Coupling Effect of Ion Channel Clusters on Calcium Signaling*

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Based on a modified intracellular Ca\textsuperscript{2+} model involving diffusive coupling of two calcium ion channel clusters, the effects of coupling on calcium signaling are numerically investigated. The simulation results indicate that the diffusive coupling of clusters together with internal noise determine the calcium dynamics of single cluster, and for either homogeneous or heterogeneous coupled clusters, the synchronization of clusters, which is important to calcium signaling, is enhanced by the coupling effect.

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Intracellular Ca\textsuperscript{2+} is a primary regulator with a variety of cell functions, for example, early response to injury of brain tissue,\textsuperscript{[1]} neurotransmitter release,\textsuperscript{[2]} synaptic plasticity,\textsuperscript{[3]} gene expression,\textsuperscript{[4]} and cell death,\textsuperscript{[5]} etc. Most of the Ca\textsuperscript{2+} ions that constitute the signal are released from intracellular stores such as the endoplasmic reticulum (ER) into the intracellular space. Inositol triphosphate receptors (IP\textsubscript{3}Rs) represent the corresponding intracellular calcium release channels.

It is widely accepted that the release channels are spatially organized in clusters,\textsuperscript{[6]} and there are only a few tens of intact IP\textsubscript{3} receptor channels in \(\mu\text{m}^{2}\)-sized clusters. Single release channel, named Ca\textsuperscript{2+} blips, has been observed in HeLa cells.\textsuperscript{[6]} Collective opening and closing of serval Ca\textsuperscript{2+} channels in a cluster, named puffs, has been also observed in Xenopus oocytes.\textsuperscript{[7]} That suggests a hierarchy of calcium signaling events from small blips to larger puffs,\textsuperscript{[6, 8]} which function as elementary building blocks through which various Ca\textsuperscript{2+} waves can be generated. Improved spatial and temporal resolution in recording reveals that there is not a clear distinction between fundamental blips and elementary puffs.

In nonlinear biological systems, the multi-system coupling is ubiquitous, and essential for specific biological function. Many biological behaviours, such as calcium signals propagation,\textsuperscript{[9]} optimal signaling in coupled hormone systems,\textsuperscript{[10]} cell-cell communication in multicellular systems,\textsuperscript{[11]} result from the inter-system coupling. In coupled hormone systems, cells are coupled to each other by mutual feedback,\textsuperscript{[10]} so it is defined as direct coupling between cells. However, in the multicellular systems of Ref. [11], signal molecules diffuse between cells and extracellular environment.\textsuperscript{[11]} Multi-cell coupling is indirectly accomplished through extracellular environment, it is accordingly defined as indirect coupling. In this Letter, discretely distributed ion channel clusters can be seen as a kind of indirect coupled blocks connected through space without ion channels.

The cooperative effects of noise and coupling, which can produce synchronization between systems,\textsuperscript{[12]} on intracellular Ca\textsuperscript{2+} signaling are intensively studied\textsuperscript{[13, 14]}. For instance, Shuai et al. studied intracellular Ca\textsuperscript{2+} waves in Ref. [14], where many indirectly coupled calcium ion channel clusters distribute on the membrane of ER, it is concluded that channel noise in conjunction with spatial clustering can result in the onset of spatially and temporally extremely coherent Ca\textsuperscript{2+} signals at levels of stimulant well below the threshold of Ca\textsuperscript{2+} oscillations for homogeneously distributed channels. However previous works were focused on signaling at level of cell. In this study, based on a modified intracellular Ca\textsuperscript{2+} model\textsuperscript{[14, 15]} involving diffusive coupling of two calcium ion channel clusters, the effects of coupling on calcium signaling at the level of ion channel cluster are numerically investigated. Our results show that the diffusive coupling of clusters, together with internal noise, significantly determine the calcium dynamics of single cluster; and for either homogeneous or heterogeneous coupled clusters, the synchronization of clusters, which is important to calcium signaling, is enhanced by the coupling effect.

The model used here involves two ion channel clusters between which there is a distance of 3 \(\mu\text{m}\),\textsuperscript{[14]} which is a typical cluster distance in Xenopus oocyte. Ion channels only distribute in the clusters. Ca\textsuperscript{2+} can diffuse along the one-dimensional space between two clusters. Considering Ca\textsuperscript{2+} efflux from intracellular stores through channel, the ATP dependent Ca\textsuperscript{2+} flux from the intracellular space back to stores, and the leak flux, the Ca\textsuperscript{2+} dynamics is given by

\[
\frac{dz(x)}{dt} = [v_2 + f(x)v_3m^{\alpha}_w n^{\beta}_h h_1 h_2 h_3](z_{er} - z(x))
\]

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where \( z \) denotes the intracellular \( \text{Ca}^{2+} \) concentration, and \( D \nabla^2 z(x) \) is the diffusive term along the one-dimensional space, \( D (\mu \text{m}^2/\text{s}) \) represents the diffusive or coupling coefficient, \( x (\mu \text{m}) \) is the spatial coordinate, and the position of two clusters are set as \( x = 0, L \), where \( L = 3 \mu \text{m} \). Every channel has three identical subunits and every subunit has three binding sites: one for the messenger molecule \( \text{IP}_3 \) (m gate), an activating site (n gate) for \( \text{Ca}^{2+} \), and an inactivating site (h gate).\(^{[16,17]} \) In order for a subunit to be conducting \( \text{Ca}^{2+} \), only the \( \text{IP}_3 \) and the activating \( \text{Ca}^{2+} \) binding site need to be occupied. The entire \( \text{IP}_3 \)R channel is conducting if three subunits are conducting. It is well known that channels open and close in a stochastic way that ascribes to the stochastic molecular binding to them,\(^{[18,19]} \) so \( h_1, h_2, h_3 \), the fractions of activated sites for three subunits of channels, satisfy the same Langevin-type equation

\[
\frac{dh}{dt} = \alpha (1 - h) - \beta h + \xi(t), \quad (2)
\]

\[
\alpha = a d_2 \frac{I + d_1}{I + d_3}, \quad \beta = a z, \quad (3)
\]

\( \xi(t) \) is Gaussian white noise, and the statistical properties are given by\(^{[18]} \)

\[
\langle \xi(t) \rangle = 0, \quad \langle \xi(t) \xi(t') \rangle = \frac{\alpha (1 - h) + \beta h}{N} \delta(t - t'), \quad (4)
\]

\( N \) represents the channel number in the cluster. Because the \( \text{Ca}^{2+} \) can efflux from intracellular stores through channel only happens within clusters, the form function \( f(x) \) is defined as

\[
f(x) = \begin{cases} 
0, & 0 < x < L, \\
1, & x = 0, L.
\end{cases} \quad (5)
\]

Then the parameters \( m_\infty \) and \( n_\infty \) are calculated by

\[
m_\infty = \frac{I}{I + d_4}, \quad n_\infty = \frac{z}{z + d_5}, \quad (6)
\]

where \( I(\mu \text{M}) \) denotes \( \text{IP}_3 \) concentration, and all other constant parameters can be taken from Ref.\(^{[14]} \).

\( \text{IP}_3 \) concentration is an experimentally controllable parameter. The \( \text{Ca}^{2+} \) concentration bifurcation diagram of one of the two clusters, which is obtained by taking \( z = 0 \) or \( L \), is shown as a function of the \( \text{IP}_3 \) concentration in Fig. 1. It can be seen that the coupling effect greatly reduces the oscillatory amplitude, and the oscillatory region is shifted to higher \( \text{IP}_3 \) concentration. In addition, the extent of oscillatory region of \( \text{Ca}^{2+} \) concentration is also slightly enhanced by coupling. Thus it can be inferred from Fig. 1 that coupling effect of two clusters greatly changes the dynamical behaviour of \( \text{Ca}^{2+} \) signal. In this study, our attention is focused on diffusive coupling, so \( \text{IP}_3 \) concentration is invariably set 0.4 \( \mu \text{M} \).

![Fig. 1. Comparison of bifurcation diagrams with (the lower one with \( D = 5 \mu \text{m}^2/\text{s} \)) and without (the higher one) diffusive coupling. The solid circles indicate maximum and minimum of stable limit cycles, while the hollow circles indicate unstable periodic orbits; the solid lines indicate stable fixed points, while the dashed line indicates unstable fixed points.](image1)

![Fig. 2. Comparing time series of different conditions of (a) without both coupling and noise; (b) with coupling and without noise; (c) with both coupling and noise. \( D = 5 \mu \text{m}^2/\text{s} \) for all conditions.](image2)
involved but noise effect is neglected in Fig. 2(b), the oscillatory behaviour totally disappears. Then in Fig. 2(c), both noise and coupling effect are involved, the oscillatory behaviour is found to be similar to real cells. Thus the noise and coupling are two important factors that can not be neglected when the Ca\(^{2+}\) oscillation is studied.

Fig. 3. Compare Ca\(^{2+}\) concentration time series of two homogeneous clusters (solid and dashed lines) for different \(D\). From top to bottom: \(D = 0, 5, 10\, \mu\text{m}^2/\text{s}\).

Fig. 4. \(\tau_c\) of Ca\(^{2+}\) dynamics of two homogeneous coupled clusters for various \(D\).

The synchronized oscillatory behaviour of Ca\(^{2+}\) concentration is important to calcium signaling. In what follows, the synchronization of Ca\(^{2+}\) oscillatory behaviour for two coupled clusters is quantitatively studied. Firstly, we focus on two coupled homogeneous clusters (with the same channel number \(N = 36\)). In Fig. 3, the Ca\(^{2+}\) concentration time series of two clusters are compared for different diffusive coefficient \(D\). It can be seen that with the increasing \(D\), the Ca\(^{2+}\) dynamics of two clusters becomes more synchronous. In addition, the amplitudes and frequencies of oscillations are reduced by diffusive coupling. When \(D\) exceeds 10 \(\mu\text{m}^2/\text{s}\), no prominent oscillation is found except for some little-amplitude oscillation (10\(^{-2}\) M scale amplitude). It must be pointed out that although there is not prominent oscillation in clusters, the prominent cell-averaged Ca\(^{2+}\) oscillation can be found.

Fig. 5. Compare the PSD of Ca\(^{2+}\) dynamics of two homogeneous clusters. \(D = 0\).

Fig. 6. Compare Ca\(^{2+}\) concentration time series of two heterogeneous clusters (solid lines: \(N = 10\) and dashed lines: \(N = 500\)) for different \(D\). From top to bottom: \(D = 0, 5, 10\, \mu\text{m}^2/\text{s}\).

To quantitatively describe the synchronization of two clusters, we introduce the cross-correlation time of Ca\(^{2+}\) dynamics of two coupled clusters,

\[
\tau_c = \int_0^\infty C^2(\tau) \, d\tau ,
\]  
where

\[
C(\tau) = \frac{\langle \tilde{z}_1(t) \tilde{z}_2(t + \tau) \rangle}{\sqrt{\langle \tilde{z}_1^2 \rangle \langle \tilde{z}_2^2 \rangle}}, \quad \tilde{z}_i = z_i - \langle z_i \rangle ,
\]  
where subscripts \(i = 1, 2\) indicate the clusters; \(\tau_c\) is plotted against diffusive coefficient in Fig. 4, which quantitatively shows that the diffusive coupling can drastically enhance the cluster-cluster correlation of Ca\(^{2+}\) dynamical behaviour. Because no prominent
oscillation is found when $D$ exceed $10 \mu m^2/s$, $\tau_c$ for $D > 10 \mu m^2/s$ has not been plotted. From time revolutions of Ca$^{2+}$, we can see that although there are not larger-amplitude oscillations for $D > 10 \mu m^2/s$, two clusters are perfectly synchronized.

From a physiological point of view, the actual frequency of the oscillations is of great relevance since it encodes information. Thus power spectral densities (PSDs) of Ca$^{2+}$ dynamics of clusters are compared in Fig. 5. Although the coupling is not involved, the peak values of the two PSD diagrams are totally identical. The PSD diagrams have also been plotted for different $D$ (the data are not shown here), the results show that coupling does not affect sameness of the two PSD diagrams.

Secondly, for real cells, the ion channel numbers in clusters range from several tens to several hundreds, and the difference between clusters is experimentally proven. Thus two heterogeneous coupled clusters will be studied, as a example, two channel numbers are set to be $N = 10$ and 500. Similarly to Fig. 3, time revolutions of Ca$^{2+}$ concentrations of two heterogeneous clusters are compared for different diffusive coefficients in Fig. 6. Because of the difference of noise intensities, oscillatory amplitude of the cluster with less channels (with stronger noise) is larger than that of the one which has more channels. With the increasing diffusive coefficient $D$, the difference is reduced, and the oscillations become more synchronous. In addition, as is found in Fig. 3, the amplitudes and frequencies of oscillations are reduced by diffusive coupling. When $D$ exceeds $12.5 \mu m^2/s$, prominent oscillation totally disappears except for some little-amplitude oscillation ($10^{-2} \mu M$ scale amplitude).

The cluster-cluster correlation changing with $D$ is quantitatively depicted by the cross-correlation time $\tau_c$. The enhancement of $\tau_c$ by increasing $D$ is found again (the data are not shown here). We have also compared the peak values of the PSD of two heterogeneous clusters in Fig. 7. When the coupling effect is excluded, the two peak values of PSD are not the same, which ascribe to the heterogeneity of two clusters. With the increasing diffusive coefficient, the difference between two peak values is reduced, and finally they become identical.

In conclusion, based on a modified intracellular Ca$^{2+}$ model, two diffusively coupled ion channel clusters are studied. The numerical results show that whenever noise or coupling effect are excluded, reasonable Ca$^{2+}$ dynamics cannot be obtained. Only when they are both considered in the model, the results accorded with experiments can be obtained, i.e., the diffusive coupling of clusters, together with internal noise, significantly determine the calcium dynamics of single cluster.

Furthermore, the synchronization of two coupled clusters is studied. Firstly, two coupled homogeneous clusters (with the same channel numbers) are studied, the results indicate that coupling can drastically enhance synchronization of two clusters, which is quantitatively described by cross-correlation time $\tau_c$ in Fig. 4. Secondly, similar conclusion can be made for coupled heterogeneous clusters (with different channel numbers). Moreover, in the second context, coupling effect can enhance the sameness of inherent frequencies of two heterogeneous clusters (see Fig. 7). In one word, coupling effect of ion channel clusters is an crucial factor to determine the Ca$^{2+}$ signaling.

References

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