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Computation of postsynaptic transmembrane currents and membrane potential in the nicotinic synapse ♮

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The postsynaptic nicotinic current generated by quantal release of acetylcholine in the neuromuscular junction is modeled based on a system of Michaelis-Menten type equations that describe the kinetics of receptor activation. Assuming that the kinetic transition rates between the different receptor states are concentration-dependent, we performed simulations for different values of the kinetic parameters. The models are implemented and solved numerically in MATHEMATICA. The simulated currents are consistent with our experimental results obtained by the whole-cell patch-clamp technique performed on the nicotinic receptor expressed by TE671LH cells.

1. Introduction

Synaptic transmission involves several steps and it is triggered by an electrical impulse (or a train of impulses), called *action potential* (PA), transmitted through the presynaptic neuron to the terminal buttons of the axon. For a specific neuron, the amplitude and length of the PA is predetermined. The intensity of a stimulus is encoded by modulating the frequency of the action potentials.

The action potential leads to depolarization (inverse polarization of the two

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sides of the cellular membrane, compared to the membrane at rest membrane) of the terminal axon, with consecutive opening of voltage-dependant calcium channels and calcium influx in the neuron. This triggers the fusion between the neurotransmitter-filled vesicles (acetylcholine - ACh, in our specific case) and the membrane, followed by ACh release within the synaptic cleft [2]. After crossing the synaptic space, the ACh molecules bind to specific receptor proteins situated on the postsynaptic membrane [3], [4], [9], [10]. It is known that 2 molecules of ACh bind to 1 receptor [4], [5]. The activated receptor acts as an ionic channel, allowing passive sodium influx and potassium efflux into/from the postsynaptic neuron. Channel opening occurs through morphological changes of the receptor protein [4].

Previous studies were concerned with the ACh release and diffusion [6], [7]. This paper proposes a model for computing postsynaptic currents based on the specific receptor opening kinetics, and involving a dynamic balance between unbound states of the receptor and states where the receptor is bound to 1 or 2 molecules of ACh, and a balance between open and closed states of the receptor[1], [5], [11]. The modeling results are compared to experimentally measured currents, obtained through the patch-clamp technique.

2. Experimental Apparatus

The nicotinic currents triggered by different concentrations of ACh (10μM, 50μM, 100μM) were measured through the patch-clamp technique in the whole-cell configuration setup (Fig. 1). The membrane potential, given by the difference of the voltage of the glass electrode, attached to the cell, and the voltage of another electrode inserted in the fluid surrounding the cells, is clamped at a holding value and the measuring circuit measures the current that is injected into the cell, in order to maintain a constant value of the membrane potential. The potential of the bath electrode is conventionally considered 0 (similar to the potential of the extracellular space).

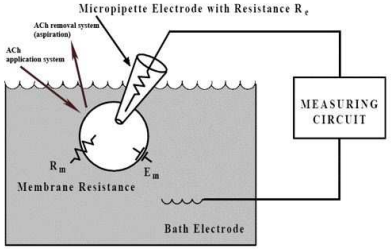


Fig. 1. Shematic of the patch-clamp setup.

The membrane voltage is fixed and the measuring circuit reads the current that is injected into the cell to hold constant the membrane voltage. The electrode voltage (potential) is set to zero (similar to the voltage outside the cell). The mea-

surements were performed on TE671LH cells, a *rhabdomiosarcoma* cell line, which natively expresses nicotinic receptors. Figure 2 shows the currents obtained for different concentrations of ACh applied to the cells.

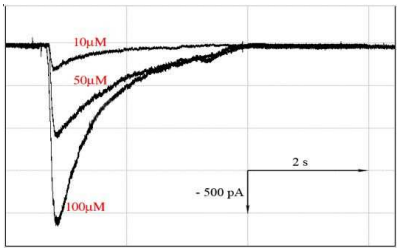


Fig. 2. Patch-clamp transmembrane currents for TE671LH cells.

3. Physical and Mathematical Models

3.1. Nicotinic receptors opening kinetics

The nicotinic receptors for ACh are chemically activated ionic channels. This means that the gating mechanism requires binding of ACh molecules to the receptor. A 5 states mechanisms of the receptor were proposed [5]: (1) R, closed non-ligated state; (2) R_1, closed state, where the receptor is bound to 1 molecule of ACh; (3) R_2, closed state where the receptor is bound to 2 molecules of ACh; (4) O_1, open state, where the receptor is bound to 1 molecule of ACh; (5) O_2, open state, where the receptor is bound to 2 molecules of ACh. The transition rates between the 5 states are k_{on}, k_{off}, α_1, α_2, β_1, β_2.

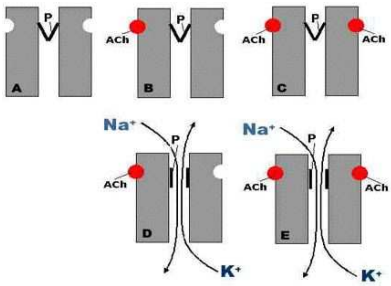


Fig. 3. The 5 states model of the ACh nicotinic receptor [5].

Figure 3 shows the kinetic diagram for the 5-states model. A, B and C are states where the P gate is locked; D and E are open states of the receptor. The gate of the receptor may open with a certain probability even if the receptor is bound to

a single molecule of ACh, and there is a certain probability for the receptor to exist in a closed state, even if is bound to 2 molecules of ACh.

The transition between these states is described by the parameters of the kinetic scheme associated to the receptor (Fig. 4).

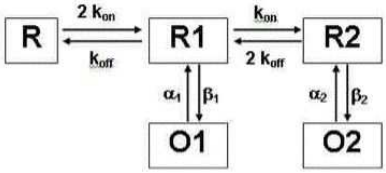


Fig. 4. Kinetics of the 5 states model of the receptor.

Table 1 presents the values of the kinetic parameters reported for the 5 states model [5]. This approach accounts for the cooperation between the ACh binding sites on the receptor – the attachment of one molecule facilitates the binding of a second molecule to the same receptor.

Table 1. Transition rates for the 5-state receptor [5]

k_{on}	0.02 ms^{-1}
k_{off}	7.6923 ms^{-1}
α_1	9.6875 ms^{-1}
α_2	0.96875 ms^{-1}
β_1	2.0667 ms^{-1}
β_2	20.667 ms^{-1}

3.2. Mathematical model

The mathematical model is described by the set of ordinary differential equations

$$\frac{\mathrm{d}R}{\mathrm{d}t} = k_{\text{off}} R_1 - 2k_{\text{on}} R, \tag{1}$$

$$\frac{\mathrm{d}R_1}{\mathrm{d}t} = 2k_{\text{on}} R + 2k_{\text{off}} R_2 + \alpha_2 O_1 - (k_{\text{off}} + k_{\text{on}} + \beta_1) R_1, \tag{2}$$

$$\frac{\mathrm{d}R_2}{\mathrm{d}t} = k_{\text{on}} R_1 + \alpha_2 O_2 - (2k_{\text{off}} + \beta_2) R_2, \tag{3}$$

$$\frac{\mathrm{d}O_1}{\mathrm{d}t} = \beta_1 R_1 - \alpha_1 O_1, \tag{4}$$

$$\frac{\mathrm{d}O_2}{\mathrm{d}t} = \beta_2 R_2 - \alpha_2 O_2, \tag{5}$$

with the initial conditions:

$$R(0) = N_{\max}, \tag{6}$$

$$R_1(0) = R_2(0) = O_1(0) = O_2(0) = 0. \tag{7}$$

The postsynaptic membrane voltage and current are obtained from the electrical circuit model of the synaptic transmission [7]:

$$C\frac{\mathrm{d}U}{\mathrm{d}t} + U\left\{\gamma\left[O_1(t) + O_2(t)\right] + \frac{1}{R_{ex}}\right\} = \frac{E}{R_{ex}}, \tag{8}$$

$$U(0) = E, \tag{9}$$

$$I_{rec}(t) = \gamma\left[O_1(t) + O_2(t)\right]U(t), \tag{10}$$

where U is the membrane voltage, C the postsynaptic membrane capacitance, R_{ex} the extracellular electrical resistance, E the holding potential of the membrane, I_{rec} the current through the open receptors, and γ the single-channel conductance. Table 2 shows the numerical values of these parameters used in our model. The capacity C and the resistance R_{ex} were computed based on reported values for the specific capacity of the cell membrane $1\,\frac{\mu\mathrm{F}}{\mathrm{cm}^2}$, and for the extracellular resistivity $100\,\Omega$cm [8], for the particular geometry of the synaptic space [7].

Table 2. Electrical circuit parameters of the model

C	4 pF	post-synaptic membrane capacitance
γ	20 pS	single receptor conductance
R_{ex}	20 MΩ	extracellular resistance in the synaptic space
E	−70 mV	membrane voltage (clamped)

The Cauchy problem for the 5 states model that we developed was implemented and solved numerically in MATHEMATICA 4.2 [12] by using the numerical differential equation solver NDSolve [11], for $N_{\max} = 1000$. Some of the results obtained for the 5 states model, and the improved 6-states model, are presented next.

4. Results

4.1. The 5-States Nicotinic Receptor Model

Figure 5 presents the membrane voltage and current resulted from the 5-states model, for the transition rates listed above.

Several other simulations for different values of kinetic parameters, based on the 5-states kinetic model (not shown here) were performed. In each case, membrane voltage and current reach a steady state, different from the initial values (-70mV voltage, 0 A current), suggesting that the experimental whole-cell currents measured by patch-clamp may not be predicted by the 5-states kinetic model for the receptor. The 5-states model can accurately predict the channel opening events in single-channel patch-clamp recordings [5], but is not suitable for modeling whole-cell currents.

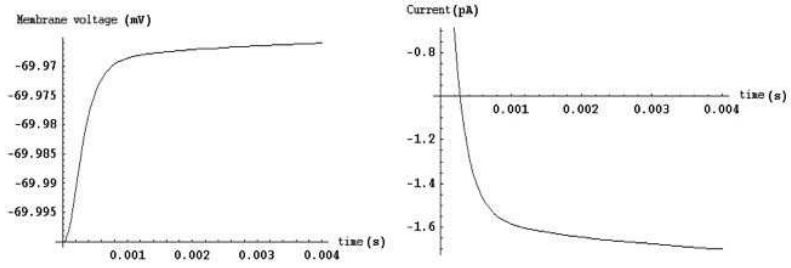


Fig. 5. Transmembrane voltage and current by the 5-states model.

4.2. The 6-States Nicotinic Receptor Model

To circumvent this difficulty, we assumed that the transition between the R_1 and O_1 receptor (which requires a conformational change of the receptor protein) occurs in two steps, rather than a single step, with different transition rates. Under this hypothesis, the kinetic transition scheme of the nicotinic receptor is completed by an intermediate, sixth state, I (Fig. 6). R, R_1, R_2 are closed states, unbounded to ACh, respectively bounded by either 1 or 2 ACh molecules. O_1 and O_2 are states bounded by either 1 or 2 ACh molecules, and I is the postulated conformational morphology; the transition rates between the 6 states are $k_{\text{on}}, k_{\text{off}}, \alpha_1, \alpha_2, \alpha_i, \beta_1, \beta_2, \beta_i$.

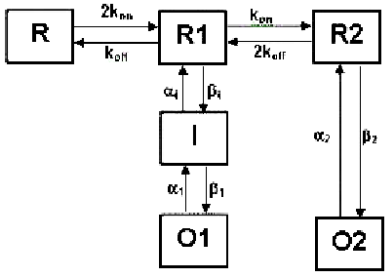
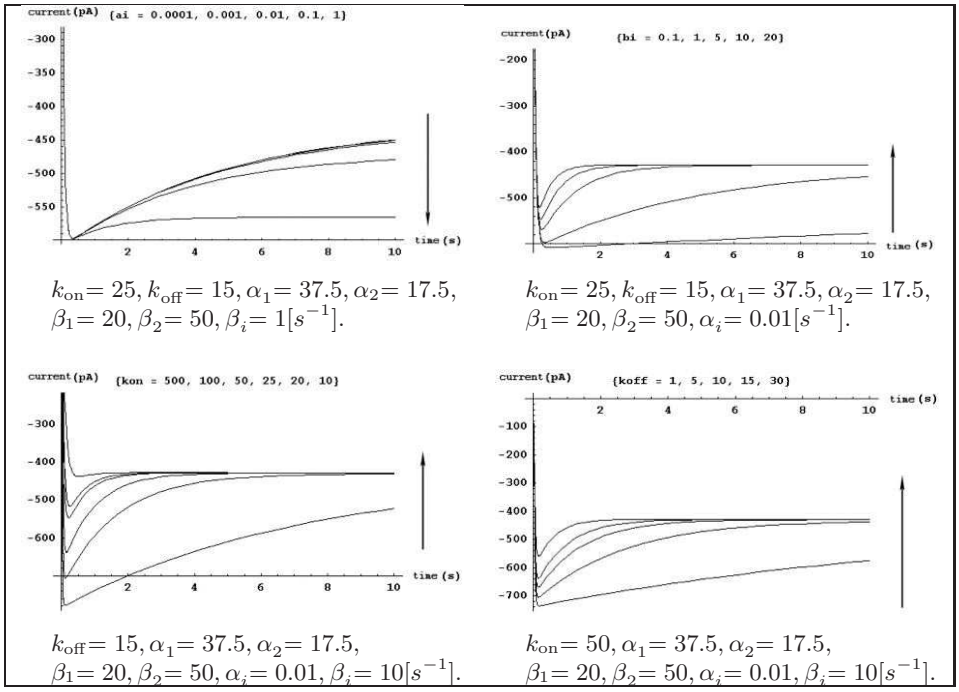


Fig. 6. The kinetic scheme of the 6-states receptor.

The number of receptors in each state was computed as for the 5-state model, based on a system of *Michaelis-Menten* equations. We performed simulations for different values of the kinetic transition rates ($k_{\text{on}}, k_{\text{off}}, \alpha_1, \alpha_2, \alpha_i, \beta_1, \beta_2, \beta_i$) and 1000 available receptors, in order to find the parameters that best match the experimental results. The results of the simulations are presented in figure 7 and figure 8.

By varying the total numbers of receptors involved, we can obtain different amplitudes for the peak currents for $k_{\text{on}} = 50$, $k_{\text{off}} = 15$, $\alpha_1 = 10000$, $\alpha_2 = 40$, $\beta_1 = 1$, $\beta_2 = 50$, $\alpha_i = 0.01$, $\beta_i = 10$ [s^{-1}].(Fig. 9a).

The best fit for the experimentally measured whole-cell patch-clamp currents for 10 μM, 50 μM and 100 μM of ACh was obtained for k_{on}=50, k_{off}=15, α_1=10000, α_2=40, β_1=1, β_2=50, α_i=0.01, β_i=10 and 800, 3500 and 12000 receptors involved



$k_{\text{on}}= 25, k_{\text{off}}= 15, \alpha_1= 37.5, \alpha_2= 17.5,$
$\beta_1= 20, \beta_2= 50, \beta_i= 1[s^{-1}].$

$k_{\text{on}}= 25, k_{\text{off}}= 15, \alpha_1= 37.5, \alpha_2= 17.5,$
$\beta_1= 20, \beta_2= 50, \alpha_i= 0.01[s^{-1}].$

$k_{\text{off}}= 15, \alpha_1= 37.5, \alpha_2= 17.5,$
$\beta_1= 20, \beta_2= 50, \alpha_i= 0.01, \beta_i= 10[s^{-1}].$

$k_{\text{on}}= 50, \alpha_1= 37.5, \alpha_2= 17.5,$
$\beta_1= 20, \beta_2= 50, \alpha_i= 0.01, \beta_i= 10[s^{-1}].$

Fig. 7.

(Fig. 9b).

4.3. Conclusions

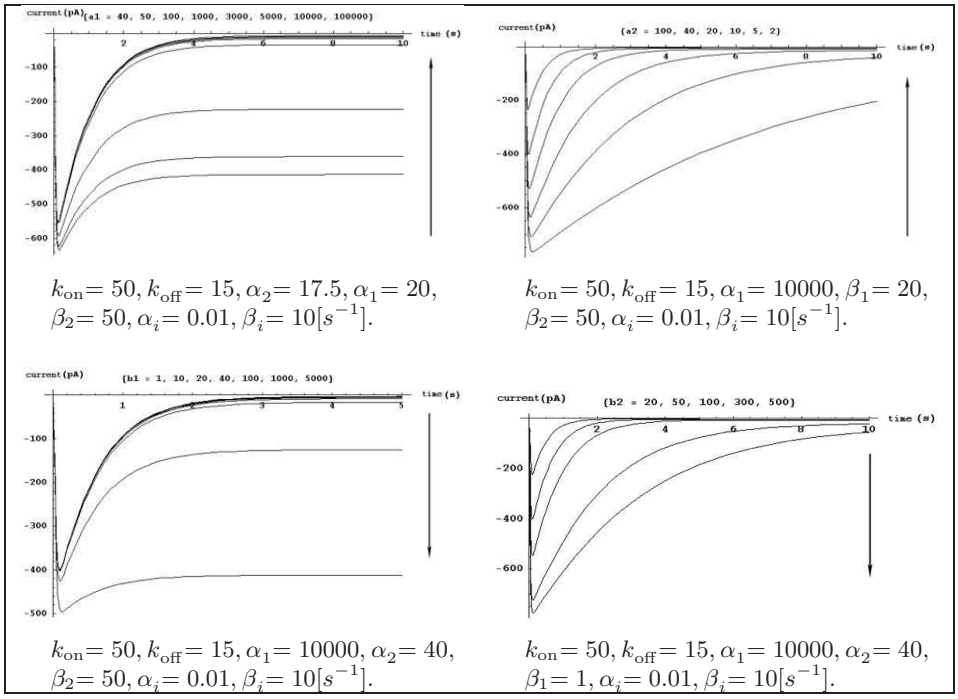
In this study we presented the physical, mathematical model for the synaptic transmission based on a 5-states kinetics mechanism, and its connection to the electrical circuit model of the synaptic transmission previously developed by us. The model is solved numerically and it is shown to provide for valuable predictive capability in assisting patch-clamp experiments performed on TE671LH cells.

Although accurate in predicting the receptor opening events for single-channel patch-clamp recordings [5], our paper suggests that the 5-states kinetic model for the nicotinic receptor may not satisfactorily predict patch-clamp whole-cell currents.

The 6-states model proposed in this paper is based on a physical and biological plausible hypothesis: the transition of the mono-ligated, closed nicotinic receptor to the open state is a 2-step process, involving a conformational intermediate.

The simulation results obtained with this 6-states model approaches within reasonable accuracy limits the experimentally measured currents.

Further studies are required in order to establish a relationship between the concentration of the ACh applied during patch-clamp experiments and the kinetic parameters of the receptor conformational changes.



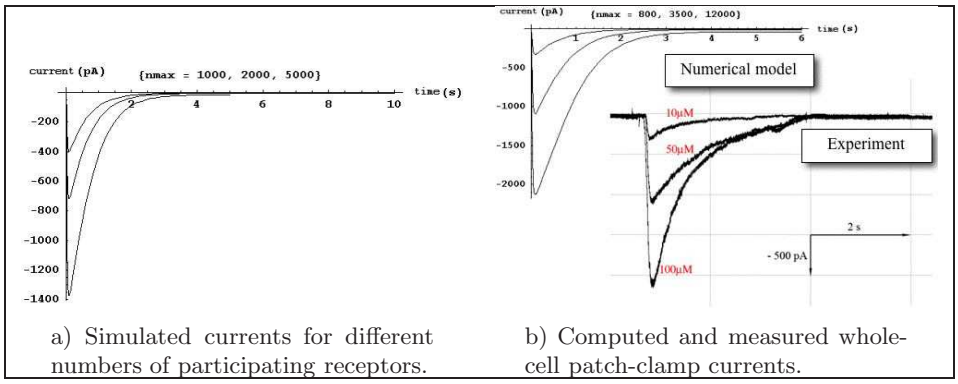
k_{on}= 50, k_{off}= 15, α_2= 17.5, α_1= 20, β_2= 50, α_i= 0.01, β_i= 10[s^{-1}].

k_{on}= 50, k_{off}= 15, α_1= 10000, β_1= 20, β_2= 50, α_i= 0.01, β_i= 10[s^{-1}].

k_{on}= 50, k_{off}= 15, α_1= 10000, α_2= 40, β_2= 50, α_i= 0.01, β_i= 10[s^{-1}].

k_{on}= 50, k_{off}= 15, α_1= 10000, α_2= 40, β_1= 1, α_i= 0.01, β_i= 10[s^{-1}].

Fig. 8.



a) Simulated currents for different numbers of participating receptors.

b) Computed and measured whole-cell patch-clamp currents.

Fig. 9.

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