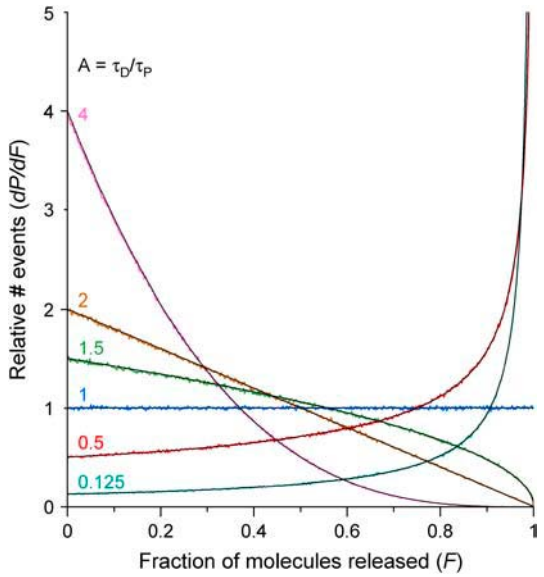

FIGURE 1 The distribution of release events. Analytical calculations from Eq. 7 (*smooth black curves*) and Monte Carlo simulations (*noisy colored curves*) for six values of *A*. For $A < 1$, values near $F = 1$ are off scale. For all *A*, the area under the curve = 1.

OTHER FACTORS

Experimentally observed distributions of miniature synaptic current amplitudes are typically either Gaussian (9) or skewed (10), and clearly do not resemble any of the curves in Fig. 1. Gaussian ''noise'' in event amplitudes can markedly affect the distributions, especially when the SD $\geq 0.1\ F$ (Supplementary Material, Fig. S1). Fig. S2 (Supplementary Material) considers a more physiological factor, random Gaussian variation in the number of molecules initially contained in each vesicle. That broadens the peak near $F = 1$ when $A < 1$. For $A \ll 1$ (near full release), the distribution approaches a Gaussian with peak at $F = 1$.

For a wide range of A, Eq. 7 predicts a substantial number of small events, which experimentally might be lost in the baseline recording noise. This would truncate the observed distribution on the left side, giving a less broad distribution. Postsynaptic factors (e.g., receptor saturation) can also affect the observed distributions.

What if fusion pore open times are not exponentially distributed (assumption No. 1)? If the pore lifetime is described by the sum of exponential components (as expected for channel-like behavior), the distribution of release events would be the sum of components described by Eq. 7, which is also not a Gaussian-like distribution. Another possibility is that the pore lifetime depends on a time-varying factor (e.g., intracellular Ca^{2+}), which could act to synchronize pore openings, producing a tighter distribution.

An exponential time course for loss of vesicle contents seems reasonable (assumption No. 2), as long as the vesicle contents are well mixed (i.e., diffusion within a vesicle, and in the extracellular space, is fast compared to exit through the fusion pore). However, dilation of the fusion pore (11) would produce a more complex time course (12), and can give a bimodal distribution of release amplitudes with maxima at $F = 0$ and $F = 1$ (Supplementary Material, Fig. S3). This can be explored further by detailed simulations of diffusion out of a vesicle (3,12,13).

SUMMARY

Exponential release of vesicle contents, through a fusion pore with exponentially distributed open times, produces a distribution of release events described by a simple analytical

expression (Eq. 7) with interesting and counterintuitive behavior (Fig. 1). It is especially noteworthy that the distribution never exhibits a maximum at a value of F other than 0 or 1. This needs to be considered in physiological situations where only a fraction of the total vesicle content is released through a fusion pore.

ACKNOWLEDGMENTS

This work was supported by National Institutes of Health grants NS24471 to S.W.J. and NS33514 to D.D.F.

REFERENCES and FOOTNOTES

1. Betz, W. J., and J. K. Angleson. 1998. The synaptic vesicle cycle. *Annu. Rev. Physiol.* 60:347–363.
2. Fernandez-Peruchena, C., S. Navas, M. A. Montes, and G. Alvarez de Toledo. 2005. Fusion pore regulation of transmitter release. *Brain Res. Rev.* 49:406–415.
3. Choi, S., J. Klingauf, and R. W. Tsien. 2003. Fusion pore modulation as a presynaptic mechanism contributing to expression of long-term potentiation. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 358:695–705.
4. Staal, R. G., E. V. Mosharov, and D. Sulzer. 2004. Dopamine neurons release transmitter via a flickering fusion pore. *Nat. Neurosci.* 7:341–346.
5. Pawlu, C., A. DiAntonio, and M. Heckmann. 2004. Postfusional control of quantal current shape. *Neuron.* 42:607–618.
6. Fulop, T., S. Radabaugh, and C. Smith. 2005. Activity-dependent differential transmitter release in mouse adrenal chromaffin cells. *J. Neurosci.* 25:7324–7332.
7. Aravanis, A. M., J. L. Pyle, and R. W. Tsien. 2003. Single synaptic vesicles fusing transiently and successively without loss of identity. *Nature.* 423:643–647.
8. Richards, D. A., J. Bai, and E. R. Chapman. 2005. Two modes of exocytosis at hippocampal synapses revealed by rate of FM1–43 efflux from individual vesicles. *J. Cell Biol.* 168:929–939.
9. Fatt, P., and B. Katz. 1952. Spontaneous subthreshold activity at motor nerve endings. *J. Physiol.* 117:109–128.
10. Bekkers, J. M., G. B. Richerson, and C. F. Stevens. 1990. Origin of variability in quantal size in cultured hippocampal neurons and hippocampal slices. *Proc. Natl. Acad. Sci. USA.* 87:5359–5362.
11. Breckenridge, L. J., and W. Almers. 1987. Final steps in exocytosis observed in a cell with giant secretory granules. *Proc. Natl. Acad. Sci. USA.* 84:1945–1949.
12. Khanin, R., H. Parnas, and L. Segel. 1994. Diffusion cannot govern the discharge of neurotransmitter in fast synapses. *Biophys. J.* 67: 966–972.
13. Stiles, J. R., D. Van Helden, T. M. Bartol, Jr., E. E. Salpeter, and M. M. Salpeter. 1996. Miniature endplate current rise times <100 μs from improved dual recordings can be modeled with passive acetylcholine diffusion from a synaptic vesicle. *Proc. Natl. Acad. Sci. USA.* 93:5747–5752.