Deterministic Limit of Intracellular Calcium Spikes

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In nonexcitable cells, global Ca\(^{2+}\) spikes emerge from the collective dynamics of clusters of Ca\(^{2+}\) channels that are coupled by diffusion. Current modeling approaches have opposed stochastic descriptions of these systems to purely deterministic models, while both paradoxically appear compatible with experimental data. Combining fully stochastic simulations and mean-field analyses, we demonstrate that these two approaches can be reconciled. Our fully stochastic model generates spike sequences that can be seen as noise-perturbed oscillations of deterministic origin, while displaying statistical properties in agreement with experimental data. These underlying deterministic oscillations arise from a phenomenological spike nucleation mechanism.

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The intracellular calcium ion Ca\(^{2+}\) is a major second messenger involved in many signaling pathways [1]. On average, the cell tends to maintain its cytosolic Ca\(^{2+}\) concentration [Ca\(^{2+}\)] to a low basal value of about 100 nM, but transient rises of [Ca\(^{2+}\)] can occur upon stimulation of the cell by an external agonist. The frequency and amplitude of these signals encode information about the nature and intensity of the physiological response [1].

In this Letter, we focus on variations of [Ca\(^{2+}\)] initiated by an increase in the concentration of inositol 1,4,5-trisphosphate (IP\(_3\)) and involving Ca\(^{2+}\) exchanges between the cytosol and the endoplasmic reticulum (ER). These exchanges are ensured by dedicated pumps and channels located in the membrane of the ER. The ER is able to sequestrate Ca\(^{2+}\) via endoplasmic reticulum calcium adenosine triphosphatases (SERCA pumps), while IP\(_3\) receptor (IP\(_3\)R) channels enable Ca\(^{2+}\) release from the ER to the cytosol. IP\(_3\)Rs are activated at low [Ca\(^{2+}\)] (resulting in Ca\(^{2+}\)-induced Ca\(^{2+}\) release, or CICR), but are inhibited at high [Ca\(^{2+}\)] [2]. IP\(_3\)Rs typically form multichannel clusters of 300–800 nm in width [3,4], separated by distances ranging from 1 to 7 μm [5,6].

The combination of CICR and Ca\(^{2+}\) diffusion leads to complex collective behaviors at different scales. The concerted release of Ca\(^{2+}\) through several channels in a single cluster can lead to a local rapid increase of [Ca\(^{2+}\)], which is known as a “puff.” Such subcellular Ca\(^{2+}\) signals occur randomly, last less than 1 s, and have a spatial extent of a few microns [3–6]. At the cell level, the coupling of clusters can result in the emergence of global [Ca\(^{2+}\)] increases, invading the whole cell and lasting at least a few seconds. These global Ca\(^{2+}\) events are called “spikes” [7,8]. While the mean interpuff time interval (IPI) usually does not exceed 5 s, the interspike time interval (ISI) ranges between 20 s and several minutes [5,8]. This difference points to the existence of different types of dynamics for the termination of spikes and puffs.

The statistical distributions of IPIs and ISIs are different. On one hand, IPIs are essentially random events and obey Poisson distributions with a mean mainly determined by the inhibition and reactivation timescales of IP\(_3\)Rs [9]. The dynamics of IP\(_3\)Rs clusters is thus intrinsically stochastic and aperiodic [8,10]. On the other hand, ISIs generally include a nonstochastic, absolute refractory period (T\(_{\text{min}}\)) that cannot exclusively be accounted for by the inhibition and recovery of channels alone and more likely results from the existence of a deterministic global process related to IP\(_3\) metabolism [11,12]. ISIs can be significantly larger than T\(_{\text{min}}\), which would not be the case if spikes were purely deterministic processes. ISIs are thus often divided into deterministic and stochastic (T\(_{\text{stoch}}\)) contributions, ISI = T\(_{\text{min}}\) + T\(_{\text{stoch}}\). Experiments show that T\(_{\text{stoch}}\) creates a linear relation between the standard deviation of the ISI (σ\(_{\text{ISI}}\)) and their mean (T\(_{av}\)),

$$\sigma_{\text{ISI}} = \alpha(T_{av} - T_{\text{min}}).$$

where the slope α varies according to the cell type and the agonist used to induce spikes [11,13].

Cell-level spikes can be surprisingly well described either by deterministic models with oscillatory dynamics...
or by stochastic models, based on the coupling of clusters whose local dynamics is aperiodic [15] or on intensity functions describing spikes from a top-down perspective [16]. Experiments [5] strongly suggest that global spikes and waves emerge from a nucleation mechanism, which is reproduced by deterministic models considering excitability clusters [17]. There is thus no consensus about the nonlinear behavior underlying global spikes [18]. In this Letter, we propose a unifying heuristic model, based on a nucleation mechanism. Combining fully stochastic simulations and bifurcation analyses of this model, we generated spike sequences corresponding to oscillations in the mean-field limit and displaying statistical properties compatible with experimental data.

The cell is modeled as a two-dimensional system with periodic boundary conditions, representing a layer of cytosol above the surface of the ER. The system is discretized in \( N_{\text{tot}} \) square compartments of side length \( \Delta x \) and volume \( V_c \), where the \( \left[ \text{Ca}^{2+} \right] \) is considered to be homogeneous, and some of these compartments contain clusters of IP\(_3\)Rs. The stochastic behavior of individual channels and the associated steep \( \text{Ca}^{2+} \) gradients were explicitly described in many previous models, but such simulations were computationally expensive and called for approximations [19–21]. Rather than simulate individual IP\(_3\)Rs, we based our approach on an extension of the phenomenological cluster model (Fig. 1), developed by Calabrese et al. [22] and validated against experimental observations.

Each cluster as a whole can be in four different states: an open state \( O \), a closed state \( C \), or one of the two inhibited states \( I_1 \) and \( I_2 \), and transitions between these states take place according to the scheme shown in Fig. 1. \( \text{Ca}^{2+} \) release exclusively occurs when the cluster is in state \( O \). In order to model the CICR mechanism, the transition probability from \( C \) to \( O \) depends on \( \left[ \text{Ca}^{2+} \right] \), which ensures that the probability to trigger cluster activity increases with the cytosolic \( \text{Ca}^{2+} \) content [23]. When the cluster is in state \( O \), two different pathways are available. The cluster can become rapidly inhibited to reach state \( I_2 \). The characteristic time of the \( I_2 \rightarrow C \) transition, \( 1/k_{2c} \approx 0.5 \) s, is compatible with the recovery time of a cluster after a puff. Once in state \( I_2 \), the cluster can recover and come back to state \( C \). At high ambient \( \left[ \text{Ca}^{2+} \right] \)—and hence very high local \( \left[ \text{Ca}^{2+} \right] \)—the cluster can also become inhibited in state \( I_1 \), from which recovery is slower than from \( I_2 \). This assumption is based on the observation [5] that when puffs have contributed to the onset of a global \( \text{Ca}^{2+} \) spike, puff activity is reduced. In the model, the dependence on \( \left[ \text{Ca}^{2+} \right] \) in the probability of \( O \rightarrow I_1 \) is hence combined to a long recovery time (see Table I).

Considering that open clusters release \( \text{Ca}^{2+} \) at a constant rate \( \Sigma \) [24], the amount of \( \text{Ca}^{2+} \) released depends on the time spent by clusters in state \( O \), which is ruled by the kinetic scheme described above. We evaluate \( \Sigma \) as \( \Sigma = I_{\text{eff}} \times 10^{-6}/2V_cF \), where \( I_{\text{eff}} = I(1-b) \) is an effective current accounting for the trapping of a fraction of ions by buffers, \( F = 96485 \) C mol\(^{-1} \) is the Faraday constant and the factor \( 10^{-6} \) ensures that \( I_{\text{eff}} \) is in picoamperes while \( \Sigma \) is in \( \mu \text{M s}^{-1} \). To be consistent with a current \( I \) between 0.12 and 0.95 pA, as reported by Bruno et al. [20], and for a buffering \( b \) of 98\% and \( V_c = 10^{-16} \) L, \( \Sigma \) ranges from 124 to 984 \( \mu \text{M s}^{-1} \) depending on the simulated stimulation level.

Contrary to the majority of previous models for global \( \text{Ca}^{2+} \) signals, we simulated the dynamics of the system with a fully stochastic approach based on Gillespie’s algorithm [25] (see Supplemental Material [26]). Fluxes associated with leak from the ER and uptake by SERCA pumps are also described as stochastic processes ruled by well-established kinetics, whose associated parameters are also given in Table I. Stochastic diffusion is introduced as a jump process between two neighboring boxes of the system.

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We thus included a total of nine different stochastic processes in our description: the five transitions relative to cluster states, \( \text{Ca}^{2+} \) fluxes due to leak from the ER and uptake by SERCA pumps in each compartment, \( \text{Ca}^{2+} \) diffusion between compartments, and release of \( \text{Ca}^{2+} \) from open clusters. The propensity functions associated with these processes are given in the Supplemental Material [26] (Table S1).

Experimentally, puff sequences are recorded in the presence of EGTA, an exogeneous slow buffer that prevents diffusion-mediated coupling between clusters [8]. To mimic

![FIG. 1. Model describing the dynamics of a cluster of IP\(_3\)Rs from Calabrese et al. [22].](https://example.com/fig1.png)
In order to gain insight into the nonlinear dynamics underlying this behavior, we turned to a deterministic version of the model. We considered an ensemble of clusters experiencing the same average [Ca$^{2+}$] and interacting globally via this effective concentration field. With such an approach, the Ca$^{2+}$ dynamics in the intercluster spaces is not described explicitly, but clusters are assumed to be distributed on the membrane of the ER according to a given cluster density $\rho_c = N_c/N_{tot}$, where $N_c$ is the number of clusters in the system. We considered $\rho_c = 0.1$, which corresponds to an average intercluster distance of 1 $\mu$m and is consistent with experimental data [6].

Evolution equations for [Ca$^{2+}$] and for the fraction of clusters $x = N_X/N_c$ in state $X = O$, $I_1$, $I_2$, or $C$ can be easily derived in this mean-field approximation (see Supplemental Material [26]). The deterministic dynamics of the system is ruled by the following evolution equations:

$$\frac{dO}{dt} = k_{co}[\text{Ca}^{2+}]^2(1 - o - i_1 - i_2) - k_{o1}[\text{Ca}^{2+}]^2 o - k_{o2} o,$$

$$\frac{di_1}{dt} = k_{o1}[\text{Ca}^{2+}]^2 o - k_{i1}i_1,$$

$$\frac{di_2}{dt} = k_{o2} o - k_{i2}i_2,$$

$$\frac{d[\text{Ca}^{2+}]}{dt} = \Sigma_{MF} o - J_{\text{SERCA}} + J_{\text{leak}},$$

where $\Sigma_{MF} = \Sigma\rho_c$ is a rescaled release rate. In these equations, only the fractions $o$, $i_1$, and $i_2$ appear because of the conservation relation $o + i_1 + i_2 + e = 1$. The SERCA and leak fluxes are given by

$$J_{\text{SERCA}} = \frac{v_p[\text{Ca}^{2+}]^2}{[\text{Ca}^{2+}]_b^2 + K_p^2}$$

and

$$J_{\text{leak}} = \frac{v_p[\text{Ca}^{2+}]_b^2}{[\text{Ca}^{2+}]_b^2 + K_p^2},$$

respectively, where $[\text{Ca}^{2+}]_b$ is a constant (Table I).

As shown in the bifurcation diagram (Fig. 3), the system admits a single low [Ca$^{2+}$] stable steady state for small values of $\Sigma_{MF}$. These parametric conditions coincide with those for which puffs were observed in the aforementioned stochastic simulations (for which $\Sigma_{MF} = 500 \times 0.01 = 5 \mu M/s$). For large values of $\Sigma_{MF} (>75 \mu M/s)$, one finds a stable steady state with high levels of [Ca$^{2+}$], in agreement with experiments [2]. For intermediate values of $\Sigma_{MF}$, the mean-field approach predicts the development of oscillations in a domain bounded by Hopf bifurcation points HB1 and HB2 (Fig. 3). The period of these oscillations is in the range of the mean ISIs reported in the literature [11]. This
suggests that the Ca\textsuperscript{2+} spikes observed experimentally could correspond to oscillations of the limit-cycle type, which would emerge whenever \( \bar{\rho}_c \) or \( \Sigma \) are increased.

We then performed stochastic simulations to track the possible emergence of spikes in multicenter systems. Starting from the puff regime, we ran stochastic simulations for gradually increasing values of \( \bar{\rho}_c \). By doing so, we enhanced the coupling strength between the clusters and increased \( \Sigma_{MF} \) to get closer to the parametric conditions leading to oscillations in deterministic simulations. Clusters of 0.5 \( \times \) 0.5 \( \mu m^2 \) were randomly distributed in a 5 \( \times \) 5 \( \mu m^2 \) system according to \( \bar{\rho}_c \), with the same other parametric conditions as in the puffs’ simulations. In this case, \( \text{[Ca}^{2+}] \) was averaged over the whole system. For \( \Sigma_{MF} < 25 \text{ \mu M s}^{-1} \), the average \( \text{[Ca}^{2+}] \) remains close to the basal state and small peaks, corresponding to puffs at the local scale, are observed. Beyond this threshold, the global dynamics transforms into global spikes with an amplitude of at least 1 \( \mu M \), which is close to the value predicted by the mean-field model. A typical sequence of such spikes is shown in Fig. 2(b). Similar to what is observed in an experimental time series [5], rapid puff activity is visible between spikes. The \( \sigma_{\text{ISI-T}_{av}} \) diagram shown in Fig. 4(a), where each point corresponds to a different realization performed with the same set of parameter values, suggests that a linear relation exists between the standard deviation and the average ISI of the Ca\textsuperscript{2+} spikes. Additionally, the line and the x axis intersect at a positive value. These statistical properties of the simulated spikes at \( \Sigma_{MF} > \text{HB1} \) are consistent with those of experimental signals [11,21]. In a small range of values of \( \Sigma_{MF} \) below HB1 (15.5 \( \text{\mu M s}^{-1} \leq \Sigma_{MF} \)), spiking can also occur but with a significantly higher coefficient of variation (CV) (see Fig. S1 in Supplemental Material [26]). This agrees with the CV measured in hepatocytes stimulated at subthreshold concentrations of hormones [28].

We next investigated the Ca\textsuperscript{2+} spiking dynamics for other parametric conditions leading to oscillations in the mean-field approximation. Spike trains were first obtained with random values of \( \Sigma_{MF} \) (with HB1 \( \leq \Sigma_{MF} \leq \text{HB2} \)) to simulate various IP\textsubscript{3} concentrations. As reported in [21], the corresponding \( \sigma_{\text{ISI-T}_{av}} \) points are positioned along the same straight line (see Fig. S2 in the Supplemental Material [26]). Besides, the slope \( \alpha \) is only slightly affected by variations of parameters like \( k_{co} \) [Fig. 4(b)] and \( v_p \) [Fig. 4(c)], which is consistent with experimental results showing the robustness of \( \alpha \) against various pharmacological perturbations, notably inhibiting the SERCA pumps [11]. The slight increment of the slope when \( k_{co} \) is decreased [Figs. 4(a) and 4(b)] can be attributed to a decrease in the ratio between the rates of “firing” and of recovery from inhibition [29].

To check that the Ca\textsuperscript{2+} spikes analyzed in Fig. 4 correspond to the noise-perturbed limit-cycle oscillations predicted by the mean-field analysis, shown in Fig. 3, we

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**FIG. 3.** Bifurcation diagram showing the stationary \([\text{Ca}^{2+}]\) (in black) and the maximal and minimal \([\text{Ca}^{2+}]\) reached during oscillations (in red) for \( k_{co} = 20 \text{ M}^{-1} \text{s}^{-1} \), \( v_p = 0.9 \text{ M} \text{s}^{-1} \), and the other parameter values given in Table I. Stable and unstable branches are in plain and dashed lines, respectively. HB and LP stand for Hopf bifurcation and fold, respectively. The slight increment of the slope when \( k_{co} \) is decreased [Figs. 4(a) and 4(b)] can be attributed to a decrease in the ratio between the rates of “firing” and of recovery from inhibition [29].

**FIG. 4.** \( \sigma_{\text{ISI-T}_{av}} \) plot obtained by numerical simulations with \( \Sigma = 500 \text{ M} \text{s}^{-1} \), \( \bar{\rho}_c = 0.1 \) (average intercluster distance of 1 \( \mu m \)), and (a) \( k_{co} = 20 \text{ M}^{-1} \text{s}^{-1} \) and \( v_p = 0.9 \text{ M} \text{s}^{-1} \), (b) \( k_{co} = 5 \text{ M}^{-1} \text{s}^{-1} \) and \( v_p = 0.9 \text{ M} \text{s}^{-1} \), and (c) \( k_{co} = 20 \text{ M}^{-1} \text{s}^{-1} \) and \( v_p = 0.7 \text{ M} \text{s}^{-1} \). The other parametric values are given in Table I. Each dot corresponds to a given spike sequence of at least ten spikes and the solid line is the linear fit through these points. \( T_{\text{min}} \) is equal to (a) 6.6 s, (b) 41.8 s, and (c) 12.59 s. The dashed line corresponds to the case \( \alpha = 1 \).
and deterministic modeling approaches of calcium, which tends to reconcile the current stochastic and experimental data. Moreover, the fully stochastic version of this model for spike nucleation that the deterministic counterpart of spiking dynamics can be oscillatory. The expected from the conditions of validity of the mean-field description, the agreement between the stochastic and expected period indeed lies in the distribution of the ISI. As expected from the conditions of validity of the mean-field description, the agreement between the stochastic and expected period indeed lies in the distribution of the ISI. As expected from the conditions of validity of the mean-field description, the agreement between the stochastic and expected period indeed lies in the distribution of the ISI. As

\[ \rho_c = 0.1 \]

In conclusion, we showed through this phenomenological model for spike nucleation that the deterministic counterpart of spiking dynamics can be oscillatory. The onset of stochastic spikes or mean-field oscillations is controlled by the parameters affecting the spatial coupling between the clusters, which is in agreement with experimental data. Moreover, the fully stochastic version of this model generates puff and spike sequences with realistic timescales and statistical properties. The results presented in this Letter thus highlight the compatibility of mean-field limit-cycle behavior with stochastic nucleation mechanisms, which tends to reconcile the current stochastic and deterministic modeling approaches of Ca^{2+} dynamics.

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[26] See Supplemental Material at http://link.aps.org/supplemental/10.1103/PhysRevLett.122.088101 for more information on the stochastic algorithm, derivation of the deterministic evolution equations, evolution of the coefficient of variation with respect to $\Sigma_{MF}$ and statistics of $\sigma_{ISI} - T_{avg}$ relation for varying $\Sigma_{MF}$.

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