Oscillations in the concentration of free cytosolic Ca\(^{2+}\) are an important and ubiquitous control mechanism in many cell types. It is thus correspondingly important to understand the mechanisms that underlie the control of these oscillations and how their period is determined. We show that Class I Ca\(^{2+}\) oscillations (i.e., oscillations that can occur at a constant concentration of inositol trisphosphate) have a common dynamical structure, irrespective of the oscillation period. This commonality allows the construction of a simple canonical model that incorporates this underlying dynamical behavior. Predictions from the model are tested, and confirmed, in three different cell types, with oscillation periods ranging over an order of magnitude. The model also predicts that Ca\(^{2+}\) oscillation period can be controlled by modulation of the rate of activation by Ca\(^{2+}\) of the inositol trisphosphate receptor. Preliminary experimental evidence consistent with this hypothesis is presented. Our canonical model has a structure similar to, but not identical to, the classic FitzHugh–Nagumo model. The characterization of variables by speed of evolution, as either fast or slow variables, changes over the course of a typical oscillation, leading to a model without globally defined fast and slow variables.

Significance

Oscillations in the concentration of free cytosolic calcium are an important control mechanism in many cell types. However, we still have little understanding of how some cells can exhibit calcium oscillations with a period of less than a second, whereas other cells have oscillations with a period of hundreds of seconds. Here, we show that one common type of calcium oscillation has a dynamic structure that is independent of the period. We thus hypothesize that cells control their oscillation period by varying the rate at which their critical internal variables move around this common dynamic structure and that this rate can be controlled by the rate at which calcium activates calcium release from the endoplasmic/sarco/endoplasmic reticulum.

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Data deposition: The Matlab figure file corresponding to Fig. 1 B, D, and F can be downloaded from https://figshare.com/87662da83a88b547d6d8, and can be used by readers with Matlab to view Fig. 1 interactively.

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physiological mechanism for how cells might generate oscillations of different periods, based on this dynamical structure.

Some early models of Ca\(^{2+}\) oscillations (14–16) were based on the assumption that IPRs are quickly activated by Ca\(^{2+}\) and more slowly inhibited by Ca\(^{2+}\). Thus, in response to a step increase in [Ca\(^{2+}\)], the open probability of the IPRs will first increase quickly (in a fast positive feedback process), and then, more slowly, decrease to a plateau (in a slower negative feedback process). As a consequence, the IPRs open and close in a periodic manner, leading to Ca\(^{2+}\) oscillations that can occur for a constant [IP\(_3\)]. Such models are called Class I models (17), and oscillations like this are known to occur in a range of cell types. Class II oscillations, in which [IP\(_3\)] and Ca\(^{2+}\) necessarily both vary (17–20) are not considered here, and neither are Ca\(^{2+}\) oscillations that are controlled primarily by Ca\(^{2+}\) influx from outside (21–25).

Subsequent detailed models of the IPR (26–30) have shown that, although the IPR exhibits fast positive feedback and slower negative feedback, the negative feedback is not the result of Ca\(^{2+}\) binding slowly to an inactivating binding site on the IPR. Instead, Ca\(^{2+}\) modulates the rate at which the IPR is activated by Ca\(^{2+}\) and does so in a time-dependent manner. The resultant behavior is qualitatively unchanged, with Ca\(^{2+}\) oscillations that occur at a constant [IP\(_3\)], but the biophysical mechanisms are quite different.

The qualitative similarities between older and more recent models suggest that a simple model might be sufficient to capture the important dynamical structures that underlie Ca\(^{2+}\) oscillations, at least in some cases. Thus, we construct a simplified model of Ca\(^{2+}\) oscillations (called the “canonical model”) and show how this model captures the essential dynamical behavior of Class I Ca\(^{2+}\) oscillations.

Predictions from the model are tested and confirmed experimentally in three different cell types, exhibiting oscillations with periods that vary over more than an order of magnitude. In addition, our model predicts that the rate at which IPR activation responds to changes in [Ca\(^{2+}\)] is a crucial parameter for controlling the period of Ca\(^{2+}\) oscillations. We provide indirect evidence consistent with this hypothesis, which remains an open question.

**Results**

The model is briefly described in Materials and Methods, with full details given in Supporting Information. Most importantly, the model contains a faster activation process and a slower inactivation process, but both of these processes operate on the rate of activation of the IPRs.

The model reproduces the following experimental results:

i) As [IP\(_3\)], \(p\), increases, a stable limit cycle first appears and then disappears. When these oscillations exist, they do so for constant \(p\).

ii) The oscillation frequency increases as \(p\) increases or as the Ca\(^{2+}\) influx increases.

iii) Ca\(^{2+}\) influx influences the frequency but is not necessary for the existence of oscillations.

**Model Predictions.** To determine whether the underlying dynamical structure can be uncovered experimentally, we use the model to predict the response of an oscillation to exogenous pulses of IP\(_3\).

The model response depends critically on the timing of the perturbation relative to the phase of the unperturbed oscillation. To verify this assertion, we compare different model responses with part of a 3D version of the bifurcation diagram, constructed by plotting \(c\) and \(h\) coordinates for equilibrium and periodic solutions and using \(p\) as the bifurcation parameter (Fig. 1). A feature of particular importance is the surface of unstable periodic solutions that, near the upper Hopf bifurcation, takes the shape of the bell of a trumpet; we call this structure a “Hopf trumpet.”

There are three qualitatively different responses to a pulse of IP\(_3\):

i) If the pulse occurs between oscillation peaks, the model shows an immediate spike followed by a rise in oscillation frequency (Fig. 1 A and B). This result has previously been shown in airway smooth muscle cells (ASMCs) (17). In this case, the pulse has carried the trajectory to a position, with a relatively large value of \(c\), from which it moves quickly toward the upper part of the surface of attracting periodic solutions. The trajectory thus continues oscillating; because the oscillation frequency is an increasing function of \(p\), the frequency is initially larger.

ii) If the pulse occurs close to the lower portions of the decreasing phase of the spike, the trajectory is carried to a position, with relatively small \(c\), from which it moves quickly to the lower part of the surface of attracting periodic solutions. In this region, \(h\) is changing slowly and the trajectory must move slowly along the surface before it can next spike (Fig. 1 C and D), which results in a delay before the next peak, but the oscillations still reappear with an increased frequency.

iii) If the pulse occurs slightly higher up the downstream of the spike, the trajectory is carried to a position from which it moves quickly toward the branch of stable steady states, resulting in small fluctuations that subsequently are
attenuated to a decreasing plateau. Once \( p \) has decreased far enough, the trajectory crosses the subcritical Hopf bifurcation and large-amplitude oscillations reappear.

Whether or not the pulsed orbit initially moves toward the surface of attracting periodic solutions or toward the curve of stable steady states depends on the orbit’s position relative to a separating manifold which lies close to the Hopf trumpet. We discuss this manifold further in Discussion but, for now, use the Hopf trumpet as a convenient approximation to the true separating manifold.

Note that there is no general theoretical relationship between the phase of the pulse and whether or not the pulse takes the trajectory into the interior of the Hopf trumpet. The effect of the pulse depends crucially on the exact spatial relationship between the unperturbed oscillation and the trumpet. Furthermore, the pulse response will depend on the magnitude of the pulse. A large enough pulse will always take the trajectory beyond the high \( p \) end of the Hopf trumpet, leading to oscillations on a raised baseline. However, these responses to large perturbations are independent of the phase of the pulse. If oscillations on a raised baseline occur only for some pulses, and depend on the phase of the pulse, then this indicates the presence of a Hopf trumpet. The model also predicts the pulse responses as a function of phase and amplitude of the pulse. However, the difficulty of determining the exact amount of IP\(_3\) released by the flash, combined with the difficulty of doing pulses at a precise phase, makes these predictions more difficult to test.

**Experimental Tests of the Predictions.** We tested these predictions in three cell types exhibiting Ca\(^{2+}\) oscillations: HSY cells (a cell line derived from human parotid epithelial cells), DT40-3KO cells (a cell line derived from chicken lymphocytes), and ASMC. In all three cell types, the Ca\(^{2+}\) oscillations occurred in the absence of Ca\(^{2+}\) influx, with a frequency that is an increasing function of agonist stimulation and, thus, presumably, of \( p \). IP\(_3\) was released by flash photolysis across multiple oscillating cells.

In HSY cells, the IP\(_3\) pulse results in three distinct types of responses. When the pulse occurs between Ca\(^{2+}\) spikes the result is an immediate spike in \( c \) followed by oscillations with increased frequency (Fig. 2A). When the pulse occurs on the downward phase of the spike, there is a short delay before the spikes resume with an increased frequency (Fig. 2B). When the pulse occurs close to the peak of a spike the response is an oscillation on a raised but decreasing plateau (Fig. 2C).

In ASMCs, an IP\(_3\) pulse between oscillation peaks gives an immediate increase in oscillation frequency (Fig. 3A), a pulse on the downward phase of the oscillation gives a short delay before oscillations resume with higher frequency (Fig. 3B), and a pulse close to the peak of a spike causes an oscillation on a raised but decreasing plateau (Fig. 3C). This third behavior is less obvious in ASMCs than in the other two cell types. The principal difficulty is that the oscillations in ASMCs are an order of magnitude faster than those in HSY or DT40 cells, making it difficult to ensure the pulse is correctly timed or short enough compared with the oscillation frequency.

In DT40-3KO cells, transfected with and stably expressing type II IPRs, all three behaviors can be clearly seen. Fig. 4A shows the increased frequency in response to a pulse of IP\(_3\) applied between Ca\(^{2+}\) spikes, Fig. 4B shows the response when the pulse occurs on the downward stroke of the spike, and Fig. 4C shows the response when the pulse occurs right on the peak of the oscillation. The small increases immediately after the pulse in the responses in Fig. 4B (indicated by * in the figure) are due to a small signal contamination, from the responses of closely neighboring cells, in the region of interest (ROI). This portion of the signal demonstrates that the IP\(_3\) pulse elicits an immediate response in the neighboring cells but not in the cell under direct observation, because the pulse occurred during the downward phase of the spike.

**Discussion**

We have shown that three different cell types, with oscillation periods ranging from seconds to minutes, all respond to pulses of IP\(_3\) in a manner that is predicted by the geometric structure shown in Fig. 1. In ASMCs, the oscillation moves around this structure quickly, giving an oscillation period of a few seconds,
whereas in HSY and DT40 cells, the movement around the same structure is slower, giving a period of over a minute.

In all of these cell types, the Ca^{2+} oscillations are of Class I, that is, they are oscillations that can occur at a constant [IP_{3}] and in the absence of Ca^{2+} influx. The model applies only to oscillations of this type and makes no statement about models that depend on simultaneous oscillations of Ca^{2+} and IP_{3} or those that depend on Ca^{2+} influx.

We therefore conclude that the simple canonical model presented here captures the underlying dynamic properties of Class I Ca^{2+} oscillators, irrespective of their period. In this sense, we claim that our canonical model can be used as a “FitzHugh-Nagumo (FHN)-like” model of Class I Ca^{2+} oscillations.

However, although we know that the Hopf trumpet must have some component that projects to the p axis (because, otherwise, the model would not reproduce the correct oscillatory behavior as p increases), curvature of the Hopf trumpet, or its angle with the axes, may affect the response to a pulse. Thus, the canonical model will not, in general, produce all possible qualitative behaviors of a more complex model.

In addition, the Hopf trumpet retains predictive power only when the variable p is slow. Preliminary computations indicate that the manifold separating orbits with qualitatively different behavior (in the full 3D system, as shown in Fig. 1) is a surface lying near the surface of unstable periodic solutions (in the reduced 2D system). When p is slow, this manifold is well approximated by the Hopf trumpet, and consideration of the relative positions of the trumpet and the pulsed orbit give good predictions. However, as p becomes faster, the manifold and the Hopf trumpet diverge, and predictions based on the location of the trumpet may not be accurate.

The fact that the underlying dynamical structure of the canonical model appears to agree with observations in three different cell types tells us nothing about whether or not our actual model assumptions are correct. For example, it is likely there are many different models, each with different expression for the sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) pumps or different expression for the IPRs, that all exhibit a Hopf trumpet and would make similar predictions. Thus, we wish also to find out what are the possible biophysical mechanisms that might be used to generate oscillations of widely different periods, all with the same dynamical structure.

Model computations show that by changing only τ_{max}, which controls the rate at which β (the rate of IPR activation by Ca^{2+}) responds to changes in [Ca^{2+}], the oscillation period can be made to vary over at least an order of magnitude (Supporting Information). Thus, the model predicts that cells with Class I Ca^{2+} oscillations can control the oscillation period by modulating (among other things) how fast the rate of Ca^{2+} activation of the IPR responds to changes in [Ca^{2+}].

We have only indirect evidence that the oscillation period is controlled by such a mechanism. We have previously reported (31, 32) that type I IPRs can be assembled from cDNA encoding two complementary peptide chains that correspond to the fragmentation products produced by the intracellular proteases caspase and calpain. Calpain cleavage occurs toward the C terminus of the IPR, resulting in an N-terminal fragment consisting of much of the cytosolic fraction of the protein (amino acids 1–1917) and a C-terminal fragment containing the channel pore (amino acids 1918–2749). Remarkably, these channels, in which peptide continuity has obviously been interrupted, are functional in terms of IP_{3}-gated Ca^{2+} release (31, 32). Nevertheless, as shown in Fig. 5A, channel function is markedly altered by fragmentation. DT40-3KO cells stably expressing type I IPRs exhibit very little ability to support Ca^{2+} oscillations in response to B-cell receptor crosslinking with anti-IgM antibody (31, 33). In contrast, cells expressing the complementary receptor peptides corresponding to calpain cleavage products exhibit robust Ca^{2+} oscillations with periods around 1–2 min (Supporting Information). Because no other change is made to the cell, the assumption is that only the properties of the IPR have been changed by the fragmentation.

This startling result can be qualitatively reproduced by the canonical model if it is assumed that cleavage of the IPR changes nothing but the parameter τ_{max} (Fig. 5B). When τ_{max} is very large, an increase in c causes h to decrease and get trapped at a low value, because the rate at which h can recover (as c recovers to its resting state) is very slow. Thus, the IPR gets trapped in a closed state, and cannot reopen to cause a second Ca^{2+} spike after the first has occurred (also see Materials and Methods). According to our preliminary analysis, it is possible that stable limit cycles still exist for large values of τ_{max} but that their period is so high as to be practically unobservable.

Although it is likely that IPR fragmentation changes a number of aspects of the IPR, the preliminary results in Fig. 5 suggest that...
relatively simple changes in IPR activation kinetics are a possible explanation for the dramatic changes in Ca\(^{2+}\) dynamics caused by fragmentation.

Photoreleased IP\(_3\) degrades more slowly than native IP\(_3\) (34), and thus the effects of the pulse persist long enough to be experimentally observable as an increase in oscillation period. Nevertheless, due to the difficulty of measuring [IP\(_3\)], the exact time course of the [IP\(_3\)] after the pulse is uncertain. The model simply assumes exponential decay of IP\(_3\), at a rate that is consistent with the experimental observations. Thus, the model makes no predictions about how long the oscillation period should remain elevated after the IP\(_3\) pulse.

Although our model is similar in many respects to the FHN model, there is one important difference. In the FHN model, there is a clear separation of time scales between the fast and slow variables. However, in our canonical Ca\(^{2+}\) oscillation model, the time scale, \(\tau\), of the “slow” variable, \(h\), is a decreasing function of \(c\). This feature, which is derived from the previous modeling work of ref. 35, results in a model that gives more realistic Ca\(^{2+}\) spikes than does the traditional FHN model. In this sense, our canonical model is similar to the models of refs. 36 and 37, both of which exhibit a similar time-scale fluidity. We note, however, that the model here, being based on specific Ca\(^{2+}\) transport mechanisms, can make experimentally testable predictions about Ca\(^{2+}\) dynamics, whereas neither the FHN model nor the models of refs. 36 and 37 can, because they are based on quite different underlying biophysical mechanisms.

In our discussion, so far, we have been treating Ca\(^{2+}\) oscillations as deterministic. However, Ca\(^{2+}\) oscillations are inherently stochastic. In stochastic models (13, 18, 38, 39), the period of the interspike interval is set by the probability that enough IPRs will simultaneously release enough Ca\(^{2+}\) to form a global Ca\(^{2+}\) spike. Changes in interspike interval are then determined by the coupling within and between IPR clusters. According to this approach, the difference between the short period of ASMCs and the long period of HSY cells is simply that of diffusive coupling between IPRs and not a result of changes in the rate of Ca\(^{2+}\) inhibition of the IPR. It is unclear how much changes in interspike interval result from purely stochastic mechanisms and how much from deterministic mechanisms. The most likely answer is that changes in both are required. Thus, our model points to an important current change in deterministic behavior, although accepting that changes in spatial structure and IPR coupling is also very likely to be important.

Materials and Methods

Model Construction. We assume a spatially homogeneous cell, with four Ca\(^{2+}\) fluxes: through the IPRs, SERCA pumps, a leak from outside, and plasma membrane Ca\(^{2+}\) ATPases. Each of these fluxes is modeled in a standard way (full details are given in Supporting Information). The IPR model is based on the model of ref. 35. In this model, \(\alpha(c, p)\) and \(\beta(c, p, t)\) are, respectively, the rates of inactivation and activation of the IPR, where \(c\) denotes [Ca\(^{2+}\)] and \(p\) denotes [P]. The open probability, \(P_o\), of the IPR is given by \(P_o = \beta/(\alpha + \beta)\).

In addition, ref. 35 shows that \(\beta = \beta_{\text{max}}(c) \cdot h^{\text{max}}(c)\), where \(\tau_\infty(c) = h_\infty(c) - h,\) and \(\tau_h\) and \(h_\infty\) are decreasing functions of \(c\). In particular, \(\tau_h = \tau_{\text{max}} K_3/c(c) + K_3\).

To understand intuitively how this model behaves, it is helpful to consider the response to a step increase in \(c\). When \(c\) is increased and held fixed, \(h_\infty\) decreases immediately, so \(\beta\) increases immediately, so \(P_o\) increases immediately. More slowly, \(h\) will decrease to \(h_\infty\), thus decreasing \(P_o\). Hence, in response to a step increase in \(c\), \(P_o\) will first increase and then decrease more slowly to a plateau. Note that the decrease in \(P_o\) will occur with rate proportional to \(1/\tau_h\), which increases when \(c\) increases.

The response to a decrease in \(c\) depends on the value of \(\tau_{\text{max}}\). A decrease in \(c\) leads to an immediate decrease in \(h_\infty\) and thus a decrease in \(P_o\). If \(\tau_{\text{max}}\) is not too large, \(h\) then recovers to its high resting value, allowing the IPRs to open in response to a subsequent increase in \(c\). However, if \(\tau_{\text{max}}\) is large enough, \(h\) cannot recover quickly to its resting value and so remains low for a long time, preventing any subsequent increase in \(P_o\).

Hence, when \(\tau_{\text{max}}\) is large enough, a spike in \(c\) can lock the IPR into a closed state; the increase in \(c\) closes the IPR, which is then unable to escape quickly from the closed state, even when \(c\) decreases again. In this way, changes in \(\tau_h\) can effectively control the existence and the period of oscillations (Fig. 5; also see Fig. S3).

Our canonical model has a similar mathematical form to the earlier models of refs. 14–16, in that the open probability is a function of an auxiliary variable, \(h\), that, as \(c\) increases, decreases \(P_o\) on a slow time scale. However, it is important to note that the biophysical interpretation is entirely different. In the earlier models, it was the inactivation of the IPRs that was a slow time-dependent function of \(c\). In contrast, in the model described here, the slow time dependence on \(c\) and subsequent slow decrease in \(P_o\) is controlled by the rate of IPR activation.

Ca\(^{2+}\) Signaling and Flash Photolysis of Caged-IP\(_3\) in ASMCs. Precision cut lung slices (PCLSs) were prepared from female BALB/c mice (8–12 wk) as previously described (7). HBSS was supplemented with 20 mM Hepes buffer (SHBS) and adjusted to pH 7.4. PCLSs were initially incubated with SHBS containing 20 \(\mu\)M Oregon Green 488 1,2-bis(o-aminophenoxo)ethane-N,N,N’,N’-tetraacetic acid tetra(acetylamino) ester, 0.1% Pluronic F-127 and 200 \(\mu\)M sulfobromophthalein (SB) in the dark at \(30^\circ\)C for \(\approx 1\) h, and subsequently incubated with SHBS containing 4 \(\mu\)M caged-IP\(_3\), 0.1% Pluronic F-127 and 200 \(\mu\)M SB for 1 h, followed by de-esterification in HBBS containing 200 \(\mu\)M SB for 30 min. The fluorescence intensity of ASMCs in PCLSs was examined with a custom-built, video-rate scanning confocal microscope. To facilitate the flash photolysis, a pulse of UV light was generated from a mercury arc lamp with a band-pass filter (330 nm) and focused to a point into the custom-built confocal microscope as described before (40). The fluorescence intensity in a ROI (8 \(\times\) 10 pixels) within an ASMC was determined using custom-written software. Relative fluorescence intensity was expressed as \(F/F_0\), a ratio of the fluorescence intensity at a particular time (\(F_{\text{t}}\)) normalized to the initial time (\(F_0\)). Animal maintenance and experimental procedures complied with the requirements of the Animal Welfare Act, US Public Health Service Policy, and NIH guidelines and were approved by the Institutional Animal Care and Use Committee of the University of Massachusetts Medical School.

DT40-3KO Cell Culture and Constructs. DT40-3KO cells, an IPR-null background cell line, were grown in Roswell Park Memorial Institute 1640 medium supplemented with 1% chicken serum, 10% (vol/vol) FBS, 100 units/mL penicillin, 100 \(\mu\)g/mL streptomycin at 39\(^\circ\)C with 5% (vol/vol) CO\(_2\). The method for constructing plasmid expressing calpain fragmented IPR type I was described in ref. 31. In brief, IPR type I cDNA flanked by the Nhel and NotI sites at the 5’ and 3’ ends in pcDNA3.1 were used as templates. A short nucleotide sequence comprising, in the order of, a stop codon, Nhel and NotI restriction sites, a Kozak sequence, and a start codon was inserted downstream of the sequence coding for glutamic acid 1917 by PCR. N- and C-terminal fragments were then extracted by digestions (Nhel and NotI for N-terminal fragment and NotI for C-terminal fragment) and subcloned under two separate promoters in a two-promoter vector. DT40-3KO cells expressing mouse IPR type I in ipr3KO cells were generated by stably transfecting the cells with corresponding constructs using Amaxa nucleofactor (Lonza) followed by selection with 2 mg/mL G418 (Life Technologies).

DT40-3KO Single-Cell Imaging Assay. DT40 cells were first centrifuged at 200 \(\times\)g for 5 min. For Fura-2AM calcium imaging experiments, cells were resuspended in imaging buffer (10 mM Hepes, 5.5 mM glucose, 1.26 mM Ca\(^{2+}\), 137 mM NaCl, 0.56 mM MgCl\(_2\), 4.7 mM KCl, and 1 mM NaHPO\(_4\)) (pH 7.4) containing 2 \(\mu\)M Fura-2AM (tetrabufs) for 20 min. Fura-2 loaded cells were perfused with imaging buffer to establish the recording baseline followed by stimulation with imaging buffer containing anti-IgM (Southern Biotech) to induce IPR activation. Calcium imaging was acquired using a Till Photonics imaging system. For uncaging assay, after centrifugation, cells were resuspended in imaging buffer containing 1 \(\mu\)M Fluo-8 AM (tetrabufs) and 2 \(\mu\)M iso-Ins(1,5)P\(_3\)/P3/P (caged) (enzolife) for 30 min; 50 \(\mu\)M tryanpsin was used to induce calcium response followed by a UV flash to uncage the IP\(_3\) at the indicated time points.

Ca\(^{2+}\) Signaling and Flash Photolysis of Caged-IP\(_3\) in HSY Cells. Media and cell cultures were prepared as previously described (41). IP\(_3\)-injected Ca\(^{2+}\) oscillations were examined with cell-permeable caged IP\(_3\), iso-Ins(1,5)P\(_3\)/P (caged) (Alexeks Biochemicals). In these experiments, cells were incubated with cell-permeable caged IP\(_3\) (2–10 \(\mu\)M) and Fluo-3 (2–3 \(\mu\)M) in HBBS-H containing 1% BSA for 30 min at room temperature. During monitoring Ca\(^{2+}\)
responses with 490-nm light from the Nikon mercury light source, cells were also illuminated with 400-nm light that was derived from U7773 for the photolysis of cell-permeable caged IP$_3$.


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The Model Equations

Let \( c \) denote \([Ca^{2+}]_i\), \( \bar{c} \) denote \([Ca^{2+}]_o\), and \( p \) denote \([IP_3]\). Furthermore, let \( h \) denote a variable that controls the rate at which the IPRs can be activated by \( Ca^{2+} \), as derived by refs. 30 and 35.

The model equations are

\[
\frac{dc}{dt} = J_{IPR}(c, p, h) - J_{serca} + \delta(J_m - J_{pm}), \tag{S1}
\]

\[
\frac{dc}{dt} = \gamma(J_{serca} - J_{IPR}), \tag{S2}
\]

\[
\tau_h(c) \frac{dh}{dt} = h_{\infty}(c) - h, \tag{S3}
\]

where

\[
J_{serca} = \frac{V_p(c^2 - K_{c}^2)}{c^2 + K_p^2}, \tag{S4}
\]

\[
J_m = \alpha_0 + \alpha_1 \frac{K^4_{o}}{K^2_{o} + c^2}, \tag{S5}
\]

\[
J_{pm} = \frac{V_p c^2}{K_{pm}^4 + c^2}. \tag{S6}
\]

These flux expressions are used in many previous modeling studies and are explained in detail in ref. 4. Parameter values are given in Table S1.

The expression for \( J_{IPR} \) is the most complex and important part of the model. In general, \( J_{IPR} = k_p P_o(c_e - c) \), where \( P_o \) is the open probability of the IPR and \( k_p \) is some scaling factor that determines the total IPR flux, and is thus a measure of IPR density (among other things). The IPR model is based on the schematic diagram in Fig. S1, which is itself a simplified version of the modal model of ref. 30, which gives

\[
P_o = \frac{\beta}{\beta + k_p(\beta + \alpha)}, \tag{S7}
\]

where \( k_p \approx \frac{\text{Ca}^{2+}}{2+} \approx 0.4 \). Both \( \beta \) and \( \alpha \) are functions of \( c \) and \( p \):

\[
\alpha = A_1(p) + A_2(p)(1 - \bar{m}_{\alpha}(c)\bar{h}_{\alpha}(c)), \tag{S8}
\]

\[
\beta = B_1(p) + B_2(p)\bar{m}_{\beta}(c)\bar{h}(c, t), \tag{S9}
\]

where \( h \) satisfies Eq. 3.

The details of these functions can be found in refs. 4 and 30. For our purposes, it is important to note that \( A_1 \) and \( A_2 \) are decreasing functions of \( p \), \( B_1 \) and \( B_2 \) are increasing functions of \( p \), and \( \bar{m}_{\alpha} \) and \( \bar{m}_{\beta} \) are increasing functions of \( c \), whereas \( \bar{h}_{\alpha} \) and \( \bar{h}_{\infty} \) are decreasing functions of \( c \). Thus, \( \alpha \) is an inverse bell-shaped function of \( c \), whereas, at steady state, \( \beta \) is a bell-shaped function of \( c \).

Simplifying the Rate Constants

We simplify the expressions for \( \alpha \) and \( \beta \) in an ad hoc fashion, ensuring only that the qualitative dynamic behavior is preserved. Firstly, we ignore \( A_1 \) and \( B_1 \) to get

\[
\alpha = A(p)(1 - \bar{m}_{\alpha}(c)\bar{h}_{\alpha}(c)), \tag{S10}
\]

\[
\beta = B(p)\bar{m}_{\beta}(c)\bar{h}(c, t), \tag{S11}
\]

which makes no change to the qualitative dynamics. Secondly, we assume that

\[
\bar{m}_{\alpha}(c) = \bar{m}_{\beta}(c) = \frac{c^4}{K^4_{1} + c^4}, \tag{S12}
\]

\[
\bar{h}_{\alpha}(c) = h_{\infty}(c) = \frac{K^4_{h}}{K^4_{h} + c^4}, \tag{S13}
\]

\[
1 - A(p) = B(p) = \frac{p^2}{K^2_{p} + p^2}, \tag{S14}
\]

which preserves the overall shape of the functions, using only three parameters to define six functions. Finally, we define

\[
\tau_h = \tau_{max} \frac{K^2_{3}}{K^2_{3} + c^4}. \tag{S15}
\]

The behavior of the model, for the parameters in Table S1, is summarized in the bifurcation diagram of Fig. S2. Oscillations appear and disappear in Hopf bifurcations, as \( p \) increases. The lower Hopf bifurcation leads to a complex set of bifurcations that are not shown here in detail, because they are of limited physiological interest. The upper Hopf bifurcation is subcritical and leads to a branch of unstable limit cycles that turns around in a saddle-node of periodic bifurcations to give a branch of stable limit cycles.

Computing Pulse Responses

To compute the response of the model to a pulse in \( IP_3 \) (Fig. 1), it is necessary to include an additional equation to model the dynamics of \( p \). Thus, pulse responses are computed using the above model equations together with

\[
\frac{dp}{dt} = \tau_p(p_s - p),
\]

where \( p_s \) is the steady value of \( p \) that results from the constant agonist stimulation. The parameter \( \tau_p \) models how quickly \([IP_3]\) decays to steady state after the pulse.

The Period Increases with \( \tau_{max} \)

The period of the oscillatory solution, for a fixed \( p = 0.1 \), is shown as a function of \( \tau_{max} \) in Fig. S3. As \( \tau_{max} \) increases, the period increases by more than an order of magnitude. The exact behavior as \( \tau_{max} \to \infty \), for all values of \( p \), is as yet unknown, although preliminary computations suggest that the oscillations may persist as \( \tau_{max} \to \infty \), with a period that continues to increase. Furthermore, if the oscillations exist, their domain of attraction is not known, and thus it is not yet clear whether or not the model solution shown in the right graph of Fig. 5B corresponds to a periodic orbit with very long period.

More Details of the Experimental Results

HSY Cells. Pulses of \( IP_3 \) were applied in 28 cells, with 3 large flashes in each cell, for a total of 84 responses. (Smaller flashes were also done, but these did not consistently evoke a response, so they have been ignored.) Of these, 25 were either too noisy or there were too few spikes before and after the flash to estimate the period reliably. In addition, 16 gave a response of raised oscillations on a plateau. For the remaining 43 traces, we let \( t_f \) denote the time of the flash, \( t_b \) denote the time of the \( Ca^{2+} \) spike immediately preceding the flash and \( t_a \) denote the time of the first \( Ca^{2+} \) spike immediately after the flash. We let \( T_b \) denote the
oscillation period before the flash and define $\Delta t_b = (t_f - t_b)/T_b$ and $\Delta t_a = (t_a - t_f)/T_b$.

Because $\Delta t_a$ is expected to be a function of $\Delta t_b$, we write

$$\Delta t_a = f(\Delta t_b),$$

for some function $f$, which we can determine by plotting $\Delta t_a$ against $\Delta t_b$.

If the flash has no effect on the oscillation, we know that $f(\Delta t_b) = 1 - \Delta t_b$, so that $t_a - t_b = T_b$ always. In this case, a plot of $\Delta t_a$ against $\Delta t_b$ gives the straight line $\Delta t_a = dt$. In this case, $dt$ can be either small or large, corresponding to, respectively, an immediate response or a delayed response.

The value of this function $f(\Delta t_b)$ was computed in the model for various values of $\Delta t_b$, and the results are shown in Fig. S4A, where it can be seen that $f(\Delta t_b)$ is not necessarily monotonic but generally takes on higher values when $\Delta t_b$ is smaller. This model prediction is consistent with the data (Fig. S4A).

Now let $T_a$ denote the average period of the first three oscillations after the flash, and let $T = T_a/T_b$. A plot of $T$ from the 43 HSY cell responses is shown in Fig. S4B.

**DT40-3KO Cells.** Pulses of IP$_3$ were applied in 57 cells, of which 5 exhibited the response of raised oscillations on a plateau. For each response, $\Delta t_a$ as a function of $\Delta t_b$ was calculated as for HSY cells (Fig. S4A). The periods before and after the flash could be reliably determined (an average of 3 periods) in only 16 responses, and $T$ for those responses was calculated as above (Fig. S4B).

**ASMCs.** A total of 26 IP$_3$ pulses of width either 50 or 100 ms were applied in 8 different cells. (Results from pulses of width 200 or 500 ms were not used in the analysis because the width of the pulse was a significant fraction of the period.) Both $\Delta t_a$ as a function of $\Delta t_b$ (Fig. S4A) and $T$ (Fig. S4B) were calculated for each cell.

In each cell type, the plot of $\Delta t_a$ as a function of $\Delta t_b$ is consistent with the model prediction. Similarly, in each cell type, $T < 1$ on average, as predicted by the model. The reason for the anomalous data point (with $T > 2$) in the DT40-3KO cells is unclear.

Plots of the time traces from each cell type are shown in Figs. S5-S7. From these traces, it can be seen that the three different types of responses, although clear in the model predictions, are somewhat more variable in the cells. For example, in HSY cells, in the right graphs of Fig. S5A and C, the pulse appears to occur just before the peak of the Ca$^{2+}$ spike. However, the responses in Fig. S5A show increased frequency oscillations on a unchanged baseline, whereas the responses in Fig. S5C show increased frequency oscillations on a raised baseline. However, these quantitative discrepancies with the model predictions are not unexpected. The model predicts that timing of the IP$_3$ pulse is critical, and thus very small changes in pulse timing can result in qualitatively different responses. Limited resolution of the experimental recordings, as well as uncertainty about the exact time course of the IP$_3$ release, contributes to the inability to predict the experimental results exactly, for every pulse.

**Cleaved DT40-3KO Cells.** The percentage of oscillatory DT40-3KO cells, with cleaved type I IPRs, compared with normal type I IPRs is shown in Fig. S8. An oscillation is defined as more than five transients in a 10-min recording in response to anti-IgM stimulation. Based on these criteria, the percentages of oscillatory cells for each cell type are IPR type I: 7.8 ± 9.7%; and calpain fragmented IPR type I: 85.6 ± 8.8%.

\[ C_2 \xrightarrow{q_{26}} O_6 \]
\[ \beta \]
\[ \alpha \]
\[ C_4 \]

**Fig. S1.** Schematic diagram of the IPR model of ref. 35, which is based on a simplification of the model of ref. 29. Here, $\beta$ and $\alpha$ are functions of $c$ and $r$, determined by fitting to single-channel data. $\beta$ is also a function of time, via the variable $h$, but the parameters governing $h$ were not determined by fitting to single-channel data. Instead, they were estimated from ref. 42.
Fig. S2. Bifurcation diagram of the model. HB denotes a Hopf bifurcation; black curves are steady states (solid for stable, dashed for unstable); and red curves show the maximum and minimum values of \( c \) on limit cycles (solid for stable, dashed for unstable).

Fig. S3. Oscillation period as a function of \( \tau_{\text{max}} \), plotted at a constant \( p = 0.1 \). As \( \tau_{\text{max}} \) is increased from 1, oscillations appear in a Hopf bifurcation at \( \tau_{\text{max}} = 30.2 \).
Fig. S4. (A) Plots of \((t_a - t_f)/T_b\) against \((t_f - t_b)/T_b\), as defined in the text, for three different cell types, compared with the model prediction. Both the data and the model show a clear decreasing trend, with hyperbolic shape. (B) Plot of \(T = T_p/T_a\), the postflash oscillation period divided by the preflash oscillation period, determined from those cells where it was possible to determine the pre and postflash periods.

Fig. S5. (A–C) Plots of experimental time traces from HSY cells (Fig. 2) redrawn on an expanded time scale, so the positioning of the flash is clearer. The red curve shows the timing of the flash that releases IP\(_3\), and the black curve shows the fluorescence ratio. In A and B, the sharp spike in the black curve, which occurs at exactly the same time as the pulse, is not \([Ca^{2+}]\) but is an artifact of the flash.
Fig. S6. (A–C) Plots of experimental time traces from ASMCs (Fig. 3) redrawn on an expanded time scale, so the positioning of the flash is clearer.

Fig. S7. (A–C) Plots of experimental time traces from DT40-3KO cells (Fig. 4) redrawn on an expanded time scale, so the positioning of the flash is clearer.
Fig. S8.  Percentage of oscillatory DT40-3KO cells with cleaved type I IPRs compared with normal type I IPRs.

Table S1. Parameter values

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