Calcium waves in a grid of clustered channels with synchronous IP$_3$ binding and unbinding

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Abstract. Calcium signals in cells occur at multiple spatial scales and variable temporal duration. However, a physical explanation for transitions between long-lasting global oscillations and localized short-term elevations (puffs) of cytoplasmic Ca$^{2+}$ is still lacking. Here we introduce a phenomenological, coarse-grained model for the calcium variable, which is represented by ordinary differential equations. Due to its small number of parameters, and its simplicity, this model allows us to numerically study the interplay of multi-scale calcium concentrations with stochastic ion channel gating dynamics even in larger systems. We apply this model to a single cluster of inositol trisphosphate (IP$_3$) receptor channels and find further evidence for the results presented in earlier work: a single cluster may be capable of producing different calcium release types, where long-lasting events are accompanied by unbinding of IP$_3$ from the receptor (Rückl et al., PLoS Comput. Biol. 11, e1003965 (2015)). Finally, we show the practicability of the model in a grid of 64 clusters which is computationally intractable with previous high-resolution models. Here long-lasting events can lead to synchronized oscillations and waves, while short events stay localized. The frequency of calcium releases as well as their coherence can thereby be regulated by the amplitude of IP$_3$ stimulation. Finally the model allows for a new explanation of oscillating [IP$_3$], which is not based on metabolic production and degradation of IP$_3$.

1 Introduction

Calcium ions (Ca$^{2+}$) serve in many important signaling events in cells. Changes in the intracellular Ca$^{2+}$ concentration control diverse physiological processes including muscle contraction and neurotransmitter release. Cells achieve the multitude of Ca$^{2+}$-mediated functions by generating a spectrum of spatio-temporal Ca$^{2+}$ distributions, notably long-lasting cell-wide waves or oscillations and brief localized [Ca$^{2+}$] increases called puffs or sparks [1, 2]. One of the main players in the generation of these global and local signals is the IP$_3$ receptor (IP$_3$R) channel, which releases Ca$^{2+}$ from internal stores (endoplasmic reticulum, ER) into the cytosol of the cell [3]. Since the open/closed state of the IP$_3$R is modulated by positive and negative feedbacks by Ca$^{2+}$ binding to the receptor, much of the pattern complexity has been attributed to channel gating. Additionally, release dynamics depends on a number of further transport and reaction processes, controlling for instance the local concentration of Ca$^{2+}$ near an open receptor. Further complexity arises from the stochasticity of release, which is most clearly apparent from the random nature of puff initiation, and it is difficult to discern the effects of noise and nonlinear feedback processes in this system [4]. Nevertheless, elucidating the origin of spatio-temporal Ca$^{2+}$ dynamics from realistic biophysical models is a current challenge, that is fundamental to many problems in cell science, neuroscience, and medicine.

Recently it was proposed that synchronized binding and unbinding of IP$_3$ from the receptor may have a strong impact on the shape of Ca$^{2+}$ release events and explain the different durations of local and global signals [5]. IP$_3$ is a second messenger whose concentration is tied to the binding of hormones on receptors in the plasma membrane. Binding of IP$_3$ is a general prerequisite of IP$_3$R channel openings so that with higher stimulation the Ca$^{2+}$ response generally increases. In fact, simulations incorporating multiple clusters of IP$_3$Rs and their Ca$^{2+}$ release have shown that, for sufficiently high stimulation, large global releases can be nucleated from a background of local events [6, 7]. Furthermore, it has been observed for some cell types that [IP$_3$] itself oscillates in unison with intracellular [Ca$^{2+}$] [8, 9]. Therefore, periodic metabolic production and degradation of IP$_3$ is included in many models of Ca$^{2+}$ oscillations [2].

It has been shown that modulated appearance of release (i.e. sequences of puffs and waves) appears in models for coupled clusters of IP$_3$Rs without oscillatory IP$_3$ generations [6]. Further, we have discovered that the complex

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IP$_3$R gating of the DeYoung-Keizer model [10], comprising Ca$^{2+}$ and IP$_3$ binding and unbinding, allows modulated Ca$^{2+}$ release, already on a single cluster basis [5]. Importantly, in this work, changing the control parameter IP$_3$ affected both the frequency of events and their duration. Increasing IP$_3$ leads to a higher frequency, and longer event durations. We found that many channels lose IP$_3$ during long-lasting release while IP$_3$ binding is practically constant during a short release (i.e., a puff). The model thus explained the appearance of both event types as well as the extended refractory time after long-lasting events and has been found to match well experimental data of waves and puffs [5].

IP$_3$Rs are known to appear in clusters of up to several tens of channels [11] and clusters are spaced at a few micrometers. Within this heterogeneous setup we now aim to study the transition from localized to global release. We follow the general idea that release propagates in such a medium only if the advancing release amplitude is sufficiently large [12]. Since exact representation of Ca$^{2+}$ distribution within a single cluster already requires a very high number of grid points in a numerical discretization, we decided to replace the 4D Ca$^{2+}$ evolution equations treated in [5] with a set of ordinary differential equations (ODEs). We pay tribute to the intricate nature of Ca$^{2+}$ gradients within a cluster and between clusters by employing a hierarchy of [Ca$^{2+}$] scales. A similar approximation has been used in prior studies [13–16]. In particular, we will here build on the approach devised in [15, 16], in which the feedback of the open channel to itself (at a very high concentration) is distinguished from Ca$^{2+}$ effect from an open channel towards other closed channels within the cluster. We extend this model by introducing a second variable with slow dynamics, which accounts for calcium accumulation effects and mediates coupling between clusters. Because of the phenomenological nature of the introduced model, our approach allows to understand the influence of different [Ca$^{2+}$] magnitudes on the signal properties, which would not be possible from a full reaction-diffusion model.

We begin our analysis for a single cluster where we give a short qualitative overview how buffers and IP$_3$R conductivity would be reflected by our model parameters and hence their effect on event modulation. Then, the main focus is on a grid of 8 × 8 clusters and their synchronization properties. For the single cluster, we find that, at high stimulation, release events are broadly distributed in their temporal profile. In fact, the distribution of event life-time can become bimodal with a minimum of the distribution for around 3–5 second lifetime. This behavior was not seen in [5] and may be a feature of our reduced model. Still, the findings presented here provide further evidence for the major conclusion drawn in [5].

If the release events fall into two subsets, it is natural to suppose that only the long release events may propagate through the grid because of the higher Ca$^{2+}$ amount diffusing to neighboring clusters. We therefore then investigate this behavior in a regular 2D grid of clusters and study how propagation and synchronization depend on the stimulation parameter given by the IP$_3$ concentration. For an appropriate coupling strength we observe that long-lasting events propagate while short events do not, thus mimicking the dichotomy of puff and wave events also in this respect. Furthermore, waves are accompanied by substantial synchronized unbinding of IP$_3$ from the receptor channels. The latter effect can cause increases of cytosolic IP$_3$ concentration, which suggests a new interpretation of the [IP$_3$] oscillations observed in some cell types [8, 9].

The paper is organized as follows. We first introduce the ODEs for [Ca$^{2+}$] and briefly describe the gating model. We show that a satisfactory correspondence to appropriately defined measures from finite-element simulations as in [5] can be achieved. Then the results for a single cluster are described. In the subsequent sections, we characterize release events from the cluster-grid emphasizing the transition to coherent oscillation for higher stimulation. The paper concludes with a summary of our results and a comparison with experimental findings.

2 Model and methods

In this section we describe the constituents of our model for channel gating and Ca$^{2+}$ concentration dynamics. The model consists of a Markov chain for IP$_3$R channel states [17] and deterministic ODEs for the Ca$^{2+}$ concentration at each cluster. In the numerical implementation, the stochastic and deterministic parts are coupled by the hybrid method described in [18].

2.1 Deterministic Ca$^{2+}$ reaction and diffusion model

In [5] a complex system of coupled partial differential equations was used to describe spatial concentration fields of Ca$^{2+}$ and buffers. Solving this system with the finite element method (FEM) resulted in time evolutions for the spatial distribution of Ca$^{2+}$ for different kinds of collective channel events. Here, we want to reduce the computational effort that is accompanied with the FEM algorithm by coarse graining the system of coupled partial differential equations (PDEs). Based on the results of numerical simulations (see e.g. [6, 14, 19, 20]) and various different analytical approximations (e.g. [21–23]) of buffered calcium reaction diffusion equations, we can distinguish the [Ca$^{2+}$] evolution on different temporal and spatial scales. Specifically we consider the following hierarchy of scales:

A) Nanodomains. If channels open and close there are very rapid buildups and collapses of sub-micrometer Ca$^{2+}$ profiles around the open channel pores, which we call nanodomains (fig. 1A) [24]. The peak concentrations of these profiles are located at the open channels’ pores and can exceed 100 μM [20, 25]. Despite the steep spatial gradient of the nanodomains, they can still be felt by other closed channels in the cluster. This is because of the compact structure of a channel cluster, i.e. the inter-channel distance is less than 200 nm [26, 27], and the total cluster size is on the order of 0.5 μm [11, 28, 29]. Of course, the amplitude induced
The enduring efflux of Ca\(^{2+}\) through a variety of parameters like the distance between the two channels, the buffer settings, or the channel currents. This amplitude usually ranges on the order of 1–10 \(\mu\)M [15]. In this paper we will make the following approximations for the nanodomains: within a cluster a closed channel will only feel a mean field of the nanodomains of all open channels in the cluster. Since the timescales of buildups and collapses are very short, we assume that this mean field equilibrates instantaneously. Finally, because of the steep spatial gradients, we neglect the impact of nanodomains from other clusters.

**B) Microdomains.** The enduring efflux of Ca\(^{2+}\) through multiple channels of a cluster gets pooled into a single extended microdomain around that cluster. When a cluster opens, this microdomain slowly rises towards an equilibrium concentration which depends on the number of open channels. When all channels have closed, it will slowly decay back to resting concentration (see fig. 18 in [4]). Thus, one can think of the microdomain as a dynamical offset which serves as baseline for the nanodomains. It describes the well-mixed domain on the scale of the cluster (fig. 1B) and hence its spatial scale is much larger compared to nanodomains. Finally, the different temporal and spatial scales also lead to a completely different [Ca\(^{2+}\)] amplitude of the microdomain. In this work, we neglect the spatial profile of the microdomain and instead only take into account this amplitude. Because of the slower timescale we will further model this amplitude by a single ODE per cluster with a certain time constant. Finally, due to the large extent of the microdomains, we will assume that they mediate an instantaneous diffusive coupling to neighboring clusters.

Summing up, one can think of a nanodomain as a feature of an individual channel, while a microdomain is a feature of a whole cluster of channels. Together, this results in Ca\(^{2+}\) concentrations that range through 3–4 decades and timescales from instantaneous (nanodomain build up and collapse) to seconds (microdomain concentration decay). In the following, we introduce the equations of our model and we will discuss the interdependence of physiological and phenomenological parameters.

In a first equation we want to describe the instantaneous contribution of the nanodomains, which is felt by all closed channels of cluster \(i\):

\[
c_{\text{nd},i}(t) = c_{l} n_{o,i}.
\] (1)

Here, \(n_{o,i}\) denotes the fraction of open channels of cluster \(i\), and, similar as in [15], \(c_{l}\) controls the amplitude of the mean field coupling.

The second contribution to a closed channel’s [Ca\(^{2+}\)] is given by the slow dynamics of the microdomain amplitude \(c_{\text{md},i}\) of cluster \(i\) and is equal for all closed channels within this cluster. We assume that the accumulation speed of the microdomain is proportional to \(n_{o,i}\), i.e. to the total current through all open channels. The equilibration of a microdomain is captured by an ODE for \(c_{\text{md},i}\):

\[
\frac{dc_{\text{md},i}}{dt} = \gamma n_{o,i} - P_{l} \frac{c_{\text{md},i}^2}{K_{p}^2} + P_{l}.
\] (2)

The accumulation speed is controlled by the phenomenological parameter \(\gamma\). The second term on the right-hand side of eq. (2) models SERCA pumps, that remove Ca\(^{2+}\) from the bulk and are activated according to a Hill function. The last term, a constant Ca\(^{2+}\) leak, compensates for [Ca\(^{2+}\)] loss by the pumps when there are no open channels.

To achieve a resting concentration \(c_{\text{rest}}\), \(P_{l}\) is chosen as

\[
P_{l} = P_{l} \frac{c_{\text{rest}}^2}{c_{\text{md},i}^2 + K_{p}^2}.
\] (3)

Since we do not consider any changes of [Ca\(^{2+}\)] in the ER, none of the terms in eqs. (1)–(3) incorporates an ER [Ca\(^{2+}\)] dependence. Comprising coupling between clusters yields the bulk concentration of cluster \(i\):

\[
c_{\text{bulk},i} = c_{\text{md},i} + \alpha \sum_{j \in S_{i}} \text{Max}(0, c_{\text{md},j} - c_{\text{md},i}).
\] (4)
Table 1. Model parameters for eqs. (1)–(5).

| Parameter                        | Symbol | Value  | Unit |
|----------------------------------|--------|--------|------|
| Coupling coefficient             | $\alpha$ | 0.125  | 1    |
| Cytosolic resting $\text{Ca}^{2+}$ | $c_{\text{rest}}$ | 0.02   | $\mu$M |
| Instant. feedforward (others)    | $c_{\text{f}}$ | 4.8    | $\mu$M |
| Instant. feedback (self)         | $c_{\text{open}}$ | 150   | $\mu$M |
| Channel flux                     | $\gamma$ | 4.8    | $\mu$M/s |
| Pump strength                    | $P_{\text{p}}$ | 0.2    | $\mu$M/s |
| Pump diss. const.                | $K_{\text{p}}$ | 0.1    | $\mu$M |
| # of channels per cluster        | $N$ | 16     | 1    |
| # of clusters                    | $M$ | 1 or 8 x 8 | 1 |
| IP$_3$ concentration             | [IP$_3$] | 2–100  | nM  |

Here $S_i$ and $\alpha$ denote the set of neighbors of cluster $i$, and the parameter controlling coupling strength, respectively. The dependence $\text{Max}(a, b)$ is used to model the asymmetry in the coupling of clusters, where, in contrast to standard diffusive coupling, only a small fraction of $\text{Ca}^{2+}$ released at one cluster diffuses to neighboring clusters [30].

Finally, in a last step we assume that the cluster’s local instantaneous mean field from eq. (1) adds up linearly with the bulk contribution eq. (4), and we incorporate the nanodomain if the channel $g$ of cluster $i$ is open:

$$c_i^g(t) = \begin{cases} c_{\text{open}}, & \text{if } g \text{ is open}, \\ c_{\text{bulk}, i} + c_{\text{nd}, i}, & \text{if } g \text{ is closed}. \end{cases}$$

(5)

The case for a closed channel $g$ is shown in fig. 7 in appendix A. There, the blue line denotes the dynamical offset ($c_{\text{bulk}, i}$), on top of which one can see the rapid fluctuations of the gray line ($c_{\text{nd}, i}$).

The concentration from eq. (5) is then used to determine the individual gating rates of each channel (see sect. 2.2).

Table 1 denotes the values of the parameters introduced above which will be used throughout this paper. In general, the phenomenological nature of our model allows the selection of appropriate and realistic values for various cell types and situations. We first argue that the chosen parameters are at least on the correct scale (see comparison with the FEM in appendix A). Since this work does not focus on any specific cell type or experimental setup, we argue that this approach is reasonable. Still we want to provide a short discussion on the implications of the phenomenological parameters. This will facilitate the adaption of parameters for a more quantitative comparison to experiments or other highly detailed models (e.g. [5, 31, 32]).

In [15] it was shown that the instant feedback $c_{\text{f}}$ shows approximately a linear dependence on the number of open channels. A larger $c_{\text{f}}$ would e.g. reflect higher channel current, smaller inter-channel distances or lower buffer capacity. If experiments or highly detailed simulations indicate a violation of this linear approximation, it is straightforward to adapt eq. (1) to a better mean field approximation. Second, the pore calcium concentration $c_{\text{open}}$ will depend on the channel conductivity, the ER $[\text{Ca}^{2+}]$ and buffer settings. Higher $\text{Ca}^{2+}$ buffer capacity or lower ER $[\text{Ca}^{2+}]$ will diminish the nanodomain amplitudes. The parameter $\gamma$ is governed by a mixture of real world parameters. For example, a higher channel conductivity (or higher ER $[\text{Ca}^{2+}]$) could be modeled by an increased $\gamma$, while a higher $\text{Ca}^{2+}$ diffusion coefficient, increased buffer concentration, or higher buffer affinity would slow down the accumulation process. We did again choose the most simple linear relation between microdomain accumulation speed and the number of open channels, since the ODE itself already provides a saturation curve for the microdomains. However, a system of coupled ODEs incorporating bound and unbound buffer might be a reasonable extension for future work. For the SERCA pumps it is known that they activate according to a Hill function with given $K_{\text{p}}$, which leaves $P_{\text{p}}$ as undetermined parameter. We chose $P_{\text{p}}$ such that the timescale to reach resting $[\text{Ca}^{2+}]$ does approximately reflect the decay timescales from our FEM simulations [5]. At last, the coupling between different clusters via $\alpha$ will be diminished with increased buffer capacity, but also via faster SERCA pumps, or less “geometric confinement” of calcium ions.

2.2 Stochastic channel gating model

The IP$_3$R gating is modeled according to a modified version of the DeYoung-Keizer (DYK) scheme introduced in [33, 34]. Motivated by the tetrameric structure of the IP$_3$R, each channel has four subunits where each subunit has a binding place for activating $\text{Ca}^{2+}$, inhibiting $\text{Ca}^{2+}$, and IP$_3$ leading to eight different states for each subunit. A subunit is called “active” if it has bound activating $\text{Ca}^{2+}$ and IP$_3$ but not inhibiting $\text{Ca}^{2+}$. A channel is open when at least three of the four subunits are in the “active” state. The IP$_3$ binding dynamics of channels are rather slow compared to activating and inhibiting $\text{Ca}^{2+}$ binding and a channel might end up in a “non-activatable” state if it unbinds IP$_3$, because the remaining active subunits can never lead to a channel opening. For a more detailed description of the modified DYK model we refer to [5]. Compared to [5], we adjusted two rates of the model keeping the dissociation constants fixed (see Table 2. Channel gating parameters $a_i$, $b_i$, and the dissociation constants $d_i = b_i/a_i$ of the modified DYK model. Compared to [5] the coefficients $a_1$, $b_1$, $a_4$, and $b_4$ were increased by a factor of 10 leaving dissociation constants unchanged. Old and new rates satisfy a detailed balance ($d_1d_2 = d_3d_4$).

| $i$ | $a_i$ | $b_i$ | $d_i$ |
|-----|-------|-------|-------|
| IP$_3$ while not inhibited | 1 | 2 | $2 \times 10^{-3}$ | 0.001 |
| Inhibition with IP$_3$ | 2 | 0.02 | 1.56 | 78 |
| IP$_3$ while inhibited | 3 | 0.40 | 0.80 | 2 |
| Inhibition without IP$_3$ | 4 | 1 | $3.9 \times 10^{-2}$ | 0.039 |
| Activation | 5 | 100 | 25 | 0.25 |
2.3 Definition of event length, event coupling, and coherence between clusters

Because we ask whether there is a functional difference between long and short events in terms of coupling and synchronization, we first need a proper definition of a collective event at a single cluster. Generally, we want a burst of channel openings to be grouped into one long event. We therefore define an event as the interval where at least one channel of the cluster is open. In addition, if the last channel closes, but the next channel of the cluster opens within a time of $\tau_D$, the event will be prolonged. This is done repeatedly until an event end is found which is separated from the next event start by at least $\tau_P$. We chose $\tau_D = 1 \text{s}$ because according to fig. 7 this is approximately the timescale of the decay of the microdomain concentrations $c_{\text{md}, i}$.

To obtain information about synchronization between multiple clusters, we use two distinct techniques. First, we apply a symmetric version of the method called event synchronization (ES) introduced by Quirós et al. [36] on the discrete events as defined above. Second we calculate the complex coherence function [37–39] for pairs of the continuous cluster’s microdomain Ca$^{2+}$ concentrations $c_{\text{md}, i}$.

Calculating the event synchronization is a two-step process. In our case an event series for cluster $n$ ($m$) is defined by the start times $t_n^\mu$ and $t_m^\nu$ of the events, where $\mu$ and $\nu$ denote the indices of the events. With this notation, the time between the $\mu$-th event in cluster $n$ and the $\nu$-th event in cluster $m$ is given as $d_{nm}^{\mu\nu} = t_m^\nu - t_n^\mu$. First one calculates the dynamical delay $\tau_{nm}^{\mu\nu}$ between the two event series as follows:

$$
\tau_{nm}^{\mu\nu} = \frac{1}{2} \min (d_{nm}^{\mu-1,\mu}, d_{nm}^{\mu,\mu+1}, d_{nm}^{\nu-1,\nu}, d_{nm}^{\nu,\nu+1}).
$$

The synchronization matrix between two event series is then given as:

$$
J_{nm}^{\mu\nu} = \begin{cases} 
1, & \text{if } 0 < |d_{nm}^{\mu\nu}| \leq \min (\tau_{nm}^{\mu\nu}, \tau_{\text{max}}) \\
0, & \text{else}, 
\end{cases}
$$

(7)

where $\tau_{\text{max}}$ denotes a maximum delay between events. We chose $\tau_{\text{max}}$ for each simulation separately, to be half of the average inter-event distance [36]. The absolute value $|d_{nm}^{\mu\nu}|$ in eq. (7) makes the ES measure symmetric, i.e. the $\mu$-th event will count as synchronized with the $\nu$-th event, irrespective of whether $\mu$ precedes or succeeds $\nu$.

The complex coherence function $C_{ij}$ of the two bulk concentrations of cluster $i$ and $j$ is defined as a fraction of the cross spectral density $P_{ij}$ and the product of the two power spectral densities $P_{ii}$ and $P_{jj}$ of the signals:

$$
C_{ij}(\omega) = \frac{P_{ij}(\omega)}{\sqrt{P_{ii}(\omega)P_{jj}(\omega)}}.
$$

(8)

The matrix $P_{ij}$ is called the power spectral density matrix and is given by the product of the two Fourier transforms of time series of $c_{\text{md}, i}$ and $c_{\text{md}, j}$:

$$
P_{ij}(\omega) = F(c_{\text{md}, i})^* \times F(c_{\text{md}, j}).
$$

(9)

The * denotes complex conjugation. Note that $C_{ij}$ is complex except for the diagonal entries.

Because we are interested in the $C_{ij}$ for low frequencies $\omega$ (period in the range of 5s to 60s) we need a high resolution in the frequency domain (e.g. 0.01 Hz). This requires Fourier transformations of time intervals larger than 100s. To achieve a good estimate of the individual power spectral densities, we use Welch’s method described in [40].

There, the spectral density is estimated by the average of the discrete Fourier transforms of overlapping windows of the data. Hence, we create $S$ sample sequences of the bulk concentrations $c_{\text{md}, i}^{(0)}$, $c_{\text{md}, i}^{(1)}$, $c_{\text{md}, i}^{(2)}$, ..., $c_{\text{md}, i}^{(S-1)}$ where each sequence is discrete in time and given as $c_{\text{md}, i}^{(k)} = c_{\text{md}, i}^{(n,0)}$, $c_{\text{md}, i}^{(n,1)}$, ..., $c_{\text{md}, i}^{(n,L-1)}$, where $c_{\text{md}, i}^{(n,k)} = \sum_{j=1}^{N_{\text{DD}}} (c_{\text{md}, j}^{(n,k)} - \langle \Delta(t + nD) \rangle)$, where $L\Delta t$ denotes the window size and $D$ determines the overlap of the windows. Then we calculate the sample coherence function (SCF) $C_{ij}$ as follows:

$$
\hat{C}_{ij} = \frac{(F(c_{\text{md}, i}^{(n)})^* \times F(c_{\text{md}, j}^{(n)}))_S}{\sqrt{(|(F(c_{\text{md}, i}^{(n)})|^2)_S (|(F(c_{\text{md}, j}^{(n)})|^2)_S),}
$$

(10)

where the continuous Fourier transform from eq. (9) gets replaced by the discrete Fourier transform and the average is taken over the $S$ sample sequences.

The phase of the sample coherence function reflects the phase lag of $c_{\text{md}, i}$ with respect to $c_{\text{md}, j}$ at a given frequency. The absolute value of the sample coherence function ranges between 0 and 1 and denotes their synchrony (irrespective of a possible phase lag). Because we are interested in the overall synchronization and the formation of oscillations, we average $\hat{C}_{ij}$ over all pairs of clusters. Since this average can be rearranged as a sum over the pairs $\hat{C}_{ij} + \hat{C}_{ji}$, the imaginary parts, and hence the phase lags of each pair will cancel out, because $\hat{C}_{ij} = \hat{C}_{ji}^*$. To avoid this and still obtain some information about the phase between two clusters, we always flip the imaginary part to positive values. This yields the average of the absolute phase between the signals, while losing the information on which signal precedes the other. We end up with

$$
\bar{C} = \frac{1}{M^2} \sum_{i,j} \mathcal{R}(\hat{C}_{ij}) + i|\mathcal{T}(\hat{C}_{ij})|,
$$

(11)

where $M$ is the number of clusters, $i$ is the imaginary unit and $\mathcal{R}$ and $\mathcal{T}$ denote the real and imaginary parts, respectively. For fully decoherent signals the absolute phase
order of seconds or less, while for larger IP\(_3\) concentration for the parameters given in table 1. In A1 and A2 the cluster state is shown by the number of open channels (blue) and the number of activatable channels (green). For low [IP\(_3\)] the number of activatable channels grows only slowly which leads to rare, short events. In contrast to that, the fast rise of the reactivation for high [IP\(_3\)] allows to almost fully activate the cluster and promotes a higher frequency of large release events. The distributions of event durations (B and C) show a dependence on the pump strength (B: \(P_p = 0.2 \mu M/s\), C: \(P_p = 0.3 \mu M/s\)) and the microdomain accumulation speed \(\gamma\) (black, blue, and cyan). The simulations from A1 and A2 correspond to the black lines in B1 and B2, for the parameters from table 1.

Fig. 2. Comparison of events of a single cluster for 5 nM (A1, B1, C1) and 80 nM (A2, B2, C2) IP\(_3\) concentration for the parameters given in table 1. In A1 and A2 the cluster state is shown by the number of open channels (blue) and the number of activatable channels (green). For low [IP\(_3\)] the number of activatable channels grows only slowly which leads to rare, small events. In contrast to that, the fast rise of the reactivation for high [IP\(_3\)] allows to almost fully activate the cluster and promotes a higher frequency of large release events. The distributions of event durations (B and C) show a dependence on the pump strength (B: \(P_p = 0.2 \mu M/s\), C: \(P_p = 0.3 \mu M/s\)) and the microdomain accumulation speed \(\gamma\) (black, blue, and cyan). The simulations from A1 and A2 correspond to the black lines in B1 and B2, for the parameters from table 1.

differences would be distributed uniformly over the angle \([0, \pi]\) and hence \(\pi/2\) would be the average absolute phase difference.

3 Results

3.1 Single cluster simulations

The most simple scenario we study is that of a single cluster with 16 channels. Figure 2 shows representative time series of the channels' evolution of a single cluster for low (A1) and high [IP\(_3\)] (A2), the remaining parameters are given in table 1. The blue curves show the number of open channels, while the number of activatable channels is plotted in green. Here a channel is considered activatable if the number of subunits that have bound IP\(_3\) is larger than two, making it potentially available to open by binding of Ca\(^{2+}\). For small [IP\(_3\)] most events are short on the order of seconds or less, while for larger IP\(_3\) longer events occur which can last for more than 10 seconds. An important difference is that for small [IP\(_3\)], termination occurs without or after a modest decrease in the number of activatable channels while long events in A2 terminate after unbinding of IP\(_3\) from almost all channels. This confirms our earlier results reported in [5], where we concluded that the transition from puffs to waves is mediated by a qualitative change of single-cluster release regarding the IP\(_3\) dynamics.

This transition is most clearly seen in the distributions of the event lengths. Panels B and C in fig. 2 show that the distribution generally becomes broader at larger IP\(_3\) concentration. However, we also find that changing the ODE model parameters can modify the distribution in a wide variety. For example for \(\gamma = 4.8 \mu M/s\) (black curve in B2) a minimum in the distribution is apparent for high [IP\(_3\)], which distinguishes the events in two subsets. It turns out that this bimodality is a very robust feature as long as the Ca\(^{2+}\) microdomain accumulation \(\gamma\) is large enough. For instance, it does persist under changes of the pump strength \(P_p\) (compare panels B2 and C2).

Referring to the discussion on the impact of the phenomenological parameters from sect. 2.1, the above findings are in agreement with many experimental studies: In general wave formation can be suppressed by adding EGTA as exogenous buffer (e.g. [41]). Similar, the formation of nanodomains around Ca\(^{2+}\) ion channels depends on the ER Ca\(^{2+}\) uptake via SERCA pumps [42]. Both of which would mean a decrease of \(\gamma\) in our model. On the other hand, it was suggested that BAPTA (a faster buffer with similar Ca\(^{2+}\) affinity as EGTA) can promote inter-cluster coupling while diminishing intra-cluster channel coupling [41] which would correspond to an increase of \(\alpha\) and a decrease of \(c_{id}\).

Because we suspect that the presence of two populations of events has strong implications also for the synchronization related to global release events, we chose parameters of the Ca\(^{2+}\) ODE according to the black line in panels B (\(\gamma = 4.8 \mu M/s\), \(P_p = 0.2 \mu M/s\)) for the following multi-cluster simulations.

The transition from a narrow to a bimodal distribution can be interpreted in the framework of stochastic excitability: while the decaying distribution for small [IP\(_3\)] corresponds to the stochastic trajectories in a system with a single stable state, the bimodal distribution at large [IP\(_3\)] hints at a second stable state. A transition of this kind from one to two stable states has been found in a Langevin approximation of the single cluster dynamics reported in [43].

In the light of this work, a stable state of a cluster will have all channels closed. At high IP\(_3\) most of the cluster will have bound IP\(_3\) and are therefore able to open (“state A”), while at low IP\(_3\) only a few channels can open (“state B”). Hence, if a “state A” cluster opens, it will lead to a larger Ca\(^{2+}\) efflux and a microdomain will build up, which repeatedly reopens some channels of the cluster. This process will continue until enough channels have lost their bound IP\(_3\) and therefore cannot reopen anymore. In a “state B” cluster, the Ca\(^{2+}\) efflux is not sufficient to build up a significant microdomain, and hence inhibition and stochastic attrition will lead to a quick event termi-
Fig. 3. Spatiotemporal shape of events for low and high [IP$_3$]. A and B show the temporal evolution on one column of the cluster grid (top), the mean Ca$^{2+}$ concentration over all clusters calculated from eq. (5) for a hypothetical closed channel $g$ (middle), and the fraction of activatable channels (bottom), for [IP$_3$] = 10 nM (A) and [IP$_3$] = 80 nM (B). (C-H) show snapshots of the full 8 × 8 grid of clusters for the instants of time marked with red arrows in B1. The black box denotes the cluster coordinate shown in A1 and B1, i.e. the fifth column of the grid. The color bar codes the slow evolving bulk concentration given by eq. (4) in μM. While the (time) average number of activatable channels (gray dashed line, $\mu$) is higher for low [IP$_3$] (A3), the oscillation amplitude is much lower than for large [IP$_3$] (B3). The strong amplitudes of the oscillation allow to reach some “critical” number of activatable channels which allows to trigger a propagating activity through the whole cluster.

3.2 Propagation of activation in an array of 8 × 8 clusters

The results for the single cluster suggest to identify the short events with puffs and the long events with waves or global oscillations in the sense of their temporal appearance. Indeed these different temporal shapes correspond to those seen in experiments on oocytes [41]. However, because a single cluster does not possess a spatial extent necessary for waves in the original sense, this idea needs clarification. To test our claim that long-lasting events are more likely to propagate from one cluster to its neighbors, we now study release from coupled 64 clusters arranged in a two-dimensional regular 8 × 8 grid with periodic boundaries. In the following we use clusters with 16 channels and a coupling constant $\alpha = 0.125$. The simulation times decrease from about 20000 s for low IP$_3$ concentrations to about 5000 s for higher [IP$_3$].

We chose periodic boundary conditions, because they have multiple advantages over fixed or open boundaries: they are easiest to implement, they do not introduce further parameters, and most important, they truly render each cluster identical. This is important in the following analysis, where we are interested in synchronization of the IP$_3$ dynamics. Since we measure properties that are defined by pairs of clusters, special treatment would be required for a non-periodic grid which involves boundary clusters. This would complicate data analysis and presentation and hence affect conciseness of this work. Finally, there is also some biological motivation for periodic boundaries: in a large cell the ER may resemble a spherical organelle within the cytosol, i.e. waves could propagate “around” the ER. For large cells like Xenopus oocytes, the number of clusters might be significantly larger than 8×8 (diameter: 100–1000 μm [44], cluster spacing $\sim 3 \mu$m [28, 45–48]). For such cases arrays with periodic boundary conditions provide a more representative excerpt of the whole system.

Figure 3 presents results of those simulations, once for low (10 nM) and once for high (80 nM) [IP$_3$]. Top panels of A and B show the evolution on a one-dimensional cut.
through the grid. Clearly, for small IP$_3$ only short localized releases are seen, while for large IP$_3$ several long-lasting events sweep the domain. Accordingly, the panels A2 and B2 show the limited and wide ranges of evolution for the averaged Ca$^{2+}$ concentration for small and large [IP$_3$], respectively. Panels C–H show snapshots of the cluster grid for the synchronized event depicted by the red arrows in B1. While one can see the propagation of activation for some of the events in B1, this propagation is very irregular since no clear wave front can be identified from the snapshots C–H. One cause for this irregularity could be given by the long trailing low activity state of clusters, after their first heavy initial release. This trailing low level activity is very similar to the turbulent backfiring state reported in [49].

Most interestingly, for large [IP$_3$] there is also a large-amplitude oscillation of the number of activatable channels (A3 and B3), demonstrating the relevance of the IP$_3$ unbinding during long-lasting release for global oscillations. In [50] it was shown that, given stochastic attrition is an insufficient termination mechanism, inhibitory calcium binding can facilitate puff termination in two distinctive ways: either by a quasi-permanent reduction of activatable channels, or by rapid inhibition in the course of a release event. In our case, neither of the two mechanisms are capable to terminate the long events. It is the IP$_3$ unbinding that slowly reduces the number of activatable channels during release until termination can be achieved by either stochastic attrition or inhibition.

### 3.3 Synchronization properties

To elucidate the relation of temporal shape and spatial coupling, we will quantify the synchronization of release between clusters. Therefore we calculate $J_{nm}^{\mu\nu}$ as given by eq. (7) for all pairs of neighboring clusters $S_n$. Summation of $J_{nm}^{\mu\nu}$ over $\nu$ yields the number of synchronized events in cluster $\nu$ for each event $\mu$ in cluster $n$. With this sum it is straightforward to count the number of neighbors $J_n^\mu$ that have at least one synchronized event with a given event $\mu$ at $n$:

$$J_n^\mu = \sum_{m \in S_n} \begin{cases} 1, & \text{if } \sum_{\nu} J_{nm}^{\mu\nu} > 0, \\ 0, & \text{else.} \end{cases}$$

(12)

We call the quantity $J_n^\mu$ the number of synchronized clusters and calculate it for each event of each cluster. If our hypothesis of coupled waves and uncoupled puffs is correct, the majority of short events should not be synchronized, while for a long-lasting event a high number of synchronized clusters is expected.

Figure 4 shows the event length distribution from simulations. From the figure we can see that for increasing [IP$_3$] more long-lasting events occur just as in the single-cluster case. Using the definition of events as in sect. 2.3 and eq. (12), we colored events by the number of synchronized clusters: blue=0, green=1, yellow=2, orange=3, and red=4. We find that the bumps in B, C, and D are formed by events that are strongly synchronized to the neighboring clusters. It is apparent that the majority of events which last longer than 1 second are synchronized with at least one neighboring cluster. As can be seen from the insets in fig. 4, the fraction of events that are synchronized with all four adjacent clusters continuously increases with prolonging event durations. Increasing [IP$_3$] even boosts the slope of this effect which again suggests that higher [IP$_3$] promotes synchronization.

To further strengthen our discussion of induced synchrony for higher [IP$_3$], we calculated the sample coherence function as defined by eq. (11) for a set of different IP$_3$ concentrations. From fig. 5(A–D) we can clearly see that increasing [IP$_3$] leads to the formation of a peak in the coherence (black line) indicating the presence of a dominant periodicity. Further, the peak shifts to higher frequencies for increasing [IP$_3$]. Accompanying the formation of the peak, the average absolute phase lag between clusters (green line) forms a distinctive minimum at the very same frequency.

Figure 5(E) shows the inverse of the peak position of the SCF as a function of [IP$_3$] which can be used as definition for the inter wave interval (IWI). For low [IP$_3$] the peak vanishes in a broad scattering of data. In fact, for values of [IP$_3$] below 20 nM and rapidly rising IWI, the statistic fails, because either the slow timescale can no longer be captured by the window size, or there are no waves anymore. In addition, there is an optimum of
Fig. 5. Coherence functions for different $[\text{IP}_3]$ as indicated. (A-D) Absolute value (black, left axis) and phase (green, right axis) of the averaged sample coherence function defined by eq. (11). The parameters for the calculation are $\Delta t = 2\text{s}$, overlap $D = 0.75$ and a window size $L = 400\text{s}$ resulting in $S \approx T_{\text{sim}}/100 \text{s} = 300$ sequences. For each simulation we calculated the position of the peak of $|\hat{C}|$ and the width of the peak denoted as $f_{\text{peak}}$ and $\Delta f$ (full width at half maximum), respectively. In (E) the peak position $f_{\text{peak}}$ (left axis, black crosses) is plotted against $[\text{IP}_3]$. Peak positions where the quality of periodicity, $f_{\text{peak}}/\Delta f$ (right axis, blue squares), was below 1 were excluded.

synchrony (i.e. highest $f_{\text{peak}}/\Delta f$) at around 60 nM which is similar to results reported in [51]. There, an optimum of synchronous release was found for certain $\text{IP}_3$R cluster settings. However, this optimum was only present for intermediate $[\text{IP}_3]$. While the peak in the SCF and the minimum in the phase lag between signals are a clear indicator for globally synchronized release, the causal structure of this release can not be attained from fig. 5 directly. Specifically, the SCF would show a peak for both globally synchronous release and traveling waves. While there is no a priori reason for global synchronization of spatially embedded excitable systems, this effect has been reported for, e.g., FitzHugh-Nagumo elements [52] as well as in intracellular $\text{Ca}^{2+}$-dynamics [53] and is called array enhanced coherence resonance (AECR). In systems that exhibit AECR one can tune the noise parameter such that propagating waves turn into globally synchronized oscillations. In general AECR can be detected by plotting a measure of coherence against the noise-controlling parameter, which in case of AECR shows a clear peak for a certain noise value.

In our case the distribution of phase lags, i.e. $\phi(\hat{C}_{ij}(f_{\text{peak}}))$ from eq. (10), where $i$ and $j$ denote pairs of neighboring clusters, can be used as such a measure. However, since there is no clear noise-controlling parameter in our model, we cannot check for the resonance peak of AECR in a straightforward way. Still, we calculated these distributions for the simulations with different $\text{IP}_3$ concentrations. As one can see from fig. 6 the distribution for 80 nM shows a clear peak at nonzero phase lag, which supports our statement of propagating activity from the previous section. Interestingly, for 60 nM we can no longer resolve a shift of the peak towards values different from zero. This suggests the assumption, that our model exhibits AECR, and that for this intermediate $[\text{IP}_3]$ the system is close to the AECR peak. In this regime wave-like propagation of activity would be replaced by globally synchronized oscillations.

From fig. 6 we can read out an average phase lag between neighboring clusters for 80 nM: $\arg[\hat{C}(f_{\text{peak}})] \approx 0.05\pi$. With $f_{\text{peak}} = 0.05\text{Hz}$ this results in a propagation delay of about $0.05/2 \times 1/0.05\text{Hz} \approx 0.5\text{s}$. With a cluster distance of $\approx 3\mu\text{m}$ this would provide a wave speed of about $6\mu\text{m/s}$ which is close to 10–40 $\mu\text{m/s}$ for waves in Xenopus oocytes reported in [54].
4 Discussion

Our simulations show that increasing IP$_3$ induces a synchronization of slow scale release events. While this slow oscillation governs the macroscopic pattern of the calcium signal, there are still random, short events (i.e. puffs) on the single cluster scale. The main reason for this is the spatial extent between the clusters and the limited diffusion process in between, which renders the puffs unlikely to trigger adjacent clusters. This “diffusion barrier” is overcome by the long release events, which can arise for sufficiently high [IP$_3$] because of their significantly larger calcium release. Thus only the slow release type facilitates the communication between the clusters. Our model can therefore explain the different release timescales of puffs and waves as well as the different synchronizability of puffs and waves.

As described in [5], a possible termination mechanism of waves is the unbinding of IP$_3$. Our simulations for a grid of clusters support this modeling approach. From fig. 3 (B2, B3) we can clearly see that the number of activatable channels oscillates with a sizable amplitude. There will never be a complete deactivation of all channels (percentage of activatable always stays above 30%), but we can see from fig. 2 (A2) that clusters that constitute a wave are likely to end up in a completely deactivated state. Intriguingly, this mechanism directly leads to the prediction of an oscillating free IP$_3$ concentration, because the IP$_3$ which has unbound during the oscillation will increase the free [IP$_3$]. Of course, the amplitude of the oscillation in free [IP$_3$] would depend on the density of channels in the cytosolic space.

To be specific, taking the data from the simulation shown in fig. 2(B) a cluster unbinds between 15 and 30 IP$_3$ molecules per wave event (up to 2 IP$_3$ molecules must unbind for the channel to become non-activatable). Assuming a per cluster volume of (3 μm)$^3$, the free [IP$_3$] would therefore oscillate with an amplitude of 1 to 2 nM. This number is small compared to the baseline of free [IP$_3$] and no large variation in [IP$_3$] is predicted. Still, our model yields a new possible explanation for oscillating free IP$_3$, possibly at higher channel numbers. It is not due to metabolic production of IP$_3$, but due to IP$_3$ binding and unbinding from IP$_3$R. The amplitude of the [IP$_3$] oscillation should then correlate with the density of IP$_3$R channels in the cell. Because it is probably impossible to control the IP$_3$R channel density, this prediction is likely very hard to verify in experiments. Still, oscillations or at least varying [IP$_3$] have been reported in cells other than oocytes, where the density of IP$_3$R channels may be higher [8, 9]. Finally, note that we observe in the simulations a delay of the [IP$_3$] minimum with respect to the maximum in the number of open channels (fig. 3 B2 and B3), which is also observed in experiments [8, 9]. Our model can therefore reconcile the two different approaches to Ca$^{2+}$ oscillations and may be applied to cells where oscillations in IP$_3$ are either inessential or drive the Ca$^{2+}$ oscillations.

Due to its phenomenological nature, a small number of parameters, and its simplicity the proposed model provides an intuitive understanding of the interplay of multi-scale calcium concentrations with stochastic ion channel gating dynamics. While other models with more details often prohibit simulations of larger systems, we have demonstrated the usability of the model and its computational performance by simulating and analyzing a large set of coupled clusters’ dynamics, consisting of a total of 1024 IP$_3$Rs. We were able to sample trajectories covering the duration of hours and incorporating millions of individual channel transitions. This allowed us to perform extensive statistical analysis of different event properties. We do not propose this work as the state of the art for a detailed representation of intracellular calcium dynamics, such as molecular dynamic simulations or high resolution FEM calculations, we rather think that, similar to the variety of simplified neuron models in neuroscience, our proposed model can serve as a signpost to understand the complex dynamics of such systems.

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Appendix A. Reasonability of phenomenological parameters

To validate the chosen set of parameters from table 1, i.e. to check if it shows similar features and scales as our FEM simulations from [5], we rebuilt a deterministic wave from the FEM simulations (the wave at t = 115 s from fig. 2G in [5]). We then integrated the ODE from eq. (2) for a single cluster (i.e. no coupling contributions from other clusters) to obtain $c_{ad,i}$ and calculated the corresponding total Ca$^{2+}$ concentration from eq. (5) for this wave’s specific deterministic channel gating and a closed channel g. We can see from fig. 7, that the coarse-grained model is indeed able to reproduce the basic features from the FEM simulations.
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