

# MINIATURE POSTSYNAPTIC CURRENTS WITH RESTRICTED RECEPTOR AREAS

T.M. Bartol<sup>1\*</sup>, T.J. Sejnowski<sup>1</sup>, B.R. Land<sup>2</sup>, E.E. Salpeter<sup>2</sup>, M.M. Salpeter (deceased)<sup>2</sup>

1) The Salk Institute, La Jolla, CA  
2) Cornell University, Ithaca NY

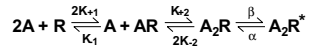


## 1. Introduction

One of the key issues in understanding synaptic transmission is the degree to which the number of neurotransmitter molecules in a quantal packet ( $N_n$ ) saturates the number of receptors ( $N_r$ ) on the postsynaptic membrane. In previous simulations of miniature postsynaptic currents (mPSCs) using MCell (www.mcell.cnl.salk.edu), a Monte Carlo simulator of cellular microphysiology, we assumed that  $N_r \gg N_n$  which is appropriate for the neuromuscular junction (NMJ). Here we explore a range of  $N_r/N_n$ , which is more appropriate for central synapses whose receptors are located within a small area.

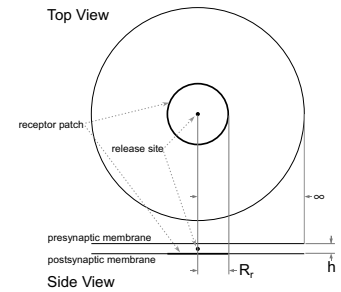
## 2. Modeling mPSCs with MCell

### 2A. Kinetic Scheme



**Neurotransmitter/receptor kinetic scheme.** We assume a slightly more general scheme than in previous NMJ simulations by allowing cooperativity in the two binding and unbinding steps.

### 2B. Synaptic Cleft Geometry



**Geometry of synaptic cleft.** We modeled a disk-shaped patch of receptors of radius  $R$ , on an infinite sheet of postsynaptic membrane. The pre- and postsynaptic membranes were separated by the cleft height distance,  $h$ .

### 2C. Model Input Parameters

#### Definitions and relationships:

$N_n$  : quantal packet size  
 $N_r$  : number of receptors in receptor patch  
 $\sigma$  : receptor density  
 $R_n$  : saturated disk radius  
 $R_r$  : receptor patch radius  
 $P$  : plateau time  
 $N_{ch}$  : peak number of open channels

$$\pi \sigma_0 R_r^2 = N_r$$

$$\pi \sigma_0 R_n^2 = N_n/2$$

$$\rho = R_r/R_n = (2N_r/N_n)^{1/2}$$

$$q_{eff} = N_{ch}/(0.5^*N_n)$$

$$r_{eff} = N_{ch}/(0.5^*N_r)$$

#### Time Scales:

$$t_{mbd} = (1 + \beta/2k_2)/\alpha$$

$$t_{bind} = 1.39h/(\sigma k_{+2})$$

$$t_{diff} = 0.8N_n/(4\pi\sigma)$$

#### Parameters held fixed:

$$N_n = 10000$$

$$h = 0.02 \mu\text{m}$$

$$\sigma_0 = 15000 \mu\text{m}^{-2} \text{ (for normal } \sigma \text{ case)}$$

$$\sigma = \sigma_0/3 \text{ (for low } \sigma \text{ case)}$$

$$t_{mbd} = 1.0 \text{ ms}$$

#### Parameters varied:

$$\beta/\alpha = [1, 10]$$

$$\beta/2k_2 = [1, 10]$$

$$D = [1, 5] \times 10^{-6} \text{ cm}^2\text{s}^{-1}$$

$$k_{+2} = [0.5, 5] \times 10^7 \text{ M}^{-1}\text{s}^{-1}$$

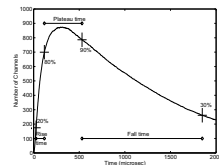
$$k_{+1}/k_{+2} = [0.1, 1, 10]$$

$$k_{-1}/k_{-2} = [0.1, 1, 10]$$

$$R_r = [0.05, 0.2, 0.75, 2.5] \mu\text{m}$$

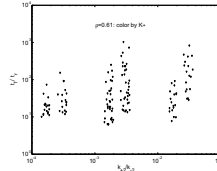
$$\text{(implies } \rho = [0.15, 0.61, 2.3, 7.7])$$

The number of parameter combinations computed was 576. Each case was repeated 20 times and averaged to reduce stochastic noise. The  $20^*576=11,520$  MCell runs were computed on BlueHorizon at the San Diego Supercomputer Center. We repeated the parameter set at  $\sigma=\sigma_0/3$ .

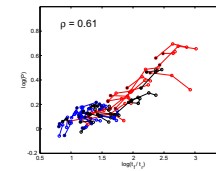


**Measurement methods.** We measured the rise time,  $t_r$ , and fall time,  $t_f$ , as shown. The plateau time is defined as the time between 80% on the rising phase and 90% on the falling phase, but we will use a normalized plateau time defined by  $P=(\text{plateau time})/(t_r t_f)^{1/2}$ .

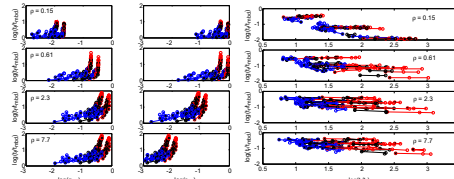
## 3. Results



**Variation of  $t_f/t_r$  with  $k_{+2}/k_{-2}$ .** Despite the large input parameter ranges the variations in  $t_f/t_r$  is surprisingly small ( $\sigma=\sigma_0$ ). The points on the plot fall into six groups (dithered horizontally to minimize overlapping points). We expected a strong positive correlation between  $t_f/t_r$  and  $k_{+2}/k_{-2}$ . The actual results show only a very weak correlation, (perhaps even the wrong sign). The correlation seems to be weakened because smaller  $k_{+2}/k_{-2}$  leads to lower efficiency,  $q_{eff}$ , which in turn leads to smaller  $t_r$ , thus raising  $t_f/t_r$ .



**Scatter plot of  $P$  vs  $t_f/t_r$ .** The normalized plateau time is a dimensionless shape parameter that varies surprisingly little across parameter sets. Colors and connections are as in the figure below. In the example shown with  $\rho=0.61$ ,  $P$  varies as  $(t_f/t_r)^s$  where  $s \sim 0.3$ .

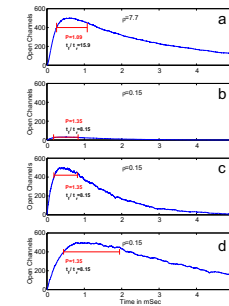


**mPSC efficiency and shape results for different receptor patch sizes.** The trends for  $t_f$  versus  $q_{eff}$ ,  $r_{eff}$ , and  $t_f/t_r$  are shown ( $\sigma=\sigma_0$ ). Each set of input parameters for which only  $k_{+1}/k_{+2}$  changes is connected by a line. The end of the line with low  $k_{+1}/k_{+2}$  is marked with an x. Each different  $k_{+1}/k_{+2}$  has a different color with 0.1, 1, 10 colored as red, black, and blue respectively. The  $q_{eff}$  decreases as  $\rho$  is decreased, while  $r_{eff}$  actually increases. These effects make sense if most of the released agonist binds to available receptor. For small  $\rho$ , the receptors are used up before the agonist, so  $q_{eff}$  is low, but  $r_{eff}$  is high because almost every available receptor is bound. The falltime also generally becomes smaller at small  $\rho$ , also decreases with decreasing  $\rho$ , roughly in proportion to  $t_r$ , so that  $t_f/t_r$  depends very little on  $\rho$ .

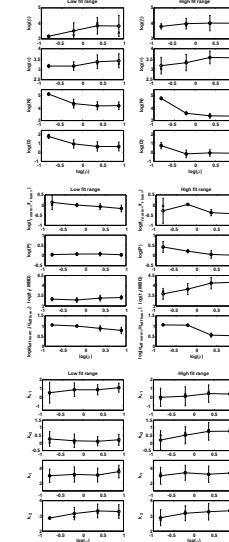
## 4. Conclusions

The simulations with  $0.1 < \rho < 10$  seem to share insensitivity of rise time and plateau time with the NMJ simulations we performed previously. Thus, there does not seem to be any shape parameter that will unambiguously resolve kinetic parameters from  $\rho$  for an unknown transmitter system under all regimes. On the other hand, if some synapse shows a plateau time appreciably different from the values in our simulations, it would indicate that a different model is required, perhaps one with slow exocytosis or a third ligand binding site.

All values of  $k_{+1}/k_{+2}$  gave about equally many fits, so that a restriction to  $k_{+1}=k_{+2}$  does not seem to lose breadth of fit. The value of  $k_{+1}/k_{+2}$ , however, does effect the resulting value of  $t_f/t_r$ , especially at large  $\rho$ . For instance, for  $\rho=7.7$ , none of the simulations which gave  $t_f/t_r$  between 5 and 12 (low fit range) came from  $k_{+1}/k_{+2}=0.1$ , whereas 15 of the 17 simulations came from  $k_{+1}/k_{+2}=10$ . Thus large values of  $k_{+1}/k_{+2}$  are required for small values of  $t_f/t_r$ . For the high fit range ( $50 < t_f/t_r < 120$ ) the situation is somewhat reversed. Only 2 of 27 simulations came from inputs with  $k_{+1}/k_{+2}=10$ . We conjecture that small values of  $k_{+1}/k_{+2}$  may be required to produce very large values of  $t_f/t_r$ .



**Removal of scaling.** To compare the of simulation results with actual data we have to remove the various scaling schemes. The model output must be converted from normalized, model units back to physical units for comparison with real data. a) mPSC computed at large  $\rho$  and scaled to fit an amplitude of 500 channels and a falltime of 3ms. b) mPSC computed at small  $\rho=0.15$ , but with the same kinetic parameters as in (a). c) mPSC,  $\rho = 0.15$ , with the amplitude scaling chosen so that the output amplitude equals the amplitude in (a). d) mPSC,  $\rho = 0.15$ , with both amplitude and time scaling chosen so that the fall time also equals that in (a). Note that  $t_r$ ,  $t_f$ , and  $N_{ch}$  all decrease as  $\rho$  decreases.



**Model output for two hypothetical classes of mPSCs.** We chose to fit only amplitude, fall time and  $t_f/t_r$  (at  $\sigma=\sigma_0$ ) because these parameters are the most likely to be available for real mPSCs. We picked an mPSC with 500 open channels at peak. Two fall times were chosen: 3 ms and 15 ms. For the 3 ms fall time we chose  $\log(t_f/t_r)=0.9 \pm 0.2$ . For the 15 ms fall time we chose  $\log(t_f/t_r)=1.9 \pm 0.2$  for the fit range. The chosen amplitude and fall time fix the model scaling parameters and the  $t_f/t_r$  becomes the single parameter that was matched to model output. The circles represent the averages of the log of each parameter, and are connected by a solid line. The error bars are the standard deviation of the log of each parameter. The x's represent the medians.  $P$  is quite insensitive to model parameters in the low fit range and decreases perhaps a factor of two with increasing  $\rho$  in the high fit range. mPSC rise time does not necessarily increase with decreasing  $\sigma$ , which is different from the NMJ. For small  $\rho$ , the rise time is almost constant with decreased  $\sigma$ , but at larger  $\rho$ , the rise time dependency on  $\sigma$  is similar to the NMJ.

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