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THE NICOTINIC SYNAPSE: MODEL OF ACETYLCHOLINE RELEASE AND DIFFUSION IN THE NEUROMUSCULAR JUNCTION

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Abstract
This paper introduces a model of the neuromuscular synapse, a particular kind of synapse where acetylcholine (ACh) acts as a neurotransmitter. The model accounts for the diffusion phenomena occurring at this level. All the important processes that influence the synaptic distribution of the neurotransmitter are considered: release of ACh in the synaptic cleft, its diffusion in the extracellular interneuronal space and degradation by a specific enzyme, the acetylcholine esterase. The model uses an exponential kinetics for ACh release therefore the presynaptic ACh vesicles are not included in the simulation space. The authors present 2D and 3D synaptic models. The mathematical model is solved numerically using the Femlab finite element software package. The simulation results are compared with available experimental data.

Key words: *acetylcholine, nicotinic synapse, numerical modeling, finite element*

INTRODUCTION

Information transmission in the human nervous system is performed through specialized structures, called interneuronal synapses. The synaptic structures are organized on three levels – the presynaptic and postsynaptic level that correspond to the pre- and postsynaptic membrane, and the extracellular space in-between, called the synaptic cleft. Transmission is unidirectional: a chemical substance that acts as a neurotransmitter is released from intracytoplasmatic vesicles in the presynaptic neuron in response to an electrical stimulus – an action potential – propagated through the presynaptic neuron. The substance diffuses through the synaptic cleft and reaches the postsynaptic membrane where it binds to specific receptors on the cell wall and triggers depolarization of the postsynaptic membrane and transmission of information to the postsynaptic neuron [1,5,6]. The subject of our paper is a particular type of synapse, the neuromuscular junction. Neuromuscular transmission is cholinergic (ACh acts as a neurotransmitter), and the postsynaptic membrane receptors are nicotinic.

Our model is based on neuromuscular models proposed by Smart et al and Tai et al [11,13], models that include the presynaptic vesicles in the simulation space. An important limitation of such an approach is that the volume changes of the vesicles during release are not being accounted for, neither are the possible active, contractile, processes involved. Recent experimental data [2,3,7,8,12] suggest that simple diffusion from the vesicles as a release mechanism does not agree with the time parameters of neurotransmission. Our model proposes a different release law, ACh reaching the synaptic cleft through presynaptic release ports. The diffusion processes are studied on 2D and 3D models, and the solutions of the mathematical model are computed by means of numerical methods – finite element, using Femlab [4].

PHYSICAL AND MATHEMATICAL MODEL

In our model we considered a synaptic space of 550 nm length; a secondary membrane fold (characteristic for the neuromuscular junction) of 800 nm depth is placed in the center of the postsynaptic membrane [6], [11], [13] (Fig.1). The thickness of the synaptic cleft is taken 50 nm [9]-[11], [13]. On the presynaptic membrane, evenly distributed, are the ACh release ports, of 18nm in diameter. Their density was computed by using reported biological experimental data [2], [6]. The acetylcholine-esterase molecules are modeled as squares (cubes) with a 20 nm edge, equally spaced between the pre- and postsynaptic membrane. Figure 1 shows the synaptic structure used in the 2D and the 3D models.

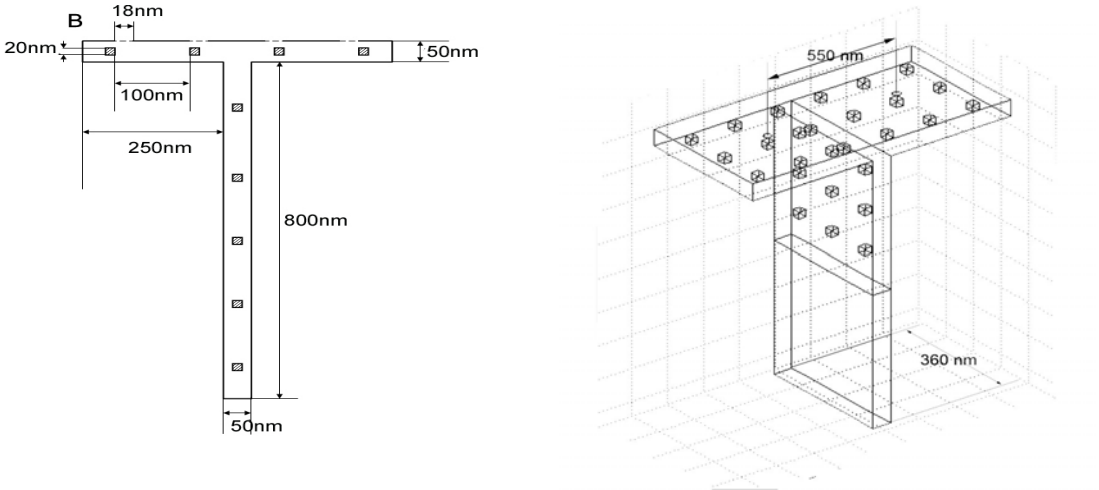


Fig. 1. The structure of the synaptic space – 2D model (A) and 3D model (B)

Acetylcholine diffusion is modeled by [9] – [11], [13]

$$\frac{du}{dt} = D\nabla^2 u \quad , \tag{1}$$

where u is the ACh concentration, and $D = 4\cdot10^{-4}$ $\mu m^2 \mu s^{-1}$ is the ACh diffusivity in the extracellular environment.

The boundary conditions corresponding to the esterase result from the kinetic equations of the chemical reaction of acetylcholine degradation [9] – [11], [13]

$$\mathbf{n}\cdot\left(D\nabla u\right) = -ku\,. \tag{2}$$

Here, k is a constant that depends on the cholinesterase enzymatic activity and has a value of $2\cdot10^{-3}\mu m\mu s^{-1}$ [9,10,11,13]. On all the other parts of the boundary, except for the ACh release ports, we used Neumann homogenous (impermeable) condition.

$$\mathbf{n}\cdot\left(D\nabla u\right) = 0 \tag{3}$$

Acetylcholine release through presynaptic ports

The flux of ACh through the presynaptic ports is the solution of the equation:

$$\int_0^{t_f} lQ(t)dt = SC_0 \,, \tag{4}$$

where l = 18 nm is the diameter of the release port (Fig.2), S = 65 nm^2 is the maximum surface section of the vesicle fused with the membrane, and C_0 the initial concentration of ACh in the vesicle, C_0 = 0.3 molecules/nm^3. In what concerns the integration time, t_f, we considered three different cases, distinguished by the the relationship between t_s and T – the time constant of the difusion process [9,10].

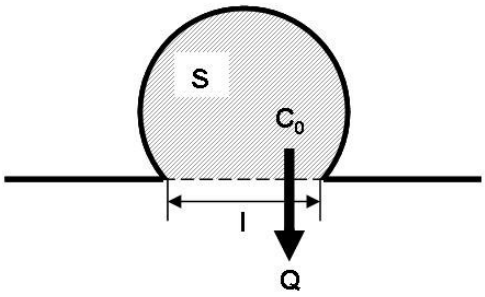


Fig. 2. Determining the ACh flux through the release ports

We assumed a solution of the form

$$Q(t) = Q_0 \exp\left(-\frac{t}{\tau}\right) \tag{5}$$

Equation (4) has the analytic solution,

$$Q(t) = \frac{SC_0}{l\tau\left[1-\exp\left(-\frac{t_f}{\tau}\right)\right]}\exp\left(-\frac{t}{\tau}\right), \tag{6}$$

therefore, the boundary condition at the release ports becomes

$$\mathbf{n}\cdot\left(D\nabla u\right) = Q(t) = \frac{SC_0}{l\tau\left[1-\exp\left(-\frac{t_f}{\tau}\right)\right]}\exp\left(-\frac{t}{\tau}\right), \tag{7}$$

where τ is the time constant of the release process. In all simulations we considered the integration time $t_f = 4\tau$.

Next, we present three simulation cases, depending on the relationship between t_f and T, the time constant of the ACh diffusion process (a parameter that results out the scaling of the equations, T = 6,25 μs – see below): I. $t_f \cong T$, II. $t_f > T$, III. $t_f < T$.

Simulations for Dirichlet boundary conditions at release ports level were also performed for all cases

$$u = u_0 e^{-\frac{t}{\tau}} \tag{8}$$

The 3D problem was solved using only Dirichlet boundary conditions on the release ports. Table 1 presents the nondimensional equations for all the six simulation cases.

	Cases I and II	**Case III**
Scaling parameters	$\tilde{u} = \frac{u - u_0}{u_{\max} - u_0}$; ($u_0$=0) $\tilde{x} = \frac{x}{x_0}$ $\tilde{t} = T = \frac{x_0^2}{D}$	$\tilde{u} = \frac{u}{u_{\max}}$ $\tilde{x} = \frac{x}{x_0}$ $\tilde{t} = \tau$
Nondimesnioal equations	$\frac{\partial \tilde{u}}{\partial \tilde{t}} = \nabla^2 \tilde{u}$ $\mathbf{n} \cdot (\nabla \tilde{u}) = -\frac{k x_o}{D} \tilde{u}$ $\mathbf{n} \cdot (\nabla \tilde{u}) = 0$ $\mathbf{n} \cdot (\nabla \tilde{u}) = Q_0 \frac{x_0}{D u_{\max}} e^{-\tilde{t} T / \tau}$	$\frac{\partial \tilde{u}}{\partial \tilde{t}} = \frac{D\tau}{{x_0}^2} \nabla^2 \tilde{u}$ $\mathbf{n} \cdot (\nabla \tilde{u}) = -\frac{k x_0}{D} \tilde{u}$ $\mathbf{n} \cdot (\nabla \tilde{u}) = 0$ $\mathbf{n} \cdot (\nabla \tilde{u}) = Q_0 \frac{x_0}{D u_{\max}} e^{-\tilde{t}}$
Dirichlet release conditions	$\tilde{u} = e^{-\tilde{t}\frac{T}{\tau}}$	$\tilde{u} = e^{-\tilde{t}}$

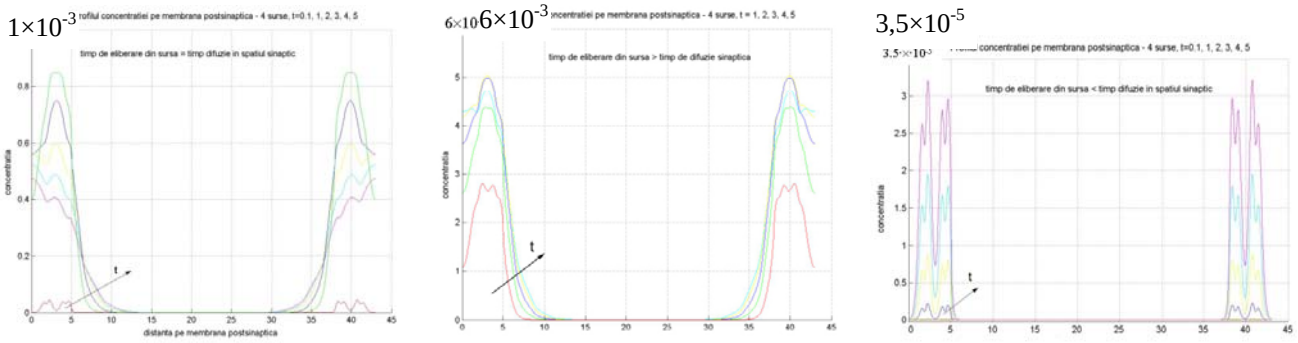
Table 1. Nondimensional models

RESULTS AND DISCUSSION

The 2D problem

Figure 3 presents the profile of the ACh concentration at postsynaptic membrane level for simulations performed using flux conditions at four release ports, for all cases distinguished by the relation between the time parameters of the release and diffusion processes.

Similarly, figure 4 presents the results of the simulations using Dirichlet release boundary conditions. The curves are at different times, varying from t = 0,1 to t = 5 (nondimensional).



Case I. $t_f = T$ **Case II.** $t_f > T$ **Case III**. $t_f < T$.

Fig. 3. ACh concentration on the postsynaptic membrane – 4 release ports, flux BCs at the release ports

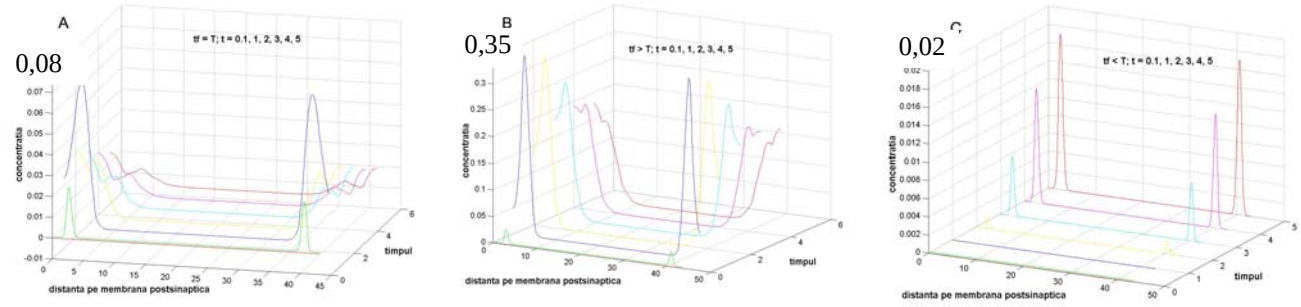


Fig. 4. ACh concentration profile on the postsynaptic membrane – 2 release ports, Dirichlet boundary condition at the release ports. A. $t_f = T$, B. $t_f > T$, C. $t_f < T$.

The 3D problem

Figure 5 displays the results of the 3D simulations using Dirichlet boundary conditions on the release ports; the time step is 0.2, and the last curve is for $t = 1$. For a better view on the process, we present the profiles on the presynaptic membrane, on a line drawn near the center of the release ports (at 10 nm).

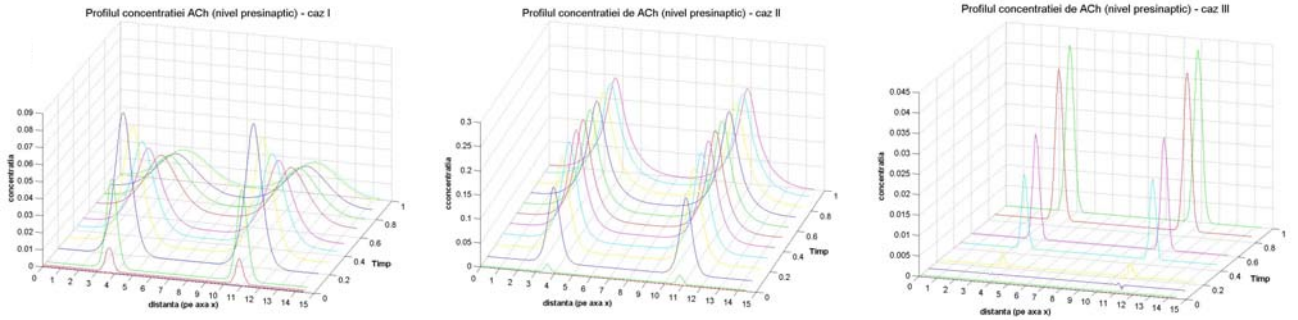


Fig. 5. ACh concentration profiles on the presynaptic membrane – 3D simulation

We computed the integral concentration of ACh in the simulation space, for both 2D and 3D models. Table 2 presents these results at $t = 1$, for 2 presynaptic ACh release ports.

	Flux BCs, 2D	Dirichlet BCs, 2D	Dirichlet BCs, 3D
Case I	0.027862	0.41586	0.40665
Case II	0.066295	1.8015	1.4497
Case III	0.0013566	0.10442	0.049384

Table 2. The results for t = 1 when two presynaptic ports are activated

CONCLUSIONS

This paper introduces an exponential release kinetic of ACh form the presynaptic vesicles, an approach that does not require the inclusion of the presynaptic vesicles in the simulation space and that accounts for the changes in the vesicles volume during release, as well as of possible active release processes (i.e., contractile proteins).

Three cases are studied, for different release times. The highest values of the ACh concentration are reached in the second case, but after a longer time than in the first case. In the third case, the peak values are increasing constantly in time, but they reach a lower maximum value than in the second case.

The value obtained for the time constant of the diffusion process is consistent with previously reported data [11], [13] and sustains the validity of the model; further studies, involving electro-chemical synaptic modeling, will allow comparing the theoretically obtained and experimental measured synaptic currents, leading to further improvement of the model and a better insight on the neurotransmission phenomena.

The paper probes the necessity and utility of 3D simulations, by evidencing significant discrepancies between the results produced by 2D and 3D models, under the same simulation conditions.

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