Calcium spikes: Chance or necessity?

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Calcium oscillations present one of the most important signaling mechanisms in cell biology. The standard paradigm for the origin of calcium oscillations is dynamic, i.e. fast release of calcium from intracellular stores is followed by slow inhibition. Yet, this very dynamic theory for these oscillations came recently into scrutiny since the building blocks of cellular calcium signals are spatially and temporally limited calcium release events through small, distinct cluster of ion channels. According to this new paradigm, a coherent wave of calcium release, triggered by stochastic release events from a group of clusters, sweeps the cell. Oscillations are believed to emerge through a spatial coherence resonance mechanism. In this paper we investigate the difference in stochastic spiking generated by a small periodic system and a small excitable system and compare with published experimental data.

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1. Introduction

Calcium signaling is one of the most important and versatile signaling mechanisms in cell biology. It describes the transient release of calcium from intracellular, calcium rich stores, most notably the endoplasmic reticulum (ER), into the cytosol. The most elemental signals are generated by the release of calcium through small clusters of release channels, called IP₃Rs or inositol trisphosphate receptor channels (for a review, see [1]), in the ER membrane. These channels are ligand gated and require the second messenger inositol trisphosphate (IP₃) and cytosolic calcium bound in order to open. Hence, the release of calcium from stores through an open channel and the subsequent local increase of cytosolic calcium can trigger the opening of nearby channels within a cluster and in turn more calcium release. This positive-feedback process has been coined calcium-induced calcium release.

Yet, this dynamic mechanism for calcium oscillations came into scrutiny since (a), the calcium concentrations around an open channel are large (for an theoretical estimate see, [3]) and inhibit channels rather rapidly, excluding oscillations on the organizational scale of channels, and (b) the clustered organization of the channels in small, spatially distinct clusters prohibits averaging, reinforcing stochasticity even on the cell-level. As a consequence, calcium spikes should be considered stochastic events ([4,5]). Periodic oscillations on the cell-level have been hypothesized by Skupin et al. [4] and Skupin and Falcke [5] through the combination of wave nucleation and a spatial form of coherence resonance, a mechanism put forward in [6] in a more general context. On the other hand, no experimental values for the domain calcium concentrations at the clusters are available, and a few IP₃Rs would also give rise to stochastic behavior even if the calcium concentrations at the cluster sites would be smaller as predicted allowing for dynamic periodicity. The question we address in this paper is whether “stochasticity” in a small oscillatory system (small numbers of channels) can be discerned from stochasticity in a non-oscillatory small system by experimentally accessible observables (see [4]).

2. The model

The starting point is a model for the average cytosolic Ca²⁺-concentration, c, (similar in the spirit than the original De Young–Keizer model [2]) where spatial gradients are not resolved, i.e.:

\[
\frac{dc}{dt} = j_{IP₃} - j_{pump} + j_{leak}.
\]

Here, \(j_{IP₃}\) describes the flux of Ca²⁺ from the ER into the cytosol through the IP₃Rs, i.e.:

\[
j_{IP₃} = v_{IP₃}f_{open}(C_{ER} - c).
\]
where \( v_{IP}\) denotes the maximum flux, \( f_{open} \) the fraction of open channels, and \( c_{ER} \) the calcium concentration in the ER. The calcium concentration in the ER is much larger than in the cytosol and is assumed to be constant. Extrusion of cytosolic calcium is facilitated by sarcoendoplasmic reticulum calcium ATPases (SERCA or calcium pumps) present on the ER membrane. We model the pump flux \( f_{pump} \) using a Hill-form with a Hill-coefficient of 2, i.e.:

\[
f_{pump} = \frac{v_{pump} C^2}{k_{pump} + C^2}.
\]

(3)

The term \( J_{leak} = v_{leak}(c_{ER} - c) \) describes nonspecific Ca\(^{2+}\) leakage from the calcium rich ER into the cytosol. The fraction of open channels, \( f_{open} \), is determined by the specific model for the IP\(_3\) receptor. We use here a recently published derivative of the De Young–Keizer model for the IP\(_3\) receptor, which generates calcium puffs consistent with experimental observations (\[7\]) (see Fig. 1). The model for the IP\(_3\) receptor sports four identical subunits, each of which has one binding site for IP\(_3\) and two binding site for calcium, one for activation, one for inhibition. Each binding site is either occupied or unoccupied. Therefore, each subunit can exist in any of eight permissible states. We force sequential binding of IP\(_3\) and Ca\(^{2+}\) to the receptor, i.e. Ca\(^{2+}\) only binds to the activation site on the subunit when IP\(_3\) is bound. Thus, if \( S_{ijk} \) denotes the state of a subunit, where \( i \) characterizes the state of the IP\(_3\) binding site (\( i = 0 \) means not bound), \( j \) the state of the Ca\(^{2+}\) activation binding site, and \( k \) the state of the inhibiting Ca\(^{2+}\) binding site, the two states \( S_{010} \) and \( S_{011} \) do not exist. Therefore we have a six state model for each subunit of the IP\(_3\)R. The subunit is activated only when the IP\(_3\) and Ca\(^{2+}\) activation sites are occupied. The channel is considered open if any three or all four subunits are activated.

The full set of rate equations describing a subunit of the IP\(_3\) receptor, introduced above, reads (see also Fig. 1)

\[
\begin{align*}
\frac{dx_{000}}{dt} &= b_1 x_{100} + b_4 x_{001} - (a_1 p + a_4 c) x_{000}, \\
\frac{dx_{001}}{dt} &= b_1 x_{101} + a_4 c x_{000} - (b_2 + a_3 p) x_{001}, \\
\frac{dx_{100}}{dt} &= a_1 p x_{000} + b_2 x_{101} + b_3 x_{110} - (b_1 + a_3 c + a_2 c) x_{100}, \\
\frac{dx_{101}}{dt} &= b_2 x_{111} + a_2 c x_{100} + a_4 c x_{001} - (a_5 c + b_2 + b_3) x_{101}, \\
\frac{dx_{110}}{dt} &= b_2 x_{111} + a_2 c x_{100} - (a_5 c + b_3) x_{110}, \\
\frac{dx_{111}}{dt} &= a_5 c x_{101} + a_2 c x_{110} - (b_5 + b_2) x_{111}.
\end{align*}
\]

(4)

where \( p \) denotes the IP\(_3\) concentration. The binding and dissociation constants are specified in Table 1. The fraction of open channels, \( f_{open} \) in Eq. (2) is given by \( f_{open} = x_{110} + 4 x_{110} (1 - x_{110}) \). Solving the set of differential equations (1) and (4) with parameter values specified in Table 1, we find stationary solutions and oscillatory solutions depending on the IP\(_3\) concentration \( p \). A bifurcation diagram is shown in Fig. 2.

For an IP\(_3\) concentration less than about 0.17 \( \mu \)M, any initial calcium concentration will approach a steady-state value of about 30 nM. Calcium oscillations, occurring for IP\(_3\) concentrations larger than 0.25 \( \mu \)M exhibit much larger maximum amplitudes of the order of 1 \( \mu \)M. The oscillations are smooth, with frequencies (typically a few Hertz) and amplitudes, depending on the IP\(_3\) concentration.

3. Calcium spikes generated by a finite number of channels

We now consider the release of calcium through a small patch of the ER membrane with an area of \( A \). \( N \) channels are assumed to be distributed uniformly over the patch with a constant areadensity of \( \rho_{\text{channel}} = N/A \). The smaller the number of channels, the smaller the patch of membrane. Calcium release events generated by the channels in the patch of any size (any number of channels recruited) are termed spikes in this paper. Note that in this paper

\[\text{Table 1}
\begin{tabular}{|l|l|l|}
\hline
Parameter & Value & Description \\
\hline
\hline
\( v_{IP} \) & 120 s\(^{-1} \) & Max channel flux \\
\( v_{leak} \) & 0.02035 s\(^{-1} \) & Calcium leak flux constant \\
\( v_{pump} \) & 4.5 \( \mu \)M s\(^{-1} \) & Max calcium uptake rate by SERCA \\
\( k_{pump} \) & 0.1 \( \mu \)M & Activation constant for SERCA \\
\( a_1 \) & 167.6 (\( \mu \)M s\(^{-1} \)) & IP\(_3\) binding rate constant \\
\( a_2 \) & 3.81 (\( \mu \)M s\(^{-1} \)) & Calcium inhibition rate constant \\
\( a_4 \) & 413.4 (\( \mu \)M s\(^{-1} \)) & IP\(_3\) binding rate constant \\
\( a_5 \) & 0.3101 (\( \mu \)M s\(^{-1} \)) & Calcium inhibition rate constant \\
\( b_1 \) & 53.9 (\( \mu \)M s\(^{-1} \)) & IP\(_3\) dissociation rate \\
\( b_2 \) & 4.099 s\(^{-1} \) & Calcium inhibition rate \\
\( b_3 \) & 188.5 s\(^{-1} \) & IP\(_3\) dissociation rate \\
\( b_4 \) & 0.096 s\(^{-1} \) & Calcium disinhibition rate \\
\( b_5 \) & 4.52 s\(^{-1} \) & Calcium inactivation rate \\
\hline
\end{tabular}
\]

Fig. 2. The bifurcation diagram for the calcium concentration \( c \) as a function of the IP\(_3\) concentration \( p \) for the model specified by Eqs. (2) and (4). The solid lines represent stable stationary states. The dashed lines represent unstable stationary states. The dotted lines represent stable periodic oscillations (calcium oscillations) while the dashed-dotted lines represent unstable periodic oscillations. For periodic solutions, maximum and minimum values of the amplitude are shown.
we only consider one patch of ER membrane. How these events propagate through the cell is not considered here. The equation of continuity for the calcium concentration, i.e.:
\[
\frac{dc}{dt} = -j_{\text{pump}}(c) + j_{\text{leak}}(c) + \rho(t),
\]
(5)
in combination with a linear relation between the cytosolic flux density \(j\) and the cytosolic calcium gradients, i.e. \(j = D \nabla c\), lead to
\[
\dot{c} = D \nabla^2 c - j_{\text{pump}}(c) + j_{\text{leak}}(c) + \rho(t),
\]
(6)
with a diffusion constant \(D\). The source density \(\rho(t)\), describing the flow of Ca\(^{2+}\) from the ER into the cytosol through the patch of membrane (the xy-plane here), is assumed to be uniform across the patch, i.e. \(\rho(t) = \rho = \delta(z) (l/A)\). Here, \(l\) denotes the total current through the entire patch of ER membrane. The total current through the patch, in turn, can be expressed as \(\sum_i c_i\). The current through each open channel is proportional to the concentration difference between cytosolic and ER-calcium, i.e. \(I_0 = g_{\text{channel}}(c_{ER} - c)\). Using \(A = N/\rho_{\text{channel}}\), we arrive at
\[
\dot{c} = D \nabla^2 c - j_{\text{pump}}(c) + j_{\text{leak}}(c) + \delta(z) \left(\frac{N_{\text{open}}}{N}\right) g_{\text{channel}} \rho_{\text{channel}} (c_{ER} - c).
\]
(7)
Since we are interested in the mechanism of generating the calcium transients and not in the emerging cytosolic concentration profiles, we neglect concentration gradients across the small patch of the membrane. The fixed calcium concentration is sometimes referred to as the domain calcium concentration and can be estimated about 10 \(\mu\text{M}\) for a cluster of 20 channels. Considering only areal calcium concentrations, i.e. \(c(t, r) = \delta(z) c\), the equation for the cytosolic calcium concentration at the patch becomes
\[
\dot{c} = \left(\frac{N_{\text{open}}}{N}\right) v_{\text{pump}} (c_{ER} - c) - j_{\text{pump}} + j_{\text{leak}},
\]
(8)
where \(v_{\text{pump}} = g_{\text{channel}} \rho_{\text{channel}}\).

The number of open channels at any instant of time is obtained through stochastic simulation of the subunits which undergo transitions between their six states, through stochastic association and dissociation of the agonist (see also the recent review [8]). Transition rates between states depend on the agonist binding rates (see Fig. 1). For sufficiently small time steps they exhibit a linear dependence on the time step. Specifically, if a subunit is in state \(S_i\) and has access to states \(S_j\) with transitions rates \(r_{ij}\), the probability of a transition \(i \rightarrow j\) is given by \(r_{ij} dt\), with \(dt\) small enough that \(r_{ij} dt \ll 1\). The probability for the subunit to remain in state \(S_i\) is given by \(1 - \sum_j r_{ij} dt\). We randomly select from these transitions with weights proportional to their probabilities.

Unlike in the deterministic model described in the previous section, where the solutions were either stationary or periodic, the stochastic model generates transient elevations (spikes) for all concentrations of IP3, regardless whether the deterministic model is periodic. In the left panel of Fig. 3, we show a simulation with 20 channels at an IP3 concentration of 0.4 \(\mu\text{M}\), i.e. in the periodic regime. In the right panel, we show sample spikes generated by a system of 20 IP3Rs at an IP3 concentration of 0.1 \(\mu\text{M}\), i.e. in the excitable regime. For a system with about 20 channels, the number of open channels is typically zero in between spikes. At the onset of a spike, the number of open channels increases rapidly through calcium-induced calcium release. The spike is terminated when increasing cytosolic calcium concentrations inhibits the channels. In the limit of \(N \rightarrow \infty\), i.e. in the limit of a large membrane patch with a spatially uniform calcium concentration, the fluctuations will decrease and the stochastic solutions must approach either a stationary state or a periodic solution, depending on the concentration of IP3 (see Fig. 2). In other words, in the oscillatory regime the spikes must become periodic, while in the steady-state regime the spike frequency must decrease to zero. Hence the regimes can be clearly distinguished in the limit of large \(N\). The main question we are addressing in this paper is whether we can distinguish between spikes from oscillatory clusters and from excitable (non-oscillatory) clusters if only few channels are present. It is important to note here that the limit \(N \rightarrow \infty\) may not be physiologically meaningful since the distribution of channels is spatially clustered and the concentration profiles around the clusters are important; but this limit allows us to classify a small cluster to be either periodic or excitable.

First, we simulate a membrane patch with \(N\) channels (and corresponding area) to obtain a time series of \(M\) (of the order of 10,000) spikes generated at times \(t_i\). We then calculate the mean time interval \(\mu(N)\) between two successive spikes and the standard deviation \(\sqrt{\sigma^2(N)}\), i.e.:
\[
\mu(N) = \frac{1}{M} \sum_{i=2}^{M} (t_i - t_{i-1}),
\]
\[
\sqrt{\sigma^2(N)} = \frac{1}{M} \sum_{i=2}^{M} (t_i - t_{i-1} - \mu(N))^2.
\]
(9)

Similar as in [4], we plot the standard deviation against the mean time interval, but for different sizes of the spike generating membrane patch. In both, the oscillatory system \((p = 0.4 \mu\text{M})\) and the excitable system \((p = 0.1 \mu\text{M})\), the resulting points (each for a

Fig. 3. In the left panel of this figure we show sample spikes generated by a system of 20 IP3Rs at an IP3 concentration of 0.4 \(\mu\text{M}\), i.e. in the periodic regime. In the right panel we show sample spikes generated by a system of 20 IP3Rs at an IP3 concentration of 0.1 \(\mu\text{M}\), i.e. in the excitable regime. Note that due to the smaller IP3 concentration the variability of the spike amplitudes in the excitable case is larger and the spike rate is lower (see also below) as in the oscillatory case since fewer channels can open by binding calcium.
certain cluster size) lie approximately on a line (see Fig. 4a,c). In the oscillatory system, the interpolating line ends (for $N \to \infty$) on the horizontal axis (i.e. no fluctuations). The mean interval and the standard deviation approaches zero (see Fig. 4b). This is the deterministic limit as described in the previous section which describes regular calcium oscillations. For the excitable cluster, the mean interval decreases with increasing cluster sizes up to about $N = 200$, and then, unlike for the oscillatory cluster, increases with further increasing cluster sizes (see Fig. 4d). The standard deviation of the spikes generated by the excitable cluster decreases for increasing cluster sizes (see inset Fig. 4d) for cluster sizes of up to about $N = 200$ and then increases for further increasing cluster sizes. The standard deviation of the spikes generated by the oscillatory cluster decreases for increasing cluster sizes and approaches zero in the limit of large cluster sizes (periodic spikes).

An important observation here is that for small cluster sizes, i.e. less than about $N = 200$, calcium spikes generated by small excitable clusters have similar features than those generated by small oscillatory clusters. Specifically, a linear relation between mean interval and standard deviation by itself, does not delineate oscillatory clusters from excitable clusters if the numbers of channels are small.

The spiking regularity in the excitable system, like in the oscillatory system, increases with increasing system size as long as the clusters remain smaller than about $N = 100$. This is demonstrated in Fig. 5, where we show the coefficients of variation as a function of the cluster size for an excitable cluster. The coefficient of variation is defined as the ratio of the standard deviation and the mean of the time intervals of successive spikes, i.e.:

$$\eta(N) = \frac{\sqrt{\sigma^2(N)}}{\mu(N)}.$$  \hspace{1cm} (10)

A coefficient of variation of unity indicates random (Poissonian) spiking, while smaller values suggest a more regular spiking behavior. Surprisingly, the coefficient of variation of spikes generated by

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**Fig. 4.** The standard deviation is plotted against the mean time interval between two successive spikes for cluster sizes ranging from one $N = 1$ to $N = 1000$ for the excitable (c) as well as for the oscillatory system (a). Except for the largest clusters, the relation between standard deviation and mean time interval is linear in the oscillatory as in the excitable system. The mean interval is plotted against the system size $N$ in the oscillatory (b) and the excitable system (d). The insets in c and d display the standard deviation against the system size.

**Fig. 5.** The coefficient of variation (see Eq. (10)) is shown for excitable membrane patches (left panel) and oscillatory membrane patches (right panel) for various sizes.
an excitable cluster decreases with increasing numbers of channels \( N \) for \( N \leq 200 \) similar as for spikes of oscillatory clusters, although the decrease is smaller in comparison to our particular oscillatory cluster. Such an increase in spiking regularity with system size has been observed in the context of action potentials, generated by clusters of sodium and potassium channels (see in [9–12]) and was associated with a coherence resonance effect ([12]).

4. Discussion

We numerically calculated the mean time intervals between two successive calcium spikes, generated by small numbers of release channels uniformly distributed across small patches of ER membrane. For \( p = 0.1 \) \( \mu M \), the cluster of channels acts like an excitable system in the thermodynamic limit of large numbers of channels, while for \( p = 0.4 \) \( \mu M \), the cluster behaves like an oscillatory system in the same limit. The goal was to compare the spiking characteristics of the oscillatory and the excitable cluster for small cluster sizes. This is a relevant question for contemporary research on calcium signaling, where we currently witness a possible shift of paradigm in the understanding of the fundamental mechanisms underlying oscillations. The new paradigm rests on the assumption that single clusters generate spikes purely stochastically (due to large calcium concentrations near open channels) and that oscillations emerge due to a spatially extended coherence resonance mechanism as first described in more general terms by Gaily and Jung [6] and then later specifically for calcium signaling by Skupin et al. [4] and Skupin and Falcke [5]. We found that for small cluster sizes (up to about \( N \approx 200 \)), the spiking statistics of excitable clusters and the oscillatory clusters are qualitatively similar. The mean interval between two successive spikes and the standard deviation of these intervals from the mean decrease with increasing cluster size. Only for large ER membrane patches with thousands of channels, the mean interval of the oscillatory cluster stabilizes towards a fixed period. The mean interval and standard deviation of the spikes generated by the excitable system increases at those system sizes and approaches purely random behavior. Specifically for calcium signaling, if we were given a time series of spiking events from an experiment with variable size clusters we would not be able to find out whether the underlying clusters are excitable or oscillatory if the spike generating clusters or groups of clusters are small. There is a big difference in the absolute values of the mean interval and the standard deviation for the two particular oscillatory and excitable clusters discussed here. But experimental recordings such as the one in [4] don’t come with reference values which could be used to draw a distinction.

Most remarkable, plotting the standard deviation of the time intervals between successive spikes versus their mean for a variety of system sizes, all points fall on a line with a slope close to unity, regardless whether the cluster was excitable or oscillatory. For very large oscillatory systems there may be a nonlinear behavior as suggested in [5] by perturbing a set of rate equations describing the thermodynamic limit, with white, additive Gaussian noise. The scatter of the experimental data in [4] is too large to resolve such a small detail.

The standard deviation grows with the mean interval regardless whether the cluster is excitable or oscillatory. A similar linear relationship was found from time series of calcium spikes, recorded from astrocytes, microglia, PLA and HEK cells by Skupin and Falcke [5] and Skupin et al. [4], although the slope seems to vary somewhat between the cell types. Each data point in their plot represents the averaged mean interval and standard deviation obtained from recordings from a single cell. Although there is a large scatter of data points (hundreds of seconds) the points can be fitted nicely by a line. In astrocytes the slope appears to be close to unity. In Fig. 4ac in this paper, each data point corresponds to a different cluster size. The line in Fig. 4a (the oscillatory cluster), intersects the horizontal axis at a mean interval of about 4 s, where the spikes are perfectly periodic. Such a value for the frequency is of the same order than periods of observed agonist induced calcium oscillations (see e.g. [13]). In the excitable cluster (see Fig. 4c) there is no intersection with the horizontal axis since the mean interval and the standard deviations increase again for large system sizes. It is worth pointing out that [4] interpreted the linear relation in the plot standard deviation versus mean value quite differently. In their model, spikes are nucleated stochastically, but the nucleation rate is time-dependent in such a way that the nucleation rate is zero immediately after a spike, but then recovers exponentially.

Similar results have been obtained in previous studies for spikes generated by clusters of voltage-gated sodium and potassium channel by Hanggi’s group (i.e. [10,14–16,12] and in [9]). This is quite remarkable as the models used in those studies (stochastic Hodgkin–Huxley models) have little similarity with the model used here for calcium signaling. In the context of calcium signaling some of these results, e.g. the increased coherence with system size in purely excitable clusters, gain renewed importance.

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