A Sensitivity Analysis of Chemical Kinetics Parameters for the Neuromuscular Junction

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Introduction

The simplest chemical kinetics scheme for the development of a miniature endplate current (MEPC) at the neuromuscular junction (NMJ) is:

$$2A + R \underset{K-}{\overset{2K+}{\rightleftharpoons}} A + AR \underset{2K-}{\overset{K+}{\rightleftharpoons}} A_2R \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} A_2R^* \qquad \text{(Rx 1)}$$

where A is the concentration of acetylcholine (ACh), R is the concentration of acetylcholine receptor (AChR), and A_2R^* is the concentration of the doubly-bound open channel configuration. Of course, ACh is free to diffuse through the NMJ, so a full description must include diffusion to make a coupled reaction-diffusion system. To estimate the values of the rate contants in Rx 1 from physiological data, we need a model which connects measured MEPC shapes with the reaction-diffusion scheme. We used MCell (www.mcell.cnl.salk.edu) to build a catalog of simulated MEPCs based on 1568 combinations of rate constants. We then searched the catalog of MEPCs for those with shape parameters (e.g. $t_{r,Normal}/t_{d,Normal}$ – see Glossary) that matched real MEPCs. The catalog of simulated MEPCs allows us to estimate the sensitivity of the model to changes in rate constants, and to invert this sensitivity to determine how well we can determine rate constants from MEPC data.

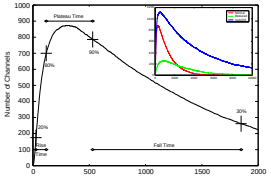
1 MCell Simulations

For the MCell simulations, four quantities were held fixed:

h = 0.05 μm
σ_o = 7500 μm^{-2}
N = 5000
mbd = 1000 μs

These values imply:

t_b = 279/K_- μs
t_d = 212/D μs



1. Anatomy of an MEPC.

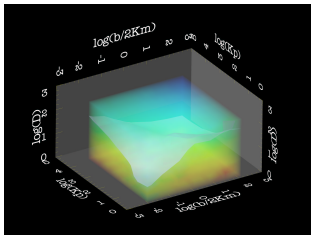
MEPCs were simulated under three conditions: (1) at normal AChR density (Normal, red), (2) with AChE deactivated (noAChE, blue), and (3) with AChE deactivated *and* AChR at 1/3 normal density (Reduced, green). The three cases shown were computed using kinetics parameters from the middle of range given below. The peak value was determined from a quadratic fit. Rise time, fall time, and plateau time were measured as the intervals between 20-80% rise, 90-30% fall, and 80% rise - 90% fall times respectively.

We carried out 1568 sets of simulations for each of the three conditions shown in figure 1. These simulations covered all combinations of:

β/α = 10, 20, 50, 100
β/2K_- = 0.125, 0.25, 0.5, 1, 2, 4, 8
t_b = 4, 9, 17, 35, 70, 139, 279
t_d = 18, 25, 35, 50, 71, 100, 141, 200

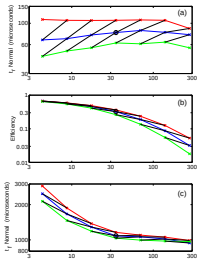
To reduce stochastic noise, each simulation case was repeated 20 times and the results averaged. The total of 94080 MCell simulations were run in about 50 hours at the San Diego Supercomputer Center on BlueHorizon, an 1152 processor IBM SP Power3 massively parallel supercomputer.

2 Model Predictions



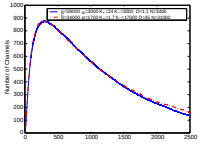
2. Volume rendering of Normal rise time as a function of log(β/2K$_-$), log(K$_-$), and log(D).

The volume colormap represents the Normal rise time values (blue = lowest rise times, red = highest rise times) at the 7x7x8 (392) combinations of β/2K$_-$, K$_-$, and D used in the simulations while holding β/α constant at 20. An iso-rise-time surface at t_r = 80 μs is shown embedded in the volume.



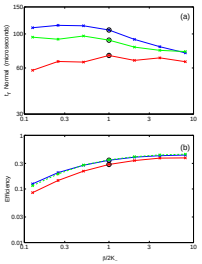
3. Normal rise time, efficiency, and fall time vs. t_b.

Simulations run in the Normal condition with t_d = 35 (green), 71 (blue), and 140 (red) μs. The thin black lines represent the effect of changing t_b and t_d by the same ratio. (a) Rise time varies remarkably little when t_b is varied considerably with constant t_d. Thus a disease which increases cleft height, h, (e.g. myasthenia gravis) would have little effect on rise time at constant σ. However if t_d is reduced by lowering σ (e.g. with αBTX or pathological condition) rise time would increase. (b) The efficiency decreases rapidly with increasing t_b when t_d is large. A disease which increases h/σ (e.g. myasthenia gravis) and thus decreases t_b would have a less serious effect on MEPC amplitude if K_- were large. (c) For medium to large t_d, the fall time is close to mbd. Only for very small t_b is the binding to receptors so efficient that (even in the presence of AChE) there is significant rebinding during the falling phase.



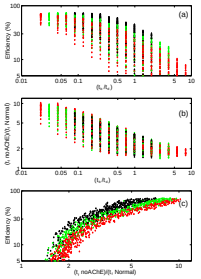
4. Differing input parameters can give similar Normal MEPCs.

The blue trace was generated with large K_- and small D which gives a high efficiency of 54%, while the red trace was generated with small K_- and large D with a quite low efficiency of 6% (requiring large N).



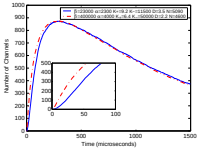
5. Normal rise time and efficiency vs. β/2K$_-$.

Simulations run in the Normal condition with β/α of 10 (blue), 20 (green), and 100 (red). Note again that the rise time changes very little with varying β/2K$_-$ especially for the most likely intermediate values of β/α. The efficiency is somewhat small when β/2K$_-$ is very small, i.e. when unbinding out weighs channel opening.



6. Scatter diagrams.

For all panels color indicates value of t_b+t_d: > 150 μs (red); 75 - 150 μs (green); < 75 μs (black). (a) Efficiency vs. t_b/t_d. Efficiency generally decreases as t_b/t_d increases but, as the color indicates, three ranges of t_b+t_d are mixed together. (b) $t_{r,noAChE}/t_{r,Normal}$ vs. t_b/t_d. (c) Efficiency vs. $t_{r,noAChE}/t_{r,Normal}$. The correlation between the two quantities is fairly strong, since both decrease with increasing t_b/t_d. The curvature is due to the fact that $t_{r,noAChE}/t_{r,Normal}$ is always greater than 1 and efficiency is always less than 100%.



7. Shape of rising phase *could* distinguish Normal MEPCs.

Input parameters can be chosen to predict the same values of t_r/ t_p and the two additional target values to within 20%. The red trace is for β/α = 100 and the blue trace is for β/α = 10. The shape of the two MEPCs is similar from the plateau to the falling phase. The rising phase is different in shape (see inset). It is almost linear for β/α = 100 but is distinctly sigmoid for smaller β/α = 10. Thus, if electrophysiological experiments could be performed with very high accuracy, the shape of the rising phase could yield information on β/α;

3 Inverting the model

Table 1a 73 best fit cases

	(β/α β/2K_-)	β	α	K_P	K_-	D	N
Smallest β	(10 0.13)	11455	1145	32.6	45819	5.3	6120
Largest β	(100 8.00)	630692	6307	5.6	39418	2.9	4922
Smallest α	(100 0.13)	95870	959	27.3	383481	2.3	6399
Largest α	(20 8.00)	183700	9185	4.1	11481	2.2	5074
Smallest Kp	(50 8.00)	356927	7139	3.2	22308	2.9	6180
Largest Kp	(10 0.13)	13972	1397	79.5	55889	7.3	4886
Smallest Km	(10 4.00)	50993	5099	4.1	6374	9.3	7584
Largest Km	(100 0.13)	109242	1092	62.0	436967	1.7	4186

Table 1b β = 60000 – 100000

	(β/α β/2K_-)	β	α	K_P	K_-	D	N
Smallest α	(100 0.13)	95870	959	27.3	383481	2.3	6399
Largest α	(20 4.00)	96101	4805	3.8	12813	3.3	5787
Smallest Kp	(20 4.00)	93210	4661	3.7	11651	5.1	6507
Largest Kp	(100 0.13)	95870	959	27.3	383481	2.3	6399
Smallest Km	(20 4.00)	93210	4661	3.7	11651	5.1	6507
Largest Km	(100 0.13)	95870	959	27.3	383481	2.3	6399

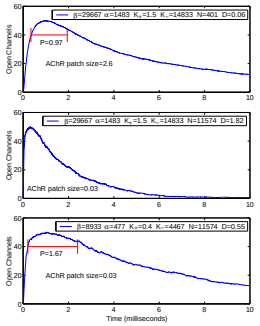
Table 1c β = 40000 – 60000

	(β/α β/2K_-)	β	α	K_P	K_-	D	N
Smallest α	(50 0.13)	52749	1055	30.0	210997	2.2	5490
Largest α	(10 4.00)	57431	5743	4.6	7179	4.0	5806
Smallest Kp	(20 2.00)	56567	2828	3.8	14142	3.7	6508
Largest Kp	(50 0.13)	54233	1085	61.7	216933	3.5	4282
Smallest Km	(10 4.00)	50993	5099	4.1	6374	9.3	7584
Largest Km	(50 0.13)	54233	1085	61.7	216933	3.5	4282

Table 1a-c. Range of models which fit the target values.

The 1568 models have been scaled so that all have 872 open channels and $t_{r,Normal}$ = 1220 μs. With this scaling the full range of parameter values represented in the MEPC catalog is: α = 880 to 61000 s^{-1}; β = 9100 to 2.7x10^6 s^{-1}; K_- = 0.62 to 430x10^3 M^{-1}s^{-1}; and K_P = 4900 to 4.9x10^3 s^{-1}. (a) 73 of the 1568 models predict the target values (see Glossary) to within 20%. With four input parameters to be chosen but only three output quantities to fit, input values cannot be determined uniquely. (b) 9 models fit the targets if β is constrained to be in the range 60000 - 100000 s^{-1}. (c) 9 models fit the targets if β is constrained to be in the range 40000 - 60000 s^{-1}.

4 Extending the MEPC catalog



8. Effect of varying AChR patch size.

Unlike the NMJ some CNS synapses have a receptor patch with fewer receptor molecules than the quantal packet has agonist. To simulate possible CNS synaptic geometry, we reduced the radius of a patch of AChR from 2.6 μm to 0.03 μm (i.e. about 10% the size of a Normal saturated disk). Simulations were run under noAChE conditions with Normal AChR density. (a) Model MEPC from extended catalog (patch size = 2.6 μm) scaled to give a small amplitude of 50 open channels and a long fall time of 6 ms. (b) Model output for small patch size scaled to the same amplitude as in (a) as if attempting to fit actual experimental data. Since the amplitude decreases by a factor of 28 with the smaller patch of receptor, N and D must be increased by the same factor. (c) Model output for small patch size scaled to the same amplitude *and* fall time as in (a). Scaling this way makes it obvious that one could separate the effects of varying receptor patch size from those of kinetic parameters on the basis of MEPC shape (at least in some ranges).

Glossary

Physical input parameters:

K_+ = binding constant (M^{-1}s^{-1})
K_- = unbinding constant (s^{-1})
β = channel opening rate (s^{-1})
α = channel closing rate (s^{-1})
D = diffusion constant (cm^{2}s^{-1})
N = number of ACh in quantal packet
(0.5N is maximum number of open channels)
h = cleft height (μm)
(bounded by two infinite planes)
σ = AChR density (μm^{-2})
σ_o = normal value of σ

Three time scales:

Binding time: t_b = 1.39h/(σK_-)
Diffusion time: t_d = 0.8N/(4πσD)
Mean burst duration: mbd = (1+β/2K_-)/α

Three target values:

($t_{r,Normal}$)/($t_{d,Normal}$)
($t_{r,noAChE}$)/($t_{d,Normal}$)
($t_{r,Reduced}$)/($t_{r,noAChE}$)

Typical experimental values of the targets:

($t_{r,Normal}$)/($t_{d,Normal}$) ≅ 13.3
($t_{r,noAChE}$)/($t_{d,Normal}$) ≅ 3.63
($t_{r,Reduced}$)/($t_{r,noAChE}$) ≅ 1.90

Conclusions

(1) To specify a model uniquely eight parameters have to be specified. In our catalog only four dimensionless parameters are varied: β/α, β/2K_-, t_b, and t_d, while four others are kept fixed. However, scaling prescriptions are given to obtain results for the other parameters without requiring further simulations.

(2) Although one would expect the rise time to be directly correlated with the binding time, t_r varies remarkably little over a wide range of t_b if t_d is held constant. This near constancy is due to some accidental cancellations – as t_b increases, the efficiency decreases, which results in the peak occurring earlier in the binding phase.

(3) With β and three target values specified to within 20%, the resultant range of uncertainty in α, K_+, and K_- is enormously larger then 20%. To obtain a moderately unique model, one has to specify the value of a second parameter (out of α, K_+, K_-, N, and D). The large range of uncertainty is connected to the cancellation noted in (2) above. A range of 20% in t_r results in virtually the whole range of t_b we simulated (see Figure 3a).

(4) The curvature of the rising phase depends on whether the ratio of β/α is moderate to large (in the range of 10 to 100). If very accurate MEPC waveforms should become available, the rising phase could give information on β/α.

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