# Simulation Analysis of the Effects of the Simultaneous Release of Quanta of Acetylcholine on the Endplate Current at the Neuromuscular Junction

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**Abstract**   Arrival of an action potential to a nerve terminal at the neuromuscular junction induces the release of a few hundred quanta of acetylcholine (ACh) into the synaptic cleft, resulting in depolarization of the muscle cell which is observed as the endplate current (EPC).   The release of each quantum of ACh invokes the miniature endplate current (MEPC), so that an EPC could be generated by summation of the MEPCs both in time during evolution of the EPC and in space for a certain area of the postsynaptic membrane.   In this study a mathematical model for EPC generation is developed as a reaction-diffusion system (RD system) which represents the dynamic behavior of ACh in the chemical transmission process with the simultaneous quantum release of ACh.   The RD system for ACh is mathematically expressed by a two-dimensional diffusion equation with nonlinear reaction terms due to the rate processes for acetylcholinesterase and ACh receptor (AChR).   Numerical solution of the governing equation with the method of lines and the Gear method yields temporal changes in relative concentrations of the open channel form of AChR which is assumed to be equivalent to the EPC.   Analysis of the behavior of the RD system with respect to the various distances between the release sites of ACh on the presynaptic membrane demonstrates that the amplitude of EPC is quite sensitive to the distances around 0.5μm, but independent of the values of the diffusion coefficient of ACh in the synaptic cleft.

## 1. INTRODUCTION

A two-dimensional compartment model for the dynamic behavior of acetylcholine (ACh), a typical neurotransmitter, in spontaneous generation of the miniature endplate current (MEPC) at the neuromuscular junction has been proposed by Naka et al. [1997] to analyze the transient process of the synaptic transmission.   The model is formulated in a polar coordinate system of the radial and transverse axes to express the respective diffusion process of ACh in the axis-symmetrical disc which represents a certain effective space in the synaptic cleft for the generation of MEPC.   It is revealed from the analysis with this model that

the radial diffusion process of ACh has more distinctive effects on spontaneous generation of the MEPC than the transverse diffusion process, so that even the homogeneous state is apparently allowed in the transverse direction.   This model is also applied to examine the functional significance of the specific structures of the junctional folds (Naka [1998]) and of the synaptic vesicles (Naka et al. [1999]) at the neuromuscular junction.   The neurotransmitter release mechanism is further analyzed with the model through evaluation of the characteristic parameters of MEPC (Naka [1999]).

In this study the compartment model is modified to delineate the process for the generation of the

endplate current (EPC) comprised of a number of MEPCs in response to the respective quantal release of ACh. Instead of the polar coordinate system, the Cartesian coordinate system with the two orthogonal axes in a square plate of the synaptic cleft is employed to represent the dynamic behavior of multiple MEPCs. Diffusion of ACh takes place in the longitudinal directions and the homogeneous concentration of ACh is assumed in the transverse direction. The effects of the density of the quantal release of ACh on generation of the EPC are analyzed with the model, revealing that the change in the distance between the release sites of ACh on the presynaptic membrane has significant effects on the amplitude of EPC regardless of the values of the diffusion coefficient of ACh in the synaptic cleft.

## 2. CONSTRUCTION OF THE MODEL

### 2.1 Mechanisms for ACh Release and Transmission in the Synaptic Cleft

An action potential arrives at the nerve terminal, inducing the release of a few hundred quanta of ACh into the synaptic cleft. The ACh molecules released diffuse in the synaptic cleft, undergoing hydrolysis by acetylcholinesterase (AChE), and binding with ACh receptor (AChR) to alter the ionic permeability of the muscle cell membrane. This results in depolarization of the muscle cell which is observed as the EPC. The EPC could be considered as the sum of the MEPCs responding to respective release of a quantum of ACh both in time during evolution of the EPC and in space for a certain area of the postsynaptic membrane. Though three dimensions in space are required to represent the summation of MEPC in time and in space, the assumption of simultaneous release of all quanta of ACh allows formulation of the EPC generation process in the two-dimensional compartment model with Cartesian coordinate system.

In a reaction-diffusion (RD) system as illustrated in Figure 1, the ACh concentration $A(x,y,t)$ is assumed to vary with time $t$ and point $(x,y)$ in a space of square plate of the synaptic cleft with the side length $2L$ and height $w$. The homogeneous concentration of ACh in the transverse direction is
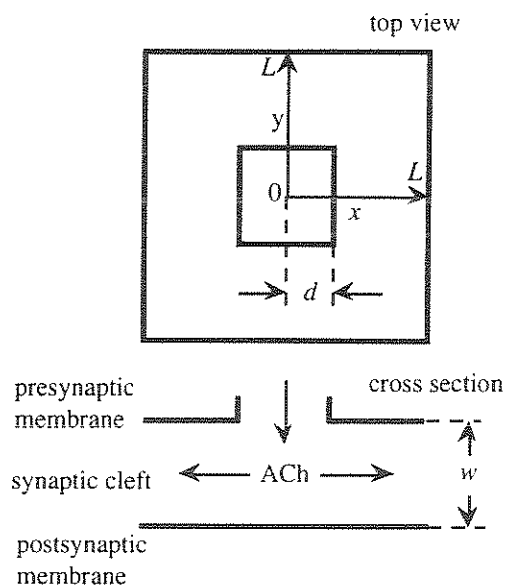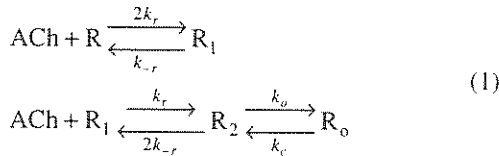


Figure 1 Reaction-diffusion system for ACh in a space of square plate of the synaptic cleft in the two-dimensional Cartesian coordinate system. A quantum of ACh molecules is released on the release area with the size of $d$ from the synaptic vesicle. AChE and AChR are uniformly distributed in the space. The grid point is located at the origin 0.

assumed, and this is justified with the analysis of the dynamic behavior of ACh previously described by Naka et al. [1997]. Instead of the polar coordinate system in an axis-symmetrical disc, which has been used to formulate the RD system of MEPC, the model is constructed with Cartesian coordinate system in a space of square plate which is suitable to fill up the space of the synaptic cleft with the multiple quantal releases of ACh corresponding to the EPC.
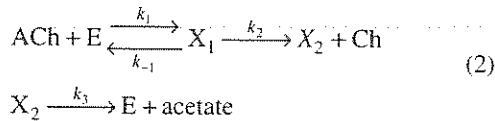
The ACh molecules are released into the synaptic cleft from the narrow pore formed by the fusion process of a synaptic vesicle with the presynaptic membrane. After the localized influx from a synaptic vesicle through a square area with the side length of $2d$ on the presynaptic membrane, the ACh molecules undergo the longitudinal diffusion in the space of the square plate of the synaptic cleft and the interactions with AChE and AChR which are distributed uniformly in the space. The side length $2L$ of the square plate is the distance between the ACh release sites on the presynaptic membrane. The localized release of ACh into the

synaptic cleft is represented with the instantaneous and impulse-wise spread of ACh into the square region of the side length $2d$. The distance $d$ between the grid point and the edge of the square plate is equal to the cleft width ($w$), and corresponds to the natural release of ACh.

The interaction of ACh with functionally dimeric AChR follows the minimal mechanism proposed by Land et al. [1984] as given in:

$$\text{ACh} + \text{R} \underset{k_{-r}}{\overset{2k_r}{\rightleftarrows}} \text{R}_1$$

$$\text{ACh} + \text{R}_1 \underset{2k_{-r}}{\overset{k_r}{\rightleftarrows}} \text{R}_2 \underset{k_c}{\overset{k_o}{\rightleftarrows}} \text{R}_o \tag{1}$$

where R and $R_1$ indicate the AChR species free and singly bound with ACh, respectively, and the AChR species doubly bound with ACh, $R_2$ and $R_o$, are associated with ion channel function of AChR so that the closed channel form $R_2$ interconverts to the open channel form $R_o$. The $k_i$'s ( i = r, -r, o, c ) are the rate constants for the respective steps in the mechanism. The reaction of AChE (EC 3.1.1.7) proceeds in the following mechanism originally proposed by Rosenberry [1975]:

$$\text{ACh} + \text{E} \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} X_1 \overset{k_2}{\longrightarrow} X_2 + \text{Ch} \tag{2}$$

$$X_2 \overset{k_3}{\longrightarrow} \text{E} + \text{acetate}$$

where E, $X_1$ and $X_2$ denote AChE species free and complexed with ACh and acetyl group, respectively, and Ch is choline. The $k_i$'s ( i = 1, -1, 2, 3) are the rate constants for the respective steps in the mechanism.

## 2.2 Reaction-Diffusion System for the ACh Behavior

The RD system for ACh in the synaptic cleft associated with the generation of EPC is thus represented by a two-dimensional diffusion equation with nonlinear reaction terms for ACh and the rate equations for AChE and AChR as follows:

$$\frac{\partial A}{\partial t} = D\left(\frac{\partial^2 A}{\partial x^2} + \frac{\partial^2 A}{\partial y^2}\right) - k_1 AE + k_{-1} X_1$$
$$- 2k_r AR + (k_{-r} - k_r A)R_1 + 2k_{-1} R_2$$

$$\frac{\partial E}{\partial t} = -k_1 AE + k_{-1} X_1 + k_3 X_2$$

$$\frac{\partial X_1}{\partial t} = k_1 AE - (k_{-1} + k_2)X_1$$

$$\frac{\partial X_2}{\partial t} = k_2 X_1 - k_3 X_2 \tag{3}$$

$$\frac{\partial R}{\partial t} = -2k_r AR + k_{-r} R_1$$

$$\frac{\partial R_1}{\partial t} = 2k_r AR - (k_{-r} - k_r A)R_1 + 2k_{-1} R_2$$

$$\frac{\partial R_2}{\partial t} = k_r AR_1 - (2k_{-r} - k_o)R_2 + k_c R_o$$

$$\frac{\partial R_o}{\partial t} = k_o R_2 - k_c R_o$$

where the italic capital letter denotes the concentration of the respective chemical species at point $(x, y)$ and time $t$. $D$ is the diffusion coefficient of ACh in the cleft. The behavior of Ch and acetate is not considered in the formulation because this analysis is mainly concerned with the dynamic behavior of ACh in the RD system which is most relevant to evolution of the EPC.

As the simultaneous release of quanta of ACh is assumed at the respective grid points distributed uniformly on the presynaptic membrane, the ACh molecules movable in the space assigned to a grid point do not diffuse beyond the edge of the space, for the exactly same processes are proceeding synchronously in the neighboring spaces. The boundary conditions closed at the edge surfaces of the space of square plate may be formulated as follows:

$$\frac{\partial A(x, y, t)}{\partial x} = 0 \quad at\ x = 0\ and\ x = L$$

$$\frac{\partial A(x, y, t)}{\partial y} = 0 \quad at\ y = 0\ and\ y = L \tag{4}$$

so that ACh cannot leak out from the respective spaces of square plate.

Similarly to our previous studies cited, it is supposed that the total number of the open channel form of AChR is linearly correlated to the MEPC. The EPC resulted from the spatial summation of the MEPC would thus be equivalent to the

spatially averaged, relative concentration of $R_o$ in the space, that is, the ratio of the open channel form to the total number of AChR, obtained by

$$C(t) = \frac{1}{R_T L^2} \int_0^L \int_0^L R_o(x, y, t) dx dy \qquad (5)$$

where $R_T$ $(= R + R_1 + R_2 + R_o)$ denotes the total concentration of AChR at a point. It should be noted in this formula that the summation for the total number of the open channel form of AChR is performed only in the first quadrant because of the axis-symmetrical feature of the process.

The initial condition corresponds to the release of a single quantal packet of ACh ($10^4$molecules), and is expressed by an impulse-wise increase in $A(x, y, 0)$ $(x \leq d$ and $y \leq d)$ from 0 to 33.2mM which is an appropriate concentration for a quantal packet of ACh. There initially exist no ACh inside the space of the square plate.

### 2.3 Simulation Method

For simulation of the RD system under the specified boundary and initial conditions, the method of lines originally proposed by Schiesser [1991] is applied to discretize the partial differential equation with respect to the space variable. The rate equations (ordinary differential equations) thus derived for ACh, AChE and AChR are numerically integrated with respect to time by the Gear method as described in MathWorks guides [1992] to yield the spatial and temporal changes in concentrations of ACh in the space of the square plate and of the open channel form of AChR.

The following values of the kinetic parameters used in Naka et al. [1997] are employed for the simulation in this study as well:

$k_r = 30\text{mM}^{-1}\text{ms}^{-1}, k_{-r} = 10\text{ms}^{-1}, k_o = 20\text{ms}^{-1},$

$k_c = 5.0\text{ms}^{-1}, k_1 = 200\text{mM}^{-1}\text{ms}^{-1}, k_{-1} = 1.0\text{ms}^{-1}$

$k_2 = 110\text{ms}^{-1}, k_3 = 20\text{ms}^{-1}$

The value of 664µM is used for $R_T$ which is evaluated from the surface density of AChR $(2 \times 10^4 \mu\text{m}^{-2})$, and dependent on the volume of discretized space (compartment) in which AChR is uniformly distributed. The value for $E_T$ (total concentration of AChE at a point; $= E + X_1 + X_2$)

is set to 74 µM. The width of the cleft $(w = 50\text{nm})$ is used to calculate the volume of the compartment of the synaptic cleft. Though $D = 1.0 \times 10^{-6} \text{cm}^2\text{s}^{-1}$ is proposed as the appropriate value for the homogeneous diffusion in Naka et al. [1997], the accurate value is still unknown. Hence, the whole analysis in this study is performed in the range of $D$ between $(0.5 \sim 4.0) \times 10^{-6} \text{cm}^2\text{s}^{-1}$.

## 3. EFFECTS OF THE SIMULTANEOUS QUANTAL RELEASE

The simulation analysis of the model with the kinetic and structural parameters given above is performed to examine the effects of the simultaneous release of quanta of ACh on the behavior of $C(t)$ (i.e., equivalent of the relative value of EPC) by variation in the values of the distance between the release sites of ACh on the presynaptic membrane.

It is demonstrated in Figure 2 that the responses of $C(t)$ to the simultaneous release of ACh with $D = 1.0 \times 10^{-6} \text{cm}^2\text{s}^{-1}$ vary according to various values of the distance between the release sites of ACh. The maximum relative concentrations are attained around 0.3ms after the release of ACh. It should be noted that the shorter distance between the release sites implies the higher concentration of ACh released into the synaptic cleft because the number of ACh molecules in a synaptic vesicle is
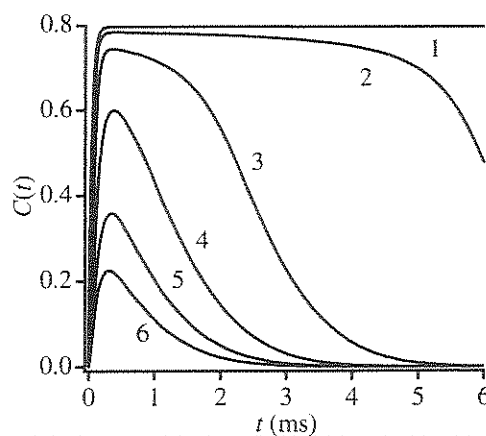


Figure 2　Effect of the distance between the ACh release sites on the response of EPC. The number on a curve corresponds to the value of $L$ (in µm): 1: 0.05, 2: 0.1, 3: 0.15, 4: 0.2, 5: 0.25, and 6: 0.3.

set at a constant value. Therefore, it is a natural consequence of this implication that the maximum values of $C(t)$ get higher with shorter distances of $L$. After reaching the peak value $C(t)$ decreases to the almost null in a certain time except for the case of $L = 0.05\mu m$ which corresponds to the homogeneous release of ACh because the size of $L$ is equal to the size $d$ of the release area of ACh. The falling phase of $C(t)$ lasts longer with closer release sites of ACh, and holds plateau in the cases of $L = 0.05$ and $0.1\mu m$.

To analyze the effects of the distance $L$ and the diffusion coefficient $D$ of ACh on the responses of $C(t)$ quantitatively, three characteristic parameters are utilized; that is, the amplitude $C_{max}$ (maximum value of $C(t)$), the rise time $t_m$ (time for $C(t)$ to increase from 20 to 80% of $C_{max}$) and the decay constant $\tau$ (relaxation time constant for exponential decay of $C(t)$). The effects of the distance between the ACh release sites in the range of $0.05 \leq L \leq 1.0\ (\mu m)$ are displayed for these characteristic parameters respectively in Figure 3. The characteristic parameters for the homogeneous release of ACh with the corresponding concentrations to respective distances between the release sites are also shown as the dashed curves to distinguish the effects of quantal releases from those of the ACh concentrations in the cleft.

Figure 3(a) and (c) illustrate the effects on the amplitude $C_{max}$ and the decay constant $\tau$, respectively. The values of the parameters monotonously decrease with larger values of $L$ and the curves have almost no differences in the shape among the examined variation of the diffusion coefficient $D$ and the homogeneous release designated as the dashed curve. On the other hand, as shown in Figure 3(b), the rise time $t_m$ increases with larger values of $L$ up to $0.2\mu m$ and then decreases to the almost constant value. The highest values are attained around $L$ of $0.2\mu m$. In contrast to the other characteristic parameters, the values of the diffusion coefficient $D$ of ACh has the substantial effects on the rise time $t_m$, which takes the lager values with the smaller values of $D$ and behave differently from the case of the homogeneous release of ACh. It thus is concluded that the change in the distance between the ACh release sites has significant effects on $C(t)$ in the range of values of $L$ between $0.2\mu m$ and
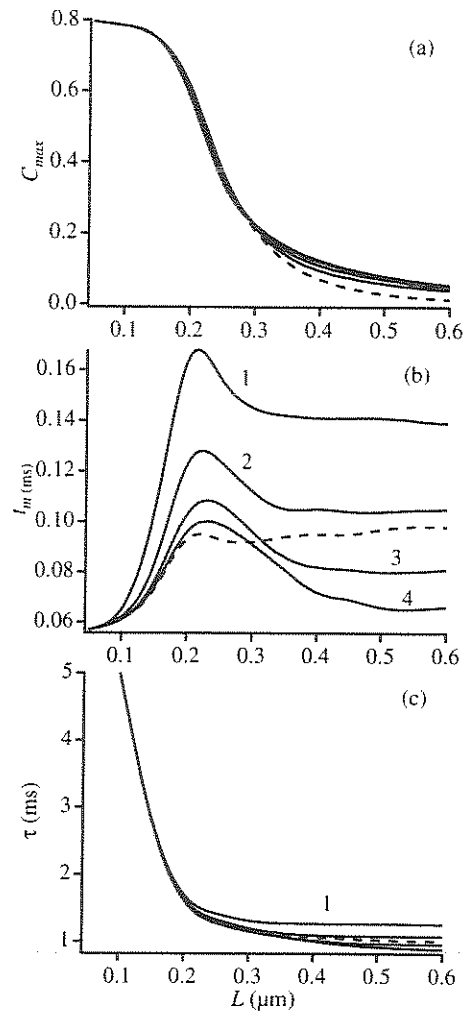


Figure 3 Effects of the distance $L$ between the ACh release sites on the response of EPC. Effects on: (a) the amplitude; (b) the rise time; and (c) the decay constant. The number on a curve corresponds to the value of $D$ ($\times 10^{-6}\,\mathrm{cm^2\,s^{-1}}$): 1: 0.5, 2: 1.0, 3: 2.0, and 4: 4.0. The dashed curves correspond to the homogeneous release of ACh.

$0.3\mu m$ and that the diffusion coefficient $D$ of ACh in the synaptic cleft affects only the rise time of the EPC.

## 4. DISCUSSION

In this study, a compartment model as an RD system of ACh in the space of square plate of the synaptic cleft is constructed to reveal the effects of the simultaneous quantal release from synaptic vesicles on generation of the EPC. The simulation analysis demonstrates that the shape of

EPC is quite sensitive to the distances around 0.5μm between the quantal release sites. This sensitive distance is in good agreement with the empirical data on electronmicrographs of a nerve terminal to be stimulated by presynaptic action potentials at the neuromuscular junction shown by Matthews [1986].

It is suggested by Naka et al. [1999] that the localized release of ACh due to the quantal characteristics of the synaptic vesicle could enlarge the amplitude of MEPC compared with the spread release like the homogeneous increase of ACh in the cleft. Such effect on the amplitude of EPC, though still detectable, is almost negligible compared with the effect of concentration of ACh due to the distance between the release sites, as described in this study. Furthermore, it is found that the localization effect is also negligible for the falling phase of EPC in contrast to the substantial effects on the rising phase of EPC. Therefore, it could be implied that the homogeneous state might be allowed in both longitudinal and transverse directions in order to analyze the amplitude and the falling phase of EPC. This characterization of the behavior of EPC obviously results from introduction of the longitudinal diffusion process of ACh in the model.

In contrast to the model for EPC constructed only with respect to the spatial summation in this study, Giniatullin et al. [1995] employs the model only with the temporal summation to analyze the relationship between the shapes of MEPC and the resulting EPC. The negligible effects of the spatial summation of the ACh quantal releases on the amplitude and the falling phase of EPC mentioned above might support the Giniatullin's model for analyzing the amplitude and the falling phase of EPC.

## 5. CONCLUSIONS

The simulation analysis with the two-dimensional compartment model is performed for characterization of EPC generated at the neuromuscular junction with respect to the various distances between the quantal release sites of ACh on the presynaptic membrane. It is demonstrated that the amplitude of EPC is quite sensitive to the distances around 0.5 μm, that is, under this condition slight change in the number of the ACh packets could have significant effects on the amplitude of EPC.

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