Chapter 1. Introduction to a random walk simulation for amperometric recordings of quantal neurotransmitter release

This chapter is modified from our review article on presynaptic mechanisms that regulate quantal size (Sulzer and Pothos, 2000).

last modified by Yvonne Schmitz and Dave Sulzer February 12, 2010

The diffusion of molecules is surprisingly non-intuitive. Crank's classic volume on diffusion (Crank, 1975) is useful (and challenging reading) for examples of how to derive formulae to describe diffusion under different conditions. One such formula was adapted by Robert Chow to estimate the relationship between distance and amperometric spike shape (Chow and von Ruden, 1995). Nevertheless, the complexity of deriving the appropriate formulae as conditions are altered is a drawback to their use.

In contrast, the random walk, discussed by H.C. Berg (Berg, 1983), provides the same answers but is simpler to manipulate. This approach was used by Schroeder et al. from Mark Wightman's group in a study on amperometric spike shape and fractional recovery (Schroeder et al., 1992). An advantage of a random walk simulation we have adapted to be easily performed on spreadsheets (even by biologists!), as shown below. We have some sample Excel files attached you can use to begin with.

The following chapters show how to extend this to amperometry and cyclic voltammetry recordings in brain slice or in vivo. What follows immediately is a first lesson on using a random walk simulation, using a "numerical analysis" for quantal recordings with amperometry, which helps in the more complex analyses to follow.

A spreadsheet modeling diffusion in one dimension over time (t) can be made with columns representing distance and rows representing time intervals (see example below). Since the vectors of diffusion are random, for a population of molecules diffusing in one dimension, one half will move to the right and one half will move to the left. Therefore, the cells in a row t (x+1) equal the average of the cells in from the two neighboring columns in row t (x).

The release site in this example is modeled as a *reflecting surface*(column a). In the spread sheet below, a reflecting surface on the left-hand side receives half of the molecules from its neighboring column and donates half of its molecules to its neighboring column. This is simulated by making a cell at t (x+1) the average of itself and its neighbor at t (x).

For modeling the electrode surface in amperometric recordings, we assume a *consuming surface*, as catecholamines are oxidized and do not return to a reduced state. A consuming surface (column g) destroys all the molecules that encounter the surface at t (x), and at t (x+1) receives half of the molecules from the cell in its neighboring column (column f) at t (x). The cell in the column next to the electrode (column f) in row t (x+1) receives only half of the molecules from the cell in its other neighboring column (column e) in row t (x), and no molecules from cells in the electrode column (column g).

	release site						**electrode**	
	0 μm	0.5 μm	1 μm	1.5 μm	2 μm	2.5 μm	3 μm	
	a	b	c	d	e	f	g	g smoothed
1	x	0	0	0	0	0	0	=avg(g1 :g4)
2	=avg(a1,b1)	=avg(a1,c1)	=avg(b1,d1)	=avc(c1,e1)	=avg(d1,f1)	=0.5*e1	=(0.5*f1)	=avg(g2 :g5)
3	=avg(a2,b2)	=avg(a2,c2)	=avg(b2,d2)	=avc(c2,e2)	=avg(d2,f2)	=0.5*e2	=(0.5*f2)	=avg(g3 :g6)
4	=avg(a3,b3)	=avg(a3,c3)	=avg(b3,d3)	=avc(c3,e3)	=avg(d3,f3)	=0.5*e3	=(0.5*f3)	=avg(g4 :g7)

"avg" is an averaging function, the columns represent distances, and the rows iterations (time). The dimensions of the distance axis can be set arbitrarily. More columns representing smaller distances will yield greater accuracy and smoother shapes.(In this case we smooth the values in column g by averaging 4 iterations.)

In the following example, 7000 molecules are released from the reflecting column a at t = 0. The diffusion of the molecules is shown for 0.5 μm bins and 20 iterations:

	a	b	c	d	e	f	g	g smoothed
	7000	0	0	0	0	0	0	0
1	3500	3500	0	0	0	0	0	0
2	3500	1750	1750	0	0	0	0	0
3	2625	2625	875	875	0	0	0	27
4	2625	1750	1750	438	438	0	0	41
5	2188	2188	1094	1094	219	219	0	82
6	2188	1641	1641	656	656	109	109	106
7	1914	1914	1148	1148	383	328	55	125
8	1914	1531	1531	766	738	191	164	141
9	1723	1723	1148	1135	479	369	96	147
10	1723	1436	1429	813	752	239	185	156
11	1579	1576	1125	1090	526	376	120	156
12	1577	1352	1333	825	733	263	188	160
13	1465	1455	1089	1033	544	367	132	156
14	1460	1277	1244	816	700	272	183	158
15	1368	1352	1047	972	544	350	136	153
16	1360	1207	1162	795	661	272	175	152
17	1284	1261	1001	911	534	330	136	148
18	1272	1143	1086	768	621	267	165	146
19	1208	1179	955	854	517	310	133	141
20	1193	1081	1016	736	582	259	155	139

The relationship between the iterations on the spread sheet and time can be determined from the diffusion coefficient (*D*) for the respective molecule and medium. The diffusion coefficients for classical transmitters in water are available from standard references and most small hydrophilic molecules like classical neurotransmitters are pretty similar.

The relationship between time (t) and distance (x) is: t = x^2/2*D*

For bins of 0.5 µm and the apparent diffusion coefficient D for dopamine in water at 34 degrees Celsius (6.9 x 10^-6 cm^2/s, Nicholson, 1996) the time t represented by each row is

$$t = (0.5 \times 10^{-5}\ \text{cm})^2 / (2 \times 6.9 \times 10^{-6}\ \text{cm}^2/\text{sec}) = 0.182\ \text{ms}$$

The amperometric current can be converted into 'number of molecules' by applying Faraday's law, which for dopamine results in 1 pCoul (=pA s)= 3.1212 x 10^6 molecules (if one assumes that two electrons are donated per dopamine molecule. This appears accurate at physiological pH for events shorter than ~ 1 sec. For longer lasting events 4 electrons can be donated. See our review for further information.)

For 182 µs time intervals as in our example, this would correspond to

3.1212 x 10^6 molecules/pA s x 182 x 10^-6 s = 568 molecules / pA

Now the iterations can be expressed as time intervals and the number of molecules as current, so that genuine amperometric recordings can be compared with simulations.

References

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Chapter 2. Introduction to a random walk simulation for amperometric and cyclic voltammetry recordings of evoked dopamine overflow in brain slices

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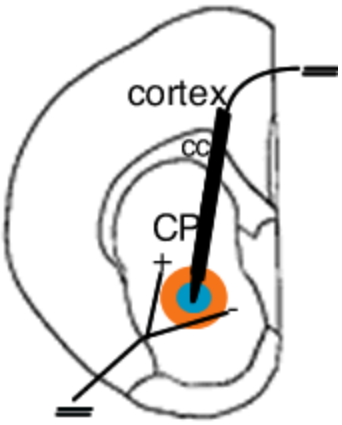
In this second lesson, we add two new concepts that help adapt the previous quantal release model to experiments in the brain, where there is release from many sites. One is the action of uptake transporters, in this example the dopamine uptake transporter, which removes dopamine from the extracellular milieu to repackage in the neuron. Thus, we have to worry about a decrease in the signal from consuming electrodes (in amperometry) that oxidize the transmitter, diffusion, and reuptake.



CP caudate putamen, cc corpus callosum

Schematic drawing: Dopamine overflow is evoked in striatal slices by a bipolar stimulation electrode. The recording carbon fiber electrode is positioned at a distance of ~ 50-100 µm from both poles of the stimulation electrode. Dopamine overflow is measured with either cyclic voltammetry, for which a triangular voltage wave (-400 mV to +1000 mV) is applied to the electrode at 10 Hz, or with continuous amperometry, for which a constant voltage of +400 mV is applied. The two methods yield recordings of dopamine overflow with very different kinetics. However, both can be simulated with the model described below.

The essentials of diffusion can be characterized by a one-dimensional random walk (Berg, 1983). A spreadsheet modeling diffusion in one dimension over time can be made with columns representing distance and rows representing time intervals. Since the vectors of diffusion are random, for a population of molecules diffusing in one dimension, one half will move to the right and one half will move to the left. The bins in a row t (x+1) equal therefore the average of the two neighboring bins in row t (x).The time steps are calculated (t=x^2/2D) for the diffusion coefficient D for dopamine in brain tissue: 2.7*10-6 cm^2/s (Nicholson, 1996). For 0.5 µm bins a time step is 0.463 ms. (See also chapter 1.)

The first row in the spreadsheets below represents the initial dopamine concentration in µM after stimulation. The middle column represents the electrode surface (located in the center of the stimulated field, represented by the orange doughnut in the schematic drawing above). An important parameter that determines the kinetics of the recorded signals is a dead space around the electrode where no dopamine release occurs (represented by the inner blue circle in the figure above).

CYCLIC VOLTAMMETRY

	a	b	c	d	e	f	g	h	i
		edge			electrode			edge	
	2 µm	1.5 µm	1 µm	0.5 µm	0	0.5 µm	1 µm	1.5 µm	2 µm
					'dead space'				
1: 0.463 ms	[DA]	[DA]	[DA]	0	0	0	[DA]	[DA]	[DA]
2: 0.926 ms	=b1*0.5	=(c1*0.5+a1*0.71)/2	=avg(b1,d1)	=avg(c1,e1)	=avg(d1/1)	=avg(e1,g1)	=avg(f1,h1)	=(g1*0.5+i1*0.71)/2	=h1*0.5
3: 1.389 ms	=b2*0.5	=(c2*0.5+a2*0.71)/2	=avg(b2,d2)	=avg(c2,e2)	=avg(d2/2)	=avg(e2,g2)	=avg(f2,h2)	=(g2*0.5+i2*0.71)/2	=h2*0.5
3: 1.852 ms	=b3*0.5	=(c3*0.5+a3*0.71)/2	=avg(b3,d3)	=avg(c3,e3)	=avg(d3/3)	=avg(e3,g3)	=avg(f3,h3)	=(g3*0.5+i3*0.71)/2	=h3*0.5

AMPEROMETRY

	a	b	c	d	e	f	g	h	i
					electrode				
	2 µm	1.5 µm	1 µm	0.5 µm	0	0.5 µm	1 µm	1.5 µm	2 µm
1: 0.463 ms	[DA]	[DA]	[DA]	0	0	0	[DA]	[DA]	[DA]
2: 0.926 ms	=b1*0.5	=(c1*0.5+a1*0.71)/2	=avg(b1,d1)	=c1^0.5	=avg(d1/1)	=g1^0.5	=avg(f1,h1)	=(g1*0.5+i1*0.71)/2	=h1*0.5
3: 1.389 ms	=b2*0.5	=(c2*0.5+a2*0.71)/2	=avg(b2,d2)	=c2^0.5	=avg(d2/2)	=g2^0.5	=avg(f2,h2)	=(g2*0.5+i2*0.71)/2	=h2*0.5
3: 1.852 ms	=b3*0.5	=(c3*0.5+a3*0.71)/2	=avg(b3,d3)	=c3^0.5	=avg(d3/3)	=g3^0.5	=avg(f3,h3)	=(g3*0.5+i3*0.71)/2	=h3*0.5

For **cyclic voltammetry**, a reflecting electrode surface is modeled, assuming that dopamine molecules are regenerated during the reducing voltage scan. The columns next to the electrode (in blue) are determined using the same formula as the other columns in the spreadsheet.

For **amperometry**, a consuming surface is modeled, assuming that dopamine molecules are oxidized at the electrode surface and that the oxidation product is not reduced back to dopamine. The bin in the column next to the electrode (in red) in row t (x+1) receives only half of the molecules from its other neighboring bin in row t (x), and no molecules from the bin in the 'electrode column'.

(Edges: We chose to model a diffusing edge: of the initial molecules 50% fall off the edge, but 50% of those return during the next iteration, and so on. This process reaches a limit, so that 71% of the molecules that reach the edge eventually return. However, it should be noted that if the release area is sufficiently large, the way the edge is modeled does not play an important role.)

Dopamine uptake

To include dopamine uptake in the simulation, the dopamine concentration is corrected after each iteration by subtracting the amount of dopamine that is taken up during a time step according to the Michaelis-Menten equation:

d[DA]/dt=Vmax*[DA]/(Km+[DA]

with the maximal uptake rate Vmax (µM/s), the apparent affinity Km (µM), and the dopamine concentration [DA] (µM)

(see also the review by Wightman and Zimmerman, 1990)

	example for column b:	
time [s]:		**b**
	1	x
0.000431	**2**	=average(a1,c1)
	3	=b2-(V_{max}*b2/(K_m+b2)*0.000463)
0.000862	**4**	=average(a3,c3)
	5	=b4-(V_{max}*b4/(K_m+b4)*0.000463)
0.001293	**6**	=average(a5,c5)

References

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Wightman RM, Zimmerman JB (1990) Control of dopamine extracellular concentration in rat striatum by impulse flow and uptake. Brain Res Brain Res Rev. 15(2):135-44

Chapter 3. Instructions for using an Excel spreadsheet for simulation of dopamine overflow in the brain

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In this last lesson, we give Excel spreadsheets you can use to begin to develop your own models and model your data.

These instructions are to be used with the Excel files from this repository.

Download the amperometry and the cyclic voltammerty spreadsheets on the download page and open it in Excel. If you have a problem with it, email me (ds43@columbia.edu) and I can send it to you as an email attachment.

To the right of the spreadsheet is a chart of the curve resulting from the simulation. There are 3 parameters that can be changed in the cells BR 3, 4 and 5: the maximal uptake rate Vmax, the apparent affinity Km, and the initial dopamine concentration. To see how the Michaelis-Menten parameters alter the curve, try changing the values for the uptake.

How the spread sheet is set up

Spreadsheet columns

This version of the simulation uses 51 columns, from column F to BD. If you decide to increase the number of bins, we recommend you use an odd number. The electrode is represented by the middle column (AE) with 25 columns on the left and right. Each column begins at time 0 with the same concentration of transmitter, which you set in cell BR5. The exception is at the electrode itself, and the 'dead space' around the electrode. If you wish to change the dimensions of the dead space, fill in "0" by hand. For most of our data, 6 µm at each side (in this case 3 bins) fit well. This seems reasonable considering a 5 µm carbon electrode enveloped by a layer of glass.

As explained in Chapter 2, the electrode can be modeled as *consuming* (as in the amperometric spreadsheet), or *reflective* (as in the cyclic voltammetry spreadsheet): this is the only difference betwee the amperometric and cyclic voltammetry spreadsheets.

The size of the release area is a separate parameter, and becomes more important with low uptake transport activity. This can be adjusted by inserting more columns.

Spreadsheet rows

The rows represent a time period that is set by the bin size, using the equation in cell A13. If one wishes to change the diffusion coefficient (set at 2.7 x 10^-6 cm^2/sec),

substitute the new value in cell A11. The spreadsheet as prepared contains 128 time rows. Each time point actually consists of two rows, one in plain font, and the other in boldface. As explained in chapter 2, the plain font first uses the random walk formula, and the boldface row then corrects for uptake according to the Michaelis-Menten equation.

The chart

The chart plots time on the X axis from column BI and the concentration on the Y axis from column BG. We used a 4 point smoothing function on column AE, which decreases the noise and makes the function appear smoother. Alternatively, the noise can be decreased by increasing the number of columns and decreasing the bin size.

Questions

1. How wide should the bin sizes be?

We prepared the spreadsheets using bin sizes of 2 µm. However, we generally use bin sizes of 0.5 µm for simulations of data from the brain slice. The spreadsheet sent as an attachment uses a larger dimension, so that the size of the file remains small enough for easy download. We recommend that you try examining smaller bin sizes, and compensate for the total volume of the simulation by pasting new columns. You will see that if the bin sizes are too large, they act as a low-pass filter, and artifactually decrease the maximum peak height. Moreover, the shape may appear jagged rather than smooth. As the bin sizes are moved to smaller values, you arrive at a limit of maximal peak height, providing a more accurate simulation. Again, for our brain slice models, 0.5 micron is a good value, as smaller bins sizes produce a negligible increase in peak height.

2. How wide should the dead volume be?

The dead volume can be adjusted by inserting 0 values in the top time row around the middle electrode column. You will see that this greatly affects the rising phase of the curve. Determine the best fit to your real data with this parameter; smaller dead volumes result in more rapid inclines in the curve.

3. How wide should the release area be?

This parameter is often significant for the fall-off phase of the curve. In slice, we often find our data is well fit by about a 50 µm radius, not far from the distance between our bipolar electrodes. You will need to experiment to find the best fit for your own parameters.

4. How to estimate 'goodness-of-fit'?

We use the R-squared (R^2) statistic for nonlinear regression. A tutorial on this subject is on the GraphPad web site. This widely-used statistic for determining the fit of non-linear functions yields a fraction between 0 and 1 and has no units. Higher values reflect a better fit. The R^2 statistic can be thought of as the fraction of the total variance that is explained by the simulation.

In short, R^2 = 1.0 - (SSreg/SStot), where SSreg is the sum-of squares of the differences between the real data and the simulation trace in units of the Y axis squared (current, concentration, or number of molecules) and SStot is the sum of squares of the differences between the data and a horizontal line through the mean of all the Y values of the data. High values R^2 result if the variance between the simulation and data is substantially lower than the variance between the data and the mean value.

5. Is there a commercial program available for running the Schmitz/Sulzer simulation?

Justin Lee from Synaptosoft has written a computer program that automates this simulation. The algorithm has been incorporated into an analysis program called 'Mini Analysis Program'. In the 'random walk menu' you can choose values for 4 parameters, which are the 'dead radius', initial transmitter concentration, Vmax and Km, and generate a curve in either amperometric or cyclic voltammetry mode. To fit the simulation curve to an actual recording trace, the program runs a simplex algorithm to find the parameters that yield the best fit according to an R^2 criterion. The size of the release area and the diffusion coefficient can be changed. The Mini Analysis Program is available for download at http://www.synaptosoft.com/.

Jill Venton, who was in Mark Wightman's lab, has also used commercial software. Download the paper here.