Amperometric post spike feet reveal most exocytosis is via extended kiss-and-run fusion: Supplementary information

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In this file of supplementary information, we detail the calculations leading to the evaluation of the initial vesicular content from pre spike and post spike foot currents. Furthermore, we provide additional figures and precisions about the analysis of the post spike feet.

1 Calculating the initial vesicular content from pre spike and post spike foot currents

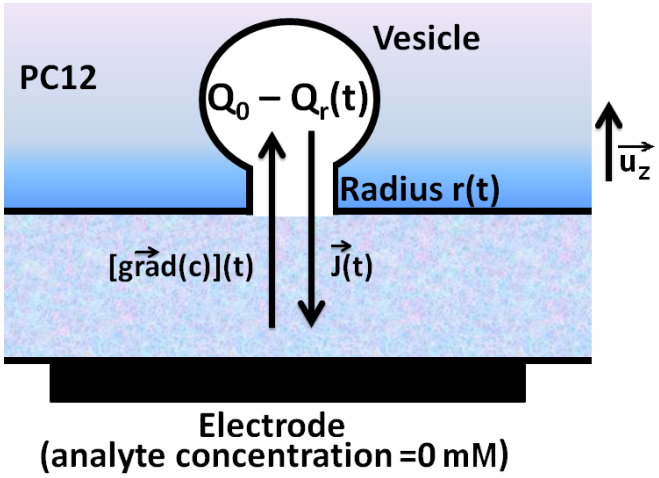

Fig. S1 Model of vesicular release defining the different parameters used in the calculations. Diffusion is assumed to be steady-state with a fixed geometry. The unitary vector $\vec{u_z}$ gives the orientation of the system. $\vec{J}(t)$ and $\left[\vec{grad}(c)\right](t)$ are the vectors representing the vesicular flux and the concentration gradient across the pore, respectively.

1.1 Simplified diffusion model

The artificial synapse has been modeled as shown on Fig. S1. For a given time t, when the pore is small enough, it is possible to describe the solution of the system using Fick's first law to relate the flux $\vec{J}$ to the gradient of the concentration c:

$$\vec{J} = -D\,\vec{grad}(c) \tag{1}$$

where D is the diffusion coefficient.
Assuming that a narrow fusion pore, *i.e.* leading to the recording of a foot, is formed and considering the geometry of the system, it is possible to assume that $\vec{J}$ is actually controlled by the pore at every instant by making the assumption that diffusion is steady-state. The system is then modeled as two semi-infinite volumes of solutions, one containg the analyte at a concentration c(t), the other one with a concentration fixed at 0 mM to model the effect of the electrode, separated by a pore of radius r(t) and length l(t). The diffusion coefficient inside the pore is D. The equation 1 can be simplified, using a first order linearization, to obtain:

$$\vec{J}(t) = -D\,\frac{c(t)-0}{l(t)}\,\vec{u_z} = -D\,\frac{c(t)}{l(t)}\,\vec{u_z} \tag{2}$$

By assuming that a steady state is reached (*i.e.* that the fusion pore is stable) and that all the molecules released are detected, we can write that the current i, in moles per second, measured across the pore, of cross-section S, and therefore at the electrode is [1]

$$i(t) = S\left|\vec{J}(t)\right| = \pi r^2(t)\left|\vec{J}(t)\right| = D\,\frac{\pi r^2(t)c(t)}{l(t)} \tag{3}$$

As the intravesicular concentration can be written as Q(t)/V where Q(t) is the intravesicular charge and V is the volume of the vesicle (assumed stable over time):

$$i(t) = D\,\frac{\pi r^2(t)Q(t)}{Vl(t)} = D\,\frac{\pi r^2(t)(Q_0 - Q_r(t))}{Vl(t)} \tag{4}$$

here Q_0 is the initial intravesicular charge related to the amount in moles and $Q_r(t)$ is the charge detected for the released amount by the vesicle between the beginning of the event and any time t. This value is the integral of the measured current i between the beginning of the event and the time t:

$$Q_r(t) = \int_0^t i(u)du \tag{5}$$

Interestingly, this relation is similar to the one describing the current flowing across a resistor of resistivity ρ, length l and

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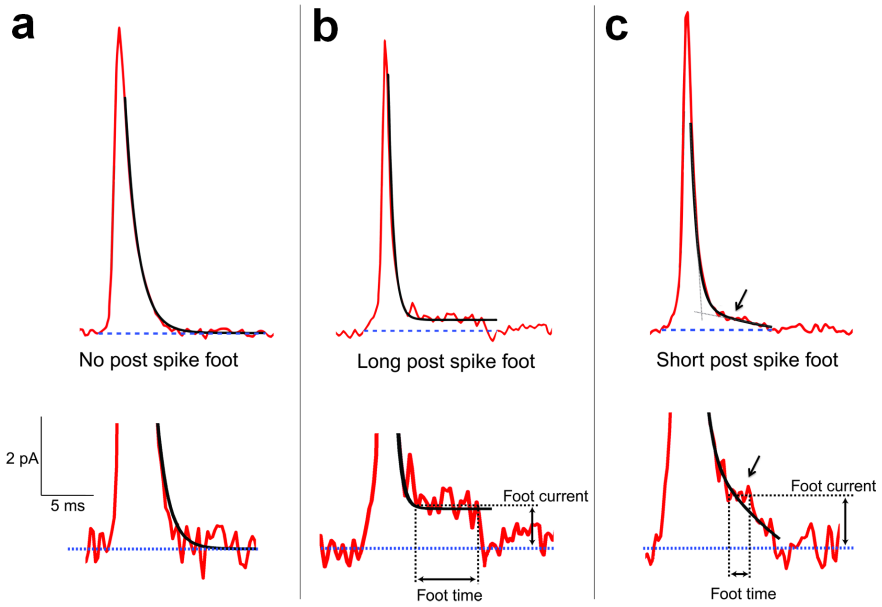

Fig. S2 Examples of peaks a) without a post spike foot, b) with a long post spike foot and c) with a short post spike foot, showing, for each case, the peak current and time.

radius r. The following equation is then obtained by defining the pore diffusional resistance R(t):

$$i(t) = \frac{1}{R(t)} \frac{(Q_0 - Q_r(t))}{V} \tag{6}$$

with

$$R(t) = \frac{l(t)}{D\pi r^2(t)} \tag{7}$$

1.2 Calculating the initial intravesicular charge

If i_{pre} and i_{post} are the pre spike and post spike foot currents, from the equation 6 we can define the current ratio β as:

$$\beta = \frac{i_{post}}{i_{pre}} = \frac{R_{pre}}{R_{post}} \frac{(Q_0 - Q_{r,post})}{(Q_0 - Q_{r,pre})} \tag{8}$$

where the indices $_{pre}$ and $_{post}$ indicate that the values are related to the pre spike or post spike foot quasi steady-states: i_{pre}, R_{pre}, $Q_{r,pre}$, r_{pre}, l_{pre} are the current, diffusional resistance, released charge, radius of the pore and length of the pore defining the quasi-steady state associated with the pre spike foot, and i_{post}, R_{post}, $Q_{r,post}$, r_{post}, l_{post} are the current, diffusional resistance, released charge, radius of the pore and length of the pore defining the quasi-steady state associated with the post spike foot.
The ratio of the pore diffusional resistances, α is defined as:

$$\alpha = \frac{R_{post}}{R_{pre}} = \frac{r_{pre}^2 l_{post}}{r_{post}^2 l_{pre}} \tag{9}$$

It is also possible to assume that $Q_{r,pre}$ can be neglected, as this value is expected to be much smaller than Q_0. These simplifications lead to:

$$\beta \approx \frac{1}{\alpha} \frac{(Q_0 - Q_{r,post})}{Q_0} \tag{10}$$

By rearranging Eq.10:

$$Q_0 = \frac{Q_{r,post}}{(1 - \alpha\beta)} \tag{11}$$

It is assumed that the intravesicular content is independent of the membrane composition. The same assumption is made for α. The experimental values used for the calculations are the ones obtained from the sub-population of spikes showing any type of foot (*i.e.* a pre spike foot, a post spike foot or both). By using the experimental values for $Q_{r,post}$ and β obtained from single cell amperometry, we find the values for the initial amount of charge in the vesicle, Q_0, and the ratio, α, to satisfy the following equations:

$$\begin{cases} Q_0 = \frac{162{,}669}{(1-0.76\ \alpha)} & (Control) \\ Q_0 = \frac{196{,}097}{(1-0.67\ \alpha)} & (LPC) \\ Q_0 = \frac{126{,}354}{(1-0.88\ \alpha)} & (PE) \end{cases} \tag{12}$$

The system was solved by calculating the three Q_0 values associated with the three equations composing the system (control, LPC and PE) for different values of α (between 0.1 and

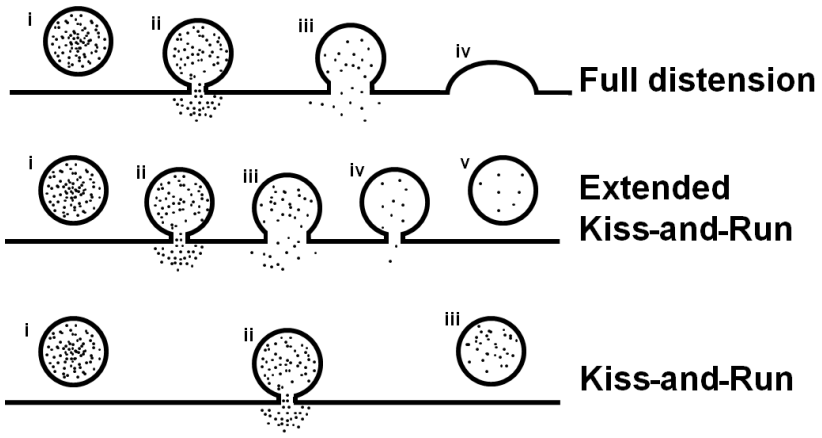

Fig. S3 Scheme of the 3 proposed mechanisms for exocytosis: **top** full distension of the vesicle into the membrane (i- docking of the vesicle, ii- formation of the fusion pore giving rise to a pre spike foot, iii- opening of the pore leading to the recording of the 'body' of the peak and iv- full integration of the vesicle into the membrane); **middle** extended kiss-and-run (i- docking of the vesicle, ii- formation of the fusion pore giving rise to a pre spike foot, iii- opening of the pore leading to the recording of the 'body' of the peak, iv- closing of the pore and formation of a fission pore giving rise to a post spike foot current and v- complete fission of the cell from the membrane); **bottom** kiss-and-run (i- docking of the vesicle, ii- formation of the fusion pore giving rise to a small, short peak and iii- complete fission of the cell from the membrane)

1.5, with 0.001 increments). For each α, the normalized standard deviation of the three Q_0 values was calculated. As Q_0 value should be the same for the 3 treatments (control, LPC and PE), the α returning the smallest normalized standard deviation was chosen as the solution of the system, and the final Q_0 value is in each case the average of the three Q_0 values associated with the three experimental conditions (control, LPC and PE) calculated for this α.

This analysis leads to a charge of 412,700 $\pm$ 18,700 molecules (average $\pm$ SEM). In the case of the control treatment, for the peaks showing a post spike foot, 39 % of the content is released, in good agreement with previous report.[2] This intravesicular content is, however, higher than the value obtained in PC12 cells. We believe the reason is that the initial content is calculated for the spikes showing a post spike foot, which are associated with a released charge significantly higher than for the total peak population. The vesicular content is therefore higher for the vesicles generating the spikes showing a post spike foot, but the fraction released (around 40 %) is similar to the results obtained with the electrochemical cytometry experiment.

The calculated Q_0 corresponds to an α of 0.79. By assuming that $l_{post} \approx l_{pre}$ as α shows a quadratic dependence on r, this corresponds to a ratio $\frac{r_{post}}{r_{pre}}$ of 1.12.

Furthermore, the calculated initial content (412,700 $\pm$ 18,700 molecules) is about 20 times higher than the amount released during the pre spike foot (about 20,000 molecules), thus supporting the assumption that $Q_{r,pre} = 0$ in Eq.10.

2 Analysis of the post spike feet

A post spike foot was identified as a quasi-stable current for at least 2 ms immediately following a release event, with a current greater than 3 times the RMS noise (measured over a 1-s period preceding the first stimulation). The foot current and foot time were estimated as shown in Fig. S2.

Out of 24 peaks with post spike feet for control cells, 19 displayed an exponential fit with a steady state current elevated from the baseline, as shown in Fig. S2B. For these feet, the foot current was measured as the difference between the steady-state current and the fitted baseline. The length of the foot was defined as the duration of this steady-state current, usually finished by a steep decrease in current.

The remaining five peaks show a short post spike foot (Fig. S2C). In this case, no long steady-state current above the baseline is observed at the end of the peak, but a significant variation in the rate of dopamine release is apparent during the decay of the peak where a steady state of at least 2 ms can be distinguished (as indicated by the arrow on Fig. S2C). These peaks have been fitted with a double exponential function to emphasize this observation.

The major factor determining which fit is applied to the peak is the length (time) of the foot. It is, however, also dependent on whether the foot has a slight slope and on how steep the slope of the peak is. For the 19 feet with exponential fits, the average foot time was 7.2 $\pm$ 1.3 ms whereas the average foot time for peaks with a double exponential fit was 3.2 $\pm$ 0.3 ms. Peaks were obtained and analyzed with an IgorPro procedure

file designed for the analysis of quantal release by the group of David Sulzer. [1]

3 Full distension, kiss-and-run and extended kiss-and-run

The three mechanisms for exocytosis discussed in the text are presented on Fig. S3. In the full distension exocytosis, the vesicular pore opens all the way and the vesicle is then integrated into the membrane. In the kiss-and-run exocytosis, the pore does not expand, and quickly closes after the beginning of the event. The result is a short, small exocytotic spike or flicker. During extended kiss-and-run, the pore expands briefly after formation of the fusion pore, and closes again. This type of event gives rise to larger events, then with a well-defined peak, and possibly pre spike and/ or post spike feet.

4 Number of events per cell

Table S1 summarizes the number of events recorded per cell, for each treatment (control, LPC, PE).

Table S1 Number of events recorded per cell

Cell Number	Control	LPC	PE
1	295	42	140
2	162	346	202
3	341	170	141
4	87	101	131
5	613	160	208
6	-	-	191
Total	1498	819	1013

5 Duration of the pre spike and post spike feet

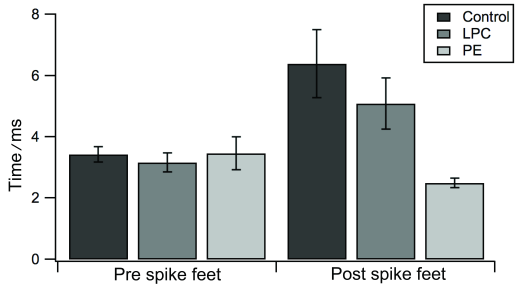

Fig. S4 Duration of the pre spike and post spike feet, for different lipid incubation conditions.

References

1 E. Mosharov and D. Sulzer, *Nat. Methods*, 2005, **2**, 651–658.

2 D. Omiatek, Y. Dong, M. Heien and A. Ewing, *ACS Chem. Neurosci.*, 2010, **1**, 234–245.