A deterministic model predicts the properties of stochastic calcium oscillations in airway smooth muscle cells

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A Deterministic Model Predicts the Properties of Stochastic Calcium Oscillations in Airway Smooth Muscle Cells

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Abstract
The inositol trisphosphate receptor (IP₃R) is one of the most important cellular components responsible for oscillations in the cytoplasmic calcium concentration. Over the past decade, two major questions about the IP₃R have arisen. Firstly, how best should the IP₃R be modeled? In other words, what fundamental properties of the IP₃R allow it to perform its function, and what are their quantitative properties? Secondly, although calcium oscillations are caused by the stochastic opening and closing of small numbers of IP₃R, is it possible for a deterministic model to be a reliable predictor of calcium behavior? Here, we answer these two questions, using airway smooth muscle cells (ASMC) as a specific example. Firstly, we show that periodic calcium waves in ASMC, as well as the statistics of calcium puffs in other cell types, can be quantitatively reproduced by a two-state model of the IP₃R, and thus the behavior of the IP₃R is essentially determined by its modal structure. The structure within each mode is irrelevant for function. Secondly, we show that, although calcium waves in ASMC are generated by a stochastic mechanism, IP₃R stochasticity is not essential for a qualitative prediction of how oscillation frequency depends on model parameters, and thus deterministic IP₃R models demonstrate the same level of predictive capability as do stochastic models. We conclude that, firstly, calcium dynamics can be accurately modeled using simplified IP₃R models, and, secondly, to obtain qualitative predictions of how oscillation frequency depends on parameters it is sufficient to use a deterministic model.

Introduction
Oscillations in cytoplasmic calcium concentration ([Ca²⁺]ᵰ), mediated by inositol trisphosphate receptors (IP₃R; a calcium channel that releases calcium ions (Ca²⁺) from the endoplasmic or sarcoplasmic reticulum (ER or SR) in the presence of inositol trisphosphate (IP₃)) play an important role in cellular function in many cell types. Hence, a thorough knowledge of the behavior of the IP₃R is a necessary prerequisite for an understanding of intracellular Ca²⁺ oscillations and waves. Mathematical and computational models of the IP₃R play a vital role in studies of Ca²⁺ dynamics. However, over the past decade, two major questions about IP₃R models have arisen.

Firstly, how best should the IP₃R be modeled? Models of the IP₃R have a long history, beginning with the heuristic models of [1–3]. With the recent appearance of single-channel data from IP₃R in vivo [4,5], a new generation of Markov IP₃R models has recently appeared [6,7]. These models show that IP₃R exist in different modes with different open probabilities. Within each mode there are multiple states, some open, some closed. Importantly, it was found [8] that time-dependent transitions between different modes are crucial for reproducing Ca²⁺ puff data from [9]. However, it is not yet clear whether transitions between states within each mode are important, or whether all the important behaviors are captured simply by inter-mode transitions.

Secondly, why do deterministic models of the IP₃R perform so well as predictive models? Deterministic models of the IP₃R have proven to be useful predictive models in a range of cell types. For example, IP₃R-based models have been developed to study Ca²⁺ oscillations in airway smooth muscle cells (ASMC) [10–13], and these models have made predictions which have been confirmed experimentally. This shows the usefulness of such models in advancing our understanding of how intracellular Ca²⁺ oscillations and waves are initiated and controlled in ASMC. However, these models are deterministic models which assume infinitely many IP₃R per unit cell volume, an assumption that contradicts experimental findings in many cell types showing that Ca²⁺ puffs and spikes occur stochastically, and that intracellular Ca²⁺ waves and oscillations arise as an emergent property of fundamental stochastic events [9,14,15]. Here, we answer these two fundamental modeling questions using data and models from ASMC. Firstly, we show that a simple
The inositol trisphosphate receptor (IP$_3$R) is one of the most important cellular components responsible for calcium oscillations. Over the past decade, two major questions about the IP$_3$R have arisen. Firstly, what fundamental properties of the IP$_3$R allow it to perform its function? Secondly, although calcium oscillations are caused by the stochastic properties of small numbers of IP$_3$R is it possible for a deterministic model to be a reliable predictor of calcium dynamics? Using airway smooth muscle cells as an example, we show that calcium dynamics can be accurately modeled using simplified IP$_3$R models, and, secondly, that deterministic models are qualitatively accurate predictors of calcium dynamics. These results are important for the study of calcium dynamics in many cell types.

A two-state model of the IP$_3$R is sufficient to reproduce function

We have previously shown [8] that the statistics of Ca$^{2+}$ puffs in SH-SY5Y cells can be reproduced by a Markov model of the IP$_3$R based on the steady-state data of [5] and the time-dependent data of [4]. In this model the IP$_3$R can exist in 6 different states, grouped into two modes, which we call Drive and Park (see Fig. 1). The Drive mode (which contains 4 states; 1 open and 3 closed) has an average open probability of around 0.7, while the Park mode (which contains the remaining two states; 1 open and 1 closed) has an open probability close to zero. Transitions between states within each mode are independent of Ca$^{2+}$ and IP$_3$; only the transitions between modes are ligand-dependent.

In our previous study on calcium puffs [8], we showed that, to reproduce the experimentally observed non-exponential interspike interval (ISI) distribution and coefficient of variation (CV) of ISI smaller than 1, the time-dependent intermodal transitions are crucial. Lack of time dependencies in the Siekmann model leads to exponential ISI distributions and CV = 1, which is not the case for calcium spikes in ASMC. Fig. 2A shows an example of Ca$^{2+}$ oscillations generated by 50 nM methacholine (MCh) by binding to a G protein-coupled receptor in the cell membrane) in ASMC. By gathering data from 14 cells in 5 mouse lung slices, we found that the average peak and duration of interspike interval, spike amplitude and spike duration.

Moreover, by looking at a more detailed comparison between the two model results (Figs. 4A, C and E) and experimental data (Figs. 4B, D and F), we found the 2-state model not only can reproduce the behaviour of the 6-state model, but can also qualitatively reproduce experimental data. The average experimental ISI shows a clear decreasing trend as MCh concentration increases (although a saturation occurs in the data for high MCh), a trend that is mirrored by the model results as the IP$_3$ concentration increases. Unfortunately, since the exact relationship between MCh concentration and IP$_3$ concentration is uncertain, a quantitative comparison is not possible. In both model and experimental results, the average peak and duration of the oscillations are nearly independent of agonist concentration. The quantitative difference in spike duration between the model results and the data in Figs. 4E and F are most likely due to choice of calcium buffering parameters. For example, adding 3 or 5 µM fast Ca$^{2+}$ buffer (see Materials and Methods) increases the average spike duration to 0.54 s or 0.7 s respectively, which are close to the levels shown in the data.

Thus, the intramodal structure of the six-state model is essentially unimportant, as the model behavior (in terms of the
statistics of puffs and oscillations) is governed almost entirely by the
time dependence of the intermode transitions, particularly the time
dependence of the rapid inhibition of the IP₃R by high [Ca²⁺],
and the slow recovery from inhibition by Ca²⁺. The multiple
states within each mode are necessary to obtain an acceptable
quantitative fit to single-channel data, but are nevertheless of
limited importance for function. Hence, even when simulating
microscopic events such as Ca²⁺ puffs it is sufficient to use a
simpler, faster, two-state model, rather than a more complex six-
state model. In the following, we will use the 2-state IP₃R model to
generate all the simulation results.

Prediction of stochastic Ca²⁺ behavior by a deterministic model
Although the data (Fig. 2) show that Ca²⁺ oscillations in ASMC
are generated by a stochastic process, not a deterministic one, we
wish to know to what extent a deterministic model can be used to
make qualitative (and experimentally testable) predictions. Our

Figure 2. Ca²⁺ oscillations in ASMC in lung slices are generated by a stochastic mechanism. A: experimental Ca²⁺ spiking in ASMC in
lung slices, stimulated with 50 nM MCh. In the upper panel we filter out baseline noise by using a low threshold of 1.42 (relative fluorescence
intensity) and then choose samples with amplitude larger than 1.75. The ISI calculated from the upper panel is shown in the lower panel. B:
relationship between the standard deviation and the mean of experimental ISIs. Data obtained from 14 ASMC in 5 mouse lung slices. The relationship
is approximately linear with a slope of 0.66, which implies that an inhomogeneous Poisson process governs the generation of oscillations. The dashed
line indicates where the coefficient of variation (CV) is 1 (as it is for a pure Poisson process). Variation in ISI is mainly caused by both use of different
doses of MCh and different sensitivities of different cells to MCh. Error bars indicate the standard errors of the means (SEM).
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Figure 3. A 2-state open/closed model quantitatively reproduces the 6-state IP₃R model. A: histograms of interspike interval (ISI)
distribution for both the 6-state and the simplified models. The ISI is defined to be the waiting time between successive spikes. Each histogram
contain an equal number of samples (180). B: comparison of average ISI, average peak value of (Ca²⁺) [c in the model] and average spike duration.
All distributions were computed at a constant [IP₃] = 0.15 μM.
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Figure 4. More detailed comparisons between the 2-state and the 6-state IP₃,R models, and a comparison to experimental data. As a function of IP₃ concentration (p), the two models give the same ISI (A), peak [Ca²⁺] (C) and spike duration (E). These results agree qualitatively with experimental data, as shown in panels B, D and F respectively. Quantitative comparisons are generally not possible as the relationship between IP₃ concentration and agonist concentration is not known. Error bars represent mean ± SEM. Data for each MCh concentration are obtained from at least three different cells from at least two different lung slices.

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simplified 2-state Markov model of the IP$_3$R can be converted to a deterministic model (see Materials and Methods). The result is a system of ordinary differential equations (ODEs) with four variables, which takes into account the increased [Ca$^{2+}$]i at an open IP$_3$R pore, as well as the increased [Ca$^{2+}$]i within a cluster of IP$_3$R; the four variables are the [Ca$^{2+}$]i outside the IP$_3$R cluster (c), the [Ca$^{2+}$]i within the IP$_3$R cluster (c$_b$), the total intracellular Ca$^{2+}$ concentration (c$_p$) and an IP$_3$R gating variable (h$_{42}$). We refer to the reduced 4D model as the deterministic model for all the results and analyses.

Note that there is no physical or geometric constraint enforcing a high local [Ca$^{2+}$]i; in this case the spatial heterogeneity arises solely from the low diffusion coefficient of Ca$^{2+}$. Our use of c$_b$ is merely a highly simplified way of introducing spatial heterogeneity of the Ca$^{2+}$ concentration. Since the IP$_3$R can only “see” c$_b$ (as well as the Ca$^{2+}$ concentration right at the mouth of an open channel, which we denote by c$_p$), but cannot be influenced directly by c (the experimentally observed Ca$^{2+}$ signal), our approach allows for the functional differentiation of the rapid local oscillatory Ca$^{2+}$ in the cluster, from the slower Ca$^{2+}$ signal in the cytoplasm, without the need for computationally intensive simulations of a partial differential equation model. Quantitative accuracy is thus sacrificed for computational convenience.

Calcium oscillations in the stochastic and deterministic models are shown in Fig. 5A. According to our previous results [8], the accuracy is thus sacrificed for computational convenience.

Dependence of oscillation frequency on IP$_3$ concentration

In many cell types a moderate increase in [IP$_3$] increases the Ca$^{2+}$ oscillation frequency (see Fig. 2A in [11], Fig. 4E in [16] and Fig. 6B in [17]), a result that is reproduced by both model types (Fig. 6A). As [IP$_3$] increases, the stochastic model increases the probability of the initial Ca$^{2+}$ release through the first open IP$_3$R and of the following Ca$^{2+}$ release, thus shortening the average ISI. Although the oscillatory region of the deterministic model is strictly confined by bifurcations which do not apply to the stochastic model, the deterministic model can successfully replicate an increasing frequency by lowering the “knee” of the red curve in Fig. 5D and shortening the time spent from the termination point c to the initiation point a (thus shortening the ISI). Hence, although the deterministic model cannot be used to predict the exact values of [IP$_3$] at which the oscillations begin and end, as stochastic effects predominate in these regions, it can be used to predict the correct qualitative trend in oscillation frequency.

Dependence of oscillation frequency on Ca$^{2+}$ influx and efflux

In many cell types, including ASMC, transmembrane fluxes modulate the total intracellular Ca$^{2+}$ load (c$_i$) on a slow time scale [16,18], and thereby modulate the oscillation frequency [19]. Experimental data can be seen in Fig. 8 in [16] and Fig. 2 in [18]. Figs. 6B and C show that both stochastic and deterministic models predict the same qualitative changes in oscillation frequency in response to changes in membrane fluxes (through membrane ATPase pumps and/or Ca$^{2+}$ influx channels such as receptor-operated channels or store-operated channels).

Dependence of oscillation frequency on SERCA expression

The level of sarco/endoplasmic reticulum calcium ATPase (SERCA) expression (or capacity) is important for airway remodeling in asthma [20] and ASMC Ca$^{2+}$ oscillations [21]. We thus investigated the predictions of the two models in response to changes in SERCA expression (V$_{r}$). As V$_{r}$ decreases, the deterministic model exhibits a decreasing frequency, in agreement with experimental data (see Figs. 3 and 4 in [21]). The same trend is seen in the stochastic model with only 20 IP$_3$R (see Fig. 6D).

Dependence of oscillation frequency on Ca$^{2+}$ buffer concentration

Calcium buffers have been shown to be able to change the ISI and spike duration, which in turn change the oscillation frequency [15,22]. We compared the effects on the two models of varying total buffer concentration (B$_i$) by adding one buffer with relatively fast kinetics to the models (see Materials and Methods for details). In both models the frequency decreases as B$_i$ increases (see Fig. 6E), which is consistent with experimental data (Fig. 2B in [18]). This is not surprising, because increasing B$_i$ can decrease the effective rates of SR Ca$^{2+}$ release and reuptake.

Dependence of oscillation baseline on Ca$^{2+}$ influx and SERCA expression

Sustained elevations of baseline during agonist-induced Ca$^{2+}$ oscillations or transients have been observed experimentally, and are believed to be a result of an increase in Ca$^{2+}$ influx caused by opening of membrane Ca$^{2+}$ channels [13,16]. Furthermore, there
is evidence showing that decreased SERCA expression could also increase the baseline (Fig. 4 in [21]). Those phenomena are successfully reproduced by both models (see Fig. 7).

**Discussion**

In this paper we address two current major questions in the field of Ca\(^{2+}\) modeling. Firstly, we show that Ca\(^{2+}\) puffs and stochastic oscillations can be reproduced quantitatively by an extremely simple model, consisting only of two states (one open, one closed), with time-dependent transitions between them. This model is obtained by removing the intramodal structure of a more complex model that was determined by fitting a Markov model to single-channel data [7]. We thus show that the internal structure of each mode is irrelevant for function and mode switching is the key mechanism for the control of calcium release. The necessity for time-dependent mode switching is shown not only by the dynamic single-channel data of [4], but also by the puff data of [9] and our ASMC data.

Secondly, we investigate the role of stochasticity of IP\(_3\)R in modeling Ca\(^{2+}\) oscillations in ASMC by comparing a stochastic IP\(_3\)R-based Ca\(^{2+}\) model and its associated deterministic version, for parameters such that both of the models exhibit Ca\(^{2+}\) spikes but the stochastic model cannot necessarily be replaced by a mean-field model. We find that a four-variable deterministic model has the same predictive power as the stochastic model, in combination.
Figure 6. Comparison of parameter-dependent frequency changes in the stochastic and deterministic models. All curves are computed at 0.12 μM IP₃ except in panel A, which uses a variety of [IP₃]. Other parameters are set at their default values given in Table 1. A: as [IP₃] increases, Ca²⁺ oscillations in both models increase in frequency. B: as Ca²⁺ influx increases (modeled by an increase in receptor-operated calcium channel flux coefficient $V_{rocc}$), so does the oscillation frequency in both models. C: as Ca²⁺ efflux increases (modeled by an increase in plasma pump expression $V_p$), oscillation frequency decreases. D: as SERCA pump expression, $V_s$, increases, so does oscillation frequency. E: as total buffer concentration, $B_t$, increases, oscillation frequency decreases.

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that it correctly reproduces the process of spike termination and predicts the same qualitative changes in oscillation frequency and baseline in response to a variety of perturbations that are commonly used experimentally. The mechanism for termination of individual spikes is fundamentally a deterministic process controlled by a rapid inhibition induced by the high local [Ca\(^{2+}\)]\(_i\) in the IP\(_3\)R cluster, whereas spike initiation is significantly affected by stochastic opening of IP\(_3\)R. Hence, repetitive Ca\(^{2+}\) cycling is primarily induced by the time-dependent gating variables governing transitions of the IP\(_3\)R from one mode to another.

Our simplified two-state model of the IP\(_3\)R is identical in structure (although not in parameter values) to the well-known model of [23]. It is somewhat ironic that after 20 years of detailed studies of the IP\(_3\)R and the construction of a plethora of models of varying complexity, the single-channel data have led us around full circle, back to these original formulations. Excitability is arising via a fast activation followed by a slower inactivation, a combination often seen in physiological single-processes [24]. Encoding of this fundamental combination results directly from the two-mode structure of the IP\(_3\)R. Although similar single-channel data have been used to construct three-mode models [6,25], neither of these models has yet been used in detailed studies of Ca\(^{2+}\) puffs and waves, and it remains unclear whether or not they have a similar underlying structure.

In contrast to previous deterministic ODE models, our four-variable Ca\(^{2+}\) model includes a more accurate IP\(_3\)R model, as well as local control of clustered IP\(_3\)R by two distinct Ca\(^{2+}\) microdomains; one at the mouth of an open IP\(_3\)R, the other inside a cluster of IP\(_3\)R. Neglect of either of these microdomains leads to models that either exhibit unphysiological cytoplasmic Ca\(^{2+}\) concentrations or fail to reproduce reasonable oscillations. This underlines the importance of taking Ca\(^{2+}\) microdomains into consideration when constructing any model. Our microdomain model is highly simplified, with the microdomain being treated simply as a well-mixed compartment. More detailed modeling of spatially-dependent microdomains is possible, and not difficult in principle, but requires far greater computational resources. It is undeniable that a more detailed model, incorporating the full spatial complexity – and possibly stochastic aspects as well – would make, overall, a better predictive tool. However, our goal is to find the simplest models that can be used as predictive tools.

An important similar study is that of Shuai and Jung [26]. They compared the use of Markov and Langevin approaches to the computation of puff amplitude distributions, compared their results with the deterministic limit, and showed that IP\(_3\)R stochasticity does not qualitatively change the type of puff amplitude distribution except for when there are fewer than 10 IP\(_3\)R. Here, we significantly extend the scope of their study by exploring the effects of IP\(_3\)R stochasticity on the dynamics of Ca\(^{2+}\) spikes, and we do this in the context of an IP\(_3\)R model that has been fitted to single-channel data. Although this is true in a general sense for the Li-Rinzel model, which is based on the DeYoung-Keizer model, which did take into account the opening time distributions of IP\(_3\)R in lipid bilayers, neither model can reproduce the more recent data obtained from on-nuclei patch clamping. When these recent data are taken into account one obtains a model with the same structure, but quite different parameters and behavior.
Table 1. Parameter values of the stochastic calcium model.

| Parameter       | Description                                             | Value/Units   |
|-----------------|---------------------------------------------------------|---------------|
| \(k_{IP3}\)     | IP, R flux coefficient                                  | 0.05 s⁻¹      |
| \(k_{diff}\)    | Ca²⁺ diffusional flux coefficient                       | 10 s⁻¹        |
| \(k_{leak}\)    | SR leak flux coefficient                                | 0.0032 s⁻¹    |
| \(V_m\)         | maximum capacity of SERCA                               | 10 \(\mu\)M s⁻¹|
| \(K_0\)         | SERCA half-maximal activating \([Ca^{2+}]_i\)           | 0.26 \(\mu\)M |
| \(n_1\)         | Hill coefficient for SERCA                              | 1.75          |
| \(J_{plakin}\)  | plasma membrane leak influx                             | 0.03115 \(\mu\)M s⁻¹|
| \(V_{RCC}\)     | ROCC flux coefficient                                   | 0.2 s⁻¹       |
| \(V_{SOCC}\)    | maximum capacity of SOCC                                | 1.6 \(\mu\)M s⁻¹|
| \(K_{ASC}\)     | SOCC dissociation constant                              | 100 \(\mu\)M  |
| \(V_p\)         | maximum capacity of plasma pump                        | 0.8 \(\mu\)M s⁻¹|
| \(\theta_p\)    | Hill coefficient for plasma pump                        | 0.5 \(\mu\)M  |
| \(\gamma_1\)    | the cytoplasmic-to-microdomain volume ratio             | 100           |
| \(\gamma_2\)    | the cytoplasmic-to-SR volume ratio                      | 10            |
| \(c_{\mu}\)     | an instantaneous high \([Ca^{2+}]_i\) at open channel    | 120 \(\mu\)M  |
| \(N_0\)         | total number of IP₃R channels                           | 20            |

We find that, in spite of a relatively large variation in spike amplitude which is partially caused by a large variation in ISI (Fig. 5B), the mechanism governing individual spike terminations is the same for both a few or infinitely many IP₃R, which explains why the one-peak type of amplitude distribution is independent of the choice of IP₃R number (see Fig. 6A in [26]).

Another important relevant study was done by Dupont et al. [27], who compared the regularity of stochastic oscillations in hepatocytes for different numbers of IP₃R clusters. They found that the impact of IP₃R stochasticity on global Ca²⁺ oscillations (in terms of CV) increases as the total cluster number decreases. Our study here extends these results, and demonstrates how well stochastic oscillations can be qualitatively described by a deterministic system, even when there is only a small number of IP₃R (which appears to be the case for ASMC, in which the wave initiation site is only 2–4 \(\mu\)m in diameter). Indeed, as we have shown, for the purposes of predictive modeling a simple deterministic model does as well as more complex stochastic simulations.

Ryanodine receptors (RyR) are another important component modulating ASMC Ca²⁺ oscillations [16,28,29] but are not included in our model. This is because the role of RyR is not fully understood and may be species-dependent; for example, in mouse or human ASMC, RyR play very little role in IP₃-induced continuing Ca²⁺ oscillations [17,30], but this appears not to be true for pigs [28]. Our study focuses on the calcium oscillations in mouse and human (as we did in our experiments) where inclusion of a deterministic model of RyR should have little effect. An understanding of the role of RyR stochasticity and how the IP₃R and the RyR interact needs a reliable RyR Markov model, exclusive to ASMC, which is not currently available. Multiple Markov models of the RyR have been developed for use in cardiac cells [31], but these are based on single-channel data from lipid bilayers, and are adapted for the specific context of cardiac cells. Their applicability to ASMC remains unclear.

Although we have not shown that the deterministic model for ASMC has the same predictive power as the stochastic model in all possible cases (which would hardly be possible in the absence of an analytical proof) the underlying similarity in phase plane structure indicates that such similarity is plausible at least. Certainly, we have not found any counterexample to this claim. However, whether or not this claim is true for all cell types is unclear. Some cell types exhibit both local Ca²⁺ puffs and global Ca²⁺ spikes (usually propagating throughout the cells in the form of traveling waves), showing that initiation of such Ca²⁺ spikes requires a synchronization of Ca²⁺ release from more than one cluster of IP₃R [14]. This type of spiking relies on the hierarchical organization of Ca²⁺ signal pathways, in particular the stochastic recruitment of both individual IP₃R and puffs at different levels [32], and therefore cannot be simply reproduced by deterministic models containing only a few ODEs. However, Ca²⁺ oscillations in ASMC, as observed in lung slices, may not be of this type, as IP₃R-dependent puffs have not been seen in these ASMC. It thus appears that, in ASMC in lung slices, every Ca²⁺ “puff” initiates a wave, resulting in periodic waves with ISI that are governed by the dynamics of individual puffs.

Materials and Methods

Ethics Statement
Animal experimentation carried out were approved by the Animal Care and Use Committee of the University of Massachusetts Medical School under approval number A-036-12.

Lung slice preparation
BALB/c mice (7–10 weeks old, Charles River Breeding Labs, Needham, MA) were euthanized via intraperitoneal injection of 0.3 ml sodium pentobarbitone (Oak Pharmaceuticals, Lake Forest,
Measurement of Ca\textsuperscript{2+} oscillations

Lung slices were incubated in shBSS containing 20 μM Oregon Green 488 BAPTA-1-AM (Invitrogen), a Ca\textsuperscript{2+}-indicator dye, 0.1% Phoramic F-127 (Invitrogen) and 200 μM sulfobromophthalein (Sigma Aldrich, St Louis, MO) in the dark at 30 °C for 1 hour. Subsequently, the slices were incubated in 200 μM sulfobromophthalein for 30 minutes. Slices were mounted on a cover-glass and held down with 200 μm mesh. A smaller cover-glass was placed on top of the mesh and sealed at the sides with silicone grease to facilitate solution exchange. Slices were examined with a custom-built 2-photon scanning laser microscope (IO Industries, Montréal, Canada). Changes in fluorescence intensity (which represent changes in [Ca\textsuperscript{2+}]) were analyzed in an ASMC of interest by averaging the grey value of a 10 × 10 pixel region using custom written software. Relative fluorescence intensity (F/F\textsubscript{0}) was expressed as a ratio of the fluorescence intensity at a particular time (F) normalized to the initial fluorescence intensity (F\textsubscript{0}).

The calcium model

Inhomogeneity of cytoplasmic Ca\textsuperscript{2+} concentration not only exists around individual channel pores of the IP\textsubscript{3}R, where a nearly instantaneous high Ca\textsuperscript{2+} concentration at the pore (denoted by \(c_p\)) leads to a very sharp concentration profile, but is also seen inside an IP\textsubscript{3}R cluster where the average cluster Ca\textsuperscript{2+} concentration (\(c_0\)) is apparently higher than that of the surrounding cytoplasm (c) [33]. This indicates that during Ca\textsuperscript{2+} oscillations each IP\textsubscript{3}R is controlled by either the pore Ca\textsuperscript{2+} concentration (when it is open) or the cluster Ca\textsuperscript{2+} concentration (when it is closed). Neither of these local concentrations influence cell membrane fluxes or the majority of SERCAs, which we assume to be distributed outside the cluster.

The scale separation between the pore Ca\textsuperscript{2+} concentration and the cluster Ca\textsuperscript{2+} concentration allows to treat \(c_p\) as a parameter, providing a simpler way of modeling local Ca\textsuperscript{2+} events (like Ca\textsuperscript{2+} puff) that has been used in several previous studies [8,34,35]. However, evolution of the cluster concentration and wide-field cytoplasm Ca\textsuperscript{2+} concentration are not always separable, so an additional differential equation for the cluster Ca\textsuperscript{2+} is necessary.

A schematic diagram of the model is shown in Fig. 8. The corresponding ODEs are

\[ \frac{dc}{dt} = J_{diff} + J_{leak} - J_{serca} + J_{in} - J_{pm}, \]  

(1)

\[ \frac{dc_0}{dt} = \gamma_1(J_{IPR} - J_{diff}). \]  

(2)

where \(c_0 = c + c_b/\gamma_1 + c_p/\gamma_2\) representing total intracellular Ca\textsuperscript{2+} concentration, and thus SR Ca\textsuperscript{2+} concentration, \(c_s\) is given by \(c_s = \gamma_3(c_1 - c - c_b/\gamma_1)\), \(\gamma_1\) and \(\gamma_2\) are the volume ratios given in Table 1. IP\textsubscript{3}R is the flux through the IP\textsubscript{3}R, \(J_{leak}\) is a background Ca\textsuperscript{2+} leak out of the SR, and \(J_{serca}\) is the uptake of Ca\textsuperscript{2+} into the SR by SERCA pumps. \(J_{pm}\) is the flux through plasma pump, and \(J_{in}\) represents a sum of main Ca\textsuperscript{2+} influxes including \(J_{rocc}\) (receptor-operated Ca\textsuperscript{2+} channel), \(J_{socc}\) (store-operated Ca\textsuperscript{2+} channel) and \(J_{leak}\) (Ca\textsuperscript{2+} leak into the cell). \(J_{diff}\) coarsely models the diffusion flux from cluster microdomain to the cytoplasm.

Details of the fluxes are

- Different formulations of \(J_{IPR}\) give different types of models:
  - For the stochastic model, \(J_{IPR} = (k_{IPR}/N_s)(c_0 - c)\) where \(k_{IPR}\) is the maximum conductance of a cluster of \(N_s\) IP\textsubscript{3}R (here \(N_s = 20\)). \(N_s\) is the number of open IP\textsubscript{3}R determined by the states of IP\textsubscript{3}R.
  - For the deterministic model we set \(J_{IPR} = k_{IPR}P_o(c_0 - c)\) where \(P_o\) is the IP\textsubscript{3}R open probability, a continuous analogue of \(N_s/N_I\).

To calculate \(N_s\) and \(P_o\), we use the IP\textsubscript{3}R model of [7,8], with minor modifications described later.

- \(J_{diff} = k_{diff}(c_b - c)\).

- \(J_{serca} = V_{serc}c_p/(K_{serc}^4 + c_p^4)\) where \(K_{serc}\) and \(n_s\) are obtained from [36].

- \(J_{leak} = k_{leak}(c_s - c)\).

- \(J_{in}\) includes a basal leak (\(J_{leak}\)), receptor-operated calcium channel (ROCC, \(J_{rocc}\)), store-operated calcium channel (SOCC, \(J_{socc}\)). By using the IP\textsubscript{3}R concentration (\(p\)) as a surrogate indicator of MCh concentration, we assume that \(J_{rocc} = V_{rocc}p\) and \(J_{socc} = V_{socc}K_{socc}^4/(K_{socc}^4 + c_p^4)\) [13].

- \(J_{pm} = V_pm c_p^2/(K_{pm}^2 + c_p^2)\).

Calcium concentration at open channel pore (\(c_p\)) does not explicitly appear in the equations but is used in the IP\textsubscript{3}R model introduced later. \(c_p\) is assumed to be proportional to SR Ca\textsuperscript{2+} concentration (\(c_s\) and is therefore simply modeled by

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c_p = c_p0(c_s/100) where c_p0 is the value corresponding to c_s = 100 μM. Alternatively, c_p can also be assumed to be a large constant (say greater than 100 μM) without fundamentally altering the model dynamics. The choice of c_p0 is not critical as long as it is sufficiently large to play a role in inactivating the open channels. All the parameter values are given in Table 1.

The data-driven IP3R model

The IP3R model used in our ASMC calcium model is an improved version of the Siekmann IP3R model which is a 6-state Markov model derived by fitting to the stationary single channel data using Markov chain Monte Carlo (MCMC) [5,7,8]. Fig. 1 has shown the structure of the IP3R model which is comprised of two modes; the drive mode, containing three closed states C1, C2, C3 and one open state Oa, and the park mode, containing one closed state C4 and one open state Oa. The transition rates in each mode are constants (shown in Table 2), but q24 and q42 which connect the two modes are Ca2+-/IP3R-dependent and are formulated as

\[ q_{24} = a_{24} + V_{24}(1 - m_{24}h_{24}), \]

where \( m_{24}, h_{24}, m_{42}, \) and \( h_{42} \) are Ca2+-/IP3R-modulated gating variables. \( a_{24}, a_{42}, V_{24} \) and \( V_{42} \) are either functions of \( p \) or constants and are given later. We assume the gating variables obey the following differential equation,

\[ \frac{dG}{dt} = \lambda_G(G_e - G), \quad (G = m_{24}, h_{24}, m_{42}, h_{42}), \]

where \( G_e \) is the equilibrium and \( \lambda_G \) is the rate at which the equilibrium is approached. These equilibria are functions of Ca2+ concentration at the cytoplasmic side of the IP3R, denoted by \( \hat{c} \) in the equations, equal to either \( c_p \) or \( c_s \) depending on the state of the channel. They are assumed to be

\[ m_{24} = \frac{\hat{c}^3}{\hat{c}^3 + k_{24}^{-1}}, \]

\[ q_{42} = a_{42} + V_{42}m_{42}h_{42}, \]

Table 2. Parameter values of the IP3R model.

| Parameter | Value/Units | Parameter | Value/Units |
|-----------|-------------|-----------|-------------|
| q_{12}    | 1240 s⁻¹   | q_{21}    | 88 s⁻¹      |
| q_{23}    | 3 s⁻¹      | q_{12}    | 69 s⁻¹      |
| q_{26}    | 10500 s⁻¹  | q_{62}    | 4010 s⁻¹    |
| H         | 11 s⁻¹     | q_{44}    | 3330 s⁻¹    |
| l_{m0}    | 100 s⁻¹    | l_{m0}    | 100 s⁻¹     |
| l_{a}     | 40 s⁻¹     |           |             |

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Figure 9. Stationary data and fits of \( q_{24} \) and \( q_{42} \). Stationary transition rates of \( q_{24} \) and \( q_{42} \), \( q_{24}^B \), and \( q_{42}^B \) as functions of Ca2+ concentration were estimated and fitted for two [IP3]ₜ, 1 μM (A) and 10 μM (B). Circles and squares represent the means of \( q_{24} \) and \( q_{42} \) distributions computed by MCMC simulation [7]. Note that MCMC failed to determine the values of \( q_{24} \) and \( q_{42} \) at \( \hat{c} = 1, 10 \) μM for 10 μM IP3, as the IP3R was almost in the drive mode for these cases. The corresponding fitting curves (solid for \( q_{42} \), dashed for \( q_{24} \)) are produced using Eqs. 7–12.

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from [5]. Data (average open probability) are constants estimated by using dynamic single channel data [4] and given in Table 2, whereas \( \lambda_{\text{ba2}} \) is not clearly revealed by experimental data. However we have shown that it should be relatively large for high \( \hat{c} \) but relatively small for low \( \hat{c} \) for reproducing experimental puff data [6] by introducing two Ca\(^{2+}\) concentrations, \( c_b \) and \( c_p \), \( \lambda_{\text{ba2}} \) and the state of the IP\(_3\)R channel become highly correlated, so that we can assume \( \lambda_{\text{ba2}} \) is a relatively large value \( H \) if the channel is open and is a relatively small value \( L \) if the channel is closed. Hence, \( \lambda_{\text{ba2}} \) is modeled by the logic function

\[
\lambda_{\text{ba2}} = \begin{cases} 
H, & \text{if the channel is open;} \\
L, & \text{if the channel is closed.}
\end{cases}
\]

Values of \( L \) and \( H \) are chosen so that simulated Ca\(^{2+}\) oscillations in ASMC are comparable to experimental observations.

**The IP\(_3\)R model reduction**

Here we reduce the 6-state model to a 2-state open/closed model. The reduction takes the following steps:

- The sum of the probabilities of \( C_1 \), \( C_3 \) and \( O_5 \) is less than 0.03 for any \( \hat{c} \), so they are either rarely visited by the IP\(_3\)R or have a very short dwell time. This implies they have very little contribution to the Ca\(^{2+}\) dynamics. Therefore, we completely remove the three states from the full model.
- Transition rates of \( q_{26} \) and \( q_{42} \) are about 2 orders larger than that of \( q_{24} \) and \( q_{42} \), which allows us to omit the fast transitions by taking a quasi-steady state approximation. This change will affect two aspects. First, we have \( O_6 = C_5 q_{26}/q_{42} \) which allows us to combine \( C_2 \) and \( O_6 \) to be a new state \( D \), which satisfies \( D = O_6(q_{26}/q_{42}) \). Although this means \( D \) is a partially open state with an open probability of \( q_{26}/(q_{26} + q_{42}) \), it can be used as an fully open state in the stochastic simulations by multiplying the maximum IP\(_3\)R flux conductance \( k_{\text{IPR}} \) by a factor of \( q_{26}/(q_{26} + q_{42}) \). Secondly, \( q_{24} \) needs to be rescaled by \( q_{24}/(q_{26} + q_{42}) \), i.e., the effective closing rate is \( q_{24} q_{42}/(q_{26} + q_{42}) \).
- Due to the combination of \( C_2 \) and \( O_6 \), \( \lambda_b \) is accordingly modified to

\[
\lambda_{\text{ba2}} = \begin{cases} 
H, & \text{if the channel is in } C_4 \\
L, & \text{if the channel is in } D \text{ (the drive mode)}
\end{cases}
\]

Hence, the reduced two-state model contains one “open” state \( D \) and one closed state \( C_4 \) with the opening transition rate of \( q_{42} \) and the closing transition rate of \( q_{24} q_{42}/(q_{26} + q_{42}) \).

**Deterministic formulation of the stochastic model**

Based on the stochastic calcium model and the reduced 2-state IP\(_3\)R model, we construct a deterministic model. We need to modify three things that are used in the stochastic model but inapplicable to fast simulations of the deterministic model. The first is the discrete number of open channels; the second is state-dependent use of \( c_b \) and \( c_p \) in calculating \( q_{42} \) and \( q_{24} \); the last is the logic expression of \( \lambda_{\text{ba2}} \). Details of the modifications are as follows,

- The fraction of open channels \( (N_o/N_t) \) is replaced by open probability \( P_o \) which is 70% of the probability of state \( D \).
In the stochastic simulations, \( q_{24} \) which only controls the IP\(_3\)R closing is primarily governed by \( \epsilon_p \), whereas \( q_{42} \) which controls IP\(_3\)R opening is mainly governed by \( \epsilon_b \). Therefore, in the deterministic model, we separate the functions of \( \epsilon_p \) and \( \epsilon_b \) by assuming \( m_{24,c} \) and \( h_{24,c} \) are functions of \( \epsilon_p \) only whereas \( m_{42,c} \) and \( h_{42,c} \) are functions of \( \epsilon_b \) only. That is, \( m_{24,c} = m_{24,c}(\epsilon_p) \), \( h_{24,c} = h_{24,c}(\epsilon_p) \), \( m_{42,c} = m_{42,c}(\epsilon_b) \) and \( h_{42,c} = h_{42,c}(\epsilon_b) \). Here \( \epsilon_p = \epsilon_p(c_i/100) \) as defined before.

To describe an average rate that infinitely many receptors are rapidly inhibited by high Ca\(^{2+}\) concentration but slowly restored from Ca\(^{2+}\)-inhibition, \( \lambda_{h_{42}} \) is proposed to be

\[
\lambda_{h_{42}} = (1 - D)L + DH.
\]

Based on the above changes, the full deterministic model containing 8 ODEs is presented as follows,

\[
\frac{dc}{dt} = J_{\text{diff}} + J_{\text{leak}} - J_{\text{serca}} + J_{\text{in}} - J_{\text{pm}},
\]

\[
\frac{dc_b}{dt} = \gamma_1(\text{J}_{\text{IPR}} - J_{\text{diff}}),
\]

\[
\frac{dc_i}{dt} = J_{\text{in}} - J_{\text{pm}},
\]

\[
\frac{dD}{dt} = q_{42}(1 - D) - \left( \frac{q_{24}q_{62}}{q_{62} + q_{26}} \right)D,
\]

\[
\frac{dm_{24}}{dt} = \lambda_{m_{24}} \left( \frac{e_p^3}{e_p + k_{24}^3} - m_{24} \right),
\]

\[
\frac{dh_{24}}{dt} = \lambda_{h_{24}} \left( \frac{k_{24}^3}{e_p + k_{24}^3} - h_{24} \right),
\]

\[
\frac{dm_{42}}{dt} = \lambda_{m_{42}} \left( \frac{e_b^3}{e_b + k_{42}^3} - m_{42} \right),
\]

\[
\frac{dh_{42}}{dt} = \lambda_{h_{42}} \left( \frac{k_{42}^3}{e_b + k_{42}^3} - h_{42} \right),
\]

where \( q_{24} \) and \( q_{42} \) are functions of the gating variables given by Eqs. 4 and 5. All the fluxes are the same as those of the stochastic model except \( \text{J}_{\text{IPR}} = k_{\text{IPR}}(D_{q_{26}}(q_{62} + q_{26})/(q_{62} + q_{26})) - (c_i - c_b) \). All the parameter values of the deterministic model are the same as those of the stochastic model and are therefore given in Tables 1 and 2.

**Reduction of the full deterministic model**

The full deterministic model contains 8 variables which make the model difficult to implement and analyze. Thus, we reduce the full model to a minimal model that still captures the crucial features of the full model. First of all, \( \lambda_{m_{24}}, \lambda_{m_{42}} \) and \( \lambda_{h_{42}} \) are sufficiently large so that we can assume they instantaneously follow their equilibrium functions. Therefore, by taking quasi-steady state approximation to \( m_{24}, h_{24}, m_{42} \) and \( h_{42} \), we remove the three time-dependent variables from the full model.

By now, the full model has been reduced to a 5D model,

\[
\frac{dc}{dt} = J_{\text{diff}} + J_{\text{leak}} - J_{\text{serca}} + J_{\text{in}} - J_{\text{pm}},
\]

\[
\frac{dc_b}{dt} = \gamma_1(\text{J}_{\text{IPR}} - J_{\text{diff}}),
\]

\[
\frac{dc_i}{dt} = J_{\text{in}} - J_{\text{pm}},
\]

\[
\frac{dD}{dt} = q_{42}(1 - D) - \left( \frac{q_{24}q_{62}}{q_{62} + q_{26}} \right)D,
\]

\[
\frac{dm_{42}}{dt} = \lambda_{m_{42}} \left( \frac{k_{42}^3}{e_b + k_{42}^3} - m_{42} \right).
\]

Second, the rate of change of \( D \) approaching its equilibrium, \( \lambda_D = (q_{24}q_{62} + q_{42}q_{26} + q_{24}q_{62})/(q_{62} + q_{26}) \) (calculated from Eq. 24), is at least one order larger than those of \( c, c_b \) and \( h_{42} \), indicating that taking the quasi-steady state approximation to Eq. 24 could not significantly affect the evolutions of \( c, c_b \) and \( h_{42} \). That is,

\[
D = \frac{q_{24}q_{62} + q_{26}}{q_{24}q_{62} + q_{42}q_{26} + q_{24}q_{62}}.
\]

We emphasize here that the theory of the quasi-steady state approximation has not yet been well established, particularly about the rigorous conditions under which such a reduction is valid. Thus, our criterion of judging the validity of the reduction is checking whether the solutions of the reduced model are capable of qualitatively reproducing that of its original model. For this model, we find the reduction works. Hence, the full model is eventually reduced to a 4D model summarized as follows,

\[
\frac{dc}{dt} = J_{\text{diff}} + J_{\text{leak}} - J_{\text{serca}} + J_{\text{in}} - J_{\text{pm}},
\]

\[
\frac{dc_b}{dt} = \gamma_1(\text{J}_{\text{IPR}} - J_{\text{diff}}),
\]

\[
\frac{dc_i}{dt} = J_{\text{in}} - J_{\text{pm}},
\]

\[
\frac{dm_{42}}{dt} = \lambda_{m_{42}} \left( \frac{k_{42}^3}{e_b + k_{42}^3} - m_{42} \right),
\]

where \( D \) is given by Eq. 26.
Inclusion of calcium buffers

To check the effect of calcium buffers on oscillation frequency, we introduce a stationary buffer (no buffer diffusion), as mobile buffers are too complicated to be included in the current deterministic model. Since we have two different cytoplasmic Ca\(^{2+}\) concentrations, \(c\) and \(c_b\), two pools of buffer with the same kinetics should be considered. Hence, the inclusion of a stationary calcium buffer is modeled by the following system,

\[
\frac{dc}{dt} = J_{\text{diff}} + J_{\text{leak}} - J_{\text{sera}} + J_{\text{in}} - J_{\text{p1}} - k_+ (B_t - b) c + k_- b_1, \tag{31}
\]

\[
\frac{dc_b}{dt} = \gamma_1 (J_{\text{IPR}} - J_{\text{diff}}) - k_+ (B_t - b_2) c_b + k_- b_2, \tag{32}
\]

\[
\frac{dc_1}{dt} = J_{\text{in}} - J_{\text{p1}}, \tag{33}
\]

\[
\frac{db_1}{dt} = k_+ (B_t - b_1) c - k_- b_1, \tag{34}
\]

\[
\frac{db_2}{dt} = k_+ (B_t - b_2) c_b - k_- b_2, \tag{35}
\]

where \(b (b_1 \text{ and } b_2)\) and \(B_t\) represent the concentrations of Ca\(^{2+}\)-bound buffer and total buffer respectively. \(k_+ \text{ and } k_-\) are the rates of Ca\(^{2+}\)-binding and Ca\(^{2+}\)-dissociation, indicating how fast the time scale of the buffer dynamics is. Fast buffer refers to the buffer with relatively large \(k_+\). In the simulations, we use a fast buffer with \(k_+ = 100 \mu \text{M}^{-1} \text{s}^{-1}\) and \(k_- = 100 \text{ s}^{-1}\) and vary \(B_t\) to test if the stochastic model and the deterministic model have a qualitatively similar \(B_t\)-dependency. Results are given in Fig. 6E.

Numerical methods and tools for deterministic and stochastic simulations

For the stochastic model, Eqs. 1–3 and ODEs of the four gating variables in the IP\(_3\)R model are solved by the fourth-order Runge-Kutta method (RK4) and the stochastic states of IP\(_3\)R determined by the IP\(_3\)R model are solved by using a hybrid Gillespie method with adaptive timing [37]. The maximum time step size is set to be either \(10^{-8}\) s (for the 6-state IP\(_3\)R model) or \(10^{-3}\) s (for the reduced 2-state IP\(_3\)R model). All the computations are done with MATLAB (The MathWorks, Natick, MA) and the codes are provided in Supporting information (Text S1–S2). For the MATLAB (The MathWorks, Natick, MA) and the codes are provided in Supporting information (Text S1–S2). For the deterministic model, we use ode15s, an ODE solver in MATLAB.

Statistical analysis

Data analysis is performed on the Ca\(^{2+}\) traces with relatively stable baselines and less noise. A moving average of every 3 data points is used to improve the data by smoothing out short-term fluctuations (Fig. 2A is an improved result). Due to large variations in baseline, amplitude, and level of noise in data, we used two thresholds to get samples: a low threshold, 20\% of the amplitude of the largest spike above the baseline, to initially filter baseline noise out; and a relatively high threshold, 50\% of the amplitude of the largest spike above the baseline, to further remove small spikes that cannot initiate waves. For simulated stochastic traces of variable \(c\), we first convert it to fluorescence ratio \((F/F_0)\) by using \(F/F_0 = c(c_0 + K_d)/(c_0 c + c_0 K_d)\) where the dissociation constant of Oregon Green \(K_d = 0.17 \mu \text{M}\) and resting \([\text{Ca}^{2+}]_0 = 0.1 \mu \text{M}\). We then used the same sampling procedure mentioned above to obtain samples. After samples are chosen, ISIs and spike durations are calculated based on the low threshold. Simulated traces used to calculate average frequency are about 200–400 seconds long. All the samplings and linear least-squares fittings are implemented using MATLAB (see Text S3–S4 for Matlab codes).

Supporting Information

Dataset S1 ASMC calcium fluorescence trace data. The data files are in Excel format and compressed in a zip file. Each Excel file has a name showing their information. For example, “S2_SM6C_MCh200nM” means data are from ASMC No. 6 in lung slice No. 2 by using 200 nM MCh. In each file, there are four columns which represent (from left to right) time(s), fluorescence intensity, \(F/F_0\) and average \(F/F_0\). (ZIP)

Text S1 Matlab code for simulation using 6 state IP\(_3\)R model. (DOCX)

Text S2 Matlab code for simulation using 2 state IP\(_3\)R model. (DOCX)

Text S3 Matlab code for experimental data analysis. (DOCX)

Text S4 Matlab code for simulation analysis. (DOCX)

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Author Contributions

Conceived and designed the experiments: JS GD MJS. Performed the experiments: XT. Analyzed the data: PC. Contributed to the writing of the manuscript: PC JS. Perceived the model: PC JS GD. Performed the simulations: PC.

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