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 PII:
 S0025-5564(22)00112-2

 DOI:
 https://doi.org/10.1016/j.mbs.2022.108923

 Reference:
 MBS 108923

To appear in: Mathematical Biosciences

Please cite this article as: J. Chung, A. Tilūnaitė, D. Ladd et al., IP₃R activity increases propensity of RyR-mediated sparks by elevating dyadic [Ca²⁺], *Mathematical Biosciences* (2022), doi: https://doi.org/10.1016/j.mbs.2022.108923.

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IP₃R activity increases propensity of RyRmediated sparks by elevating dyadic [Ca²⁺]

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Abstract

Calcium (Ca²⁺) plays a critical role in the excitation contraction coupling (ECC) process that mediates the contraction of cardiomyocytes during each heartbeat. While ryanodine receptors (RyRs) are the primary Ca²⁺ channels responsible for generating the cell-wide Ca²⁺ transients during ECC, Ca²⁺ release via inositol 1,4,5-trisphosphate (IP₃) receptors (IP₃Rs) are also reported in cardiomyocytes and to elicit ECC-modulating effects. Recent studies suggest that the localization of IP₃Rs at dyads grant their ability to modify the occurrence of Ca²⁺ sparks (elementary Ca²⁺ release events that constitute cell wide Ca²⁺ releases associated with ECC) which may underlie their modulatory influence on ECC. Here, we aim to uncover the mechanism by which dyad-localized IP_3Rs influence Ca^{2+} spark dynamics. To this end, we developed a mathematical model of the dyad that incorporates the behaviour of IP₃Rs, in addition to RyRs, to reveal the impact of their activity on local Ca²⁺ handling and consequent Ca²⁺ spark occurrence and its properties. Consistent with published experimental data, our model predicts that the propensity for Ca²⁺ spark formation increases in the presence of IP₃R activity. Our simulations support the hypothesis that IP₃Rs elevate Ca²⁺ in the dyad, sensitizing proximal RyRs toward activation and hence Ca²⁺ spark formation. The stochasticity of IP₃R gating is an important aspect of this mechanism. However, dyadic IP₃R activity lowers the Ca²⁺ available in the junctional sarcoplasmic reticulum (JSR) for release, thus resulting in Ca²⁺ sparks with similar durations but lower amplitudes.

Keywords

 Ca^{2+} microdomains, Ca^{2+} sparks, calcium, cardiomyocyte, IP₃R, RyR

Abbreviations

 Ca^{2+} , calcium; $[Ca^{2+}]$, Ca^{2+} concentration; ECC, excitation contraction coupling; AP, action potential; RyR, ryanodine receptor; IP₃, inositol 1,4,5-trisphosphate; IP₃R, IP₃ receptor; IP₃R1, type 1 IP₃R; IP₃R2, type 2 IP₃R; [IP₃], IP₃ concentration; LTCC, L-type Ca^{2+} channel; SR, sarcoplasmic reticulum; JSR, junctional SR; NSR, network SR; CICR, Ca^{2+} -induced Ca^{2+} release; GPCR, G protein-coupled receptor; ET-1, endothelin-1; IICR, IP₃-induced Ca²⁺ release; CaM, calmodulin; TnC, troponin C; CSQ, calsequestrin; SERCA, sarco-endoplasmic reticulum ATPase; 1D, 1-dimensional; FDHM, full duration at half maximum

1 Introduction

Underpinning the heart's pumping action is the concerted contraction and relaxation of individual cardiomyocytes, governed by the excitation-contraction coupling (ECC) process (1). In ventricular cardiomyocytes, ECC is initiated by the depolarisation of the sarcolemma by an action potential (AP), which, through inducing opening of voltage-gated L-type Ca²⁺ channels (LTCCs), permits calcium (Ca²⁺) influx into 10 – 15 nm wide microdomains delimited by T-tubules and the junctional cisternae of the sarcoplasmic reticulum (SR) (**Figure 1**). The Ca²⁺ influx into these microdomains (henceforth dyads) induces a larger Ca²⁺ release from the SR via resident ryanodine receptors (RyRs). This Ca²⁺-induced Ca²⁺ release (CICR) raises the local dyadic Ca²⁺ concentration ([Ca²⁺]), giving rise to elementary Ca²⁺ release events that underlie ECC known as Ca²⁺ sparks (2,3). By virtue of the distribution of T-tubules at \approx 1.8 µm intervals that form dyads throughout the cell volume, the synchronous evocation of Ca²⁺ sparks at dyads by an AP facilitates the transient rise in cell-wide cytosolic Ca²⁺ levels. This Ca²⁺ transient provides sufficient Ca²⁺ to bind to troponin C (TnC) in myofilaments enabling the cross-bridge cycle that contracts the cardiomyocyte (1).

Like RyRs, inositol 1,4,5-trisphosphate (IP₃) receptors (IP₃Rs) are Ca²⁺-regulated Ca²⁺ channels that reside on the SR of cardiomyocytes (4). IP₃Rs also require IP₃ for activation (5). IP₃ is produced following phospholipase C activation and phosphatidylinositol 4,5-bisphosphate hydrolysis downstream of G protein-coupled receptors (GPCRs) as well as certain receptor growth factor receptors (6). Indeed, ventricular cardiomyocytes stimulated by G_q-associated GPCR agonists, such as endothelin-1 (ET-1), lead to IP₃-induced Ca²⁺ release (IICR) via IP₃Rs, which are shown to promote ECC-modulating effects such as arrhythmia and positive inotropy (7–12).

Despite lower expression levels (13) and Ca^{2+} conductance (5) relative to RyRs, IP₃Rs may elicit these ECC-modulating effects by their localization to functionally relevant Ca^{2+} signalling sites in the cell (14). A notable example is the colocalization of IP₃Rs and RyRs at dyads (8,15). It has been recently shown that stimulating the activity of IP₃Rs significantly increases the frequency of dyadic Ca^{2+} spark events (15). In this regard, IICR is hypothesised to elevate Ca^{2+} in the dyad, thereby priming and recruiting otherwise "silent" RyRs for future Ca^{2+} releases (8,11,14,15). The resulting increase in propensity for Ca^{2+} spark formation is then proposed to contribute to the ECC-modulating effects observed (11,16).

Here, we employed computational modelling to simulate the effects of IICR in the dyad. We developed a 1D spatial model of a dyad containing RyRs and type 2 IP₃Rs (IP₃R2). Using this model, we varied the number of IP₃Rs in the dyad and simulated its effect on the local Ca²⁺ dynamics as well as the properties of Ca²⁺ sparks generated. Our model predicts that IP₃R activity increases the baseline dyadic [Ca²⁺] at the expense of that in the junctional SR (JSR). This elevation of dyadic [Ca²⁺] then sensitizes RyRs in the vicinity toward activation, consequently increasing the propensity of Ca²⁺ spark formation. The decrease in JSR Ca²⁺ thus resulted in Ca²⁺ sparks with lower amplitudes but a similar duration.

2 Methods

2.1 Model Formulation

We model the spatiotemporal evolution of [Ca²⁺] as a system of partial differential equations (PDEs) at three interconnected compartments: cytosol, JSR, and network SR (NSR). The spatiotemporal

evolution of $[Ca^{2+}]$ in these compartments is described by the variables $[Ca^{2+}]_c$, $[Ca^{2+}]_{JSR}$, and $[Ca^{2+}]_{NSR}$ respectively. These are shown in order in the equations below.

$$\frac{\partial [Ca^{2+}]_c}{\partial t} = \mathcal{D}_c \frac{\partial^2 [Ca^{2+}]_c}{\partial x^2} + \sum_{i=1}^4 J_{B_i} + J_{RyR} + J_{IP_3R} - J_{SERCA} + J_{Leak}$$
$$\frac{\partial [Ca^{2+}]_{JSR}}{\partial t} = \mathcal{D}_{JSR} \frac{\partial^2 [Ca^{2+}]_{JSR}}{\partial x^2} + J_{B_5} - (J_{RyR} + J_{IP_3R}) + J_{Refill}$$
$$\frac{\partial [Ca^{2+}]_{NSR}}{\partial t} = \mathcal{D}_{NSR} \frac{\partial^2 [Ca^{2+}]_{NSR}}{\partial x^2} + J_{SERCA} - J_{Refill} - J_{Leak}$$

where \mathcal{D}_c , \mathcal{D}_{JSR} , and \mathcal{D}_{NSR} represent the diffusivity of Ca²⁺ in the cytosol, JSR, and NSR compartments, respectively. J_{RyR} and J_{IP_3R} correspond to the Ca²⁺ release fluxes by open RyRs and IP₃Rs respectively. J_{SERCA} corresponds to the Ca²⁺ uptake flux by sarco/endoplasmic reticulum Ca²⁺ ATPase (SERCA). J_{Refill} corresponds to the Ca²⁺ refill flux from the NSR into the JSR compartment. J_{B_i} corresponds to the flux of Ca²⁺ binding to mobile and immobile buffer species *i*.

The reaction diffusion of Ca²⁺ buffers are described by

$$\frac{\partial [CaB_i]}{\partial t} = \mathcal{D}_{B_i} \frac{\partial^2 [CaB_i]}{\partial x^2} - J_{B_i}$$

where $[CaB]_i$ corresponds to the concentration of Ca²⁺-bound buffer species *i*, with $i \in \{1,2,3,4,5\}$ representing buffers ATP, calmodulin (CaM), Fluo-4, troponin C (TnC), and calsequestrin (CSQ) respectively. \mathcal{D}_{B_i} corresponds to the diffusivity of Ca²⁺-bound buffer species *i*. Immobile buffers TnC and CSQ have $\mathcal{D}_{B_4} = \mathcal{D}_{B_5} = 0$.

2.2 Calcium Fluxes

The flux for each buffer species i is given by

$$J_{B_i} = k_{off} [CaB_i] - k_{on} [Ca^{2+}] \left(\left[B_i^{Tot} \right] - [CaB_i] \right)$$

where $[B_i^{Tot}]$ corresponds to the total concentration of buffer species *i*. k_{on} and k_{off} corresponds to the forward and backward reaction rates of buffer species *i* with Ca²⁺ respectively.

The refill flux from the NSR to the JSR compartment is given by

$$J_{refill} = g_{refill} \left([Ca^{2+}]_{NSR} - [Ca^{2+}]_{JSR} \right)$$

where g_{refill} is the refill flux rate. Its value is adjusted to achieve a realistic JSR refill time constant of ~130 ms (17–19) in simulations where the number of IP₃Rs in their element (see **Figure 1**) is 10 as we assume this to be the average number of IP₃Rs in a cluster.

The release fluxes from RyRs and IP₃Rs are given by

$$J_{RyR} = n_{RyR}g_{RyR} ([Ca^{2+}]_{JSR} - [Ca^{2+}]_c)$$

$$J_{IP_3R} = n_{IP_3R}g_{IP_3R} ([Ca^{2+}]_{JSR} - [Ca^{2+}]_c)$$

where n_{RyR} and n_{IP_3R} correspond to the number of open RyRs and IP₃Rs, respectively, whereas g_{RyR} and g_{IP_3R} correspond to the flux rate of RyR and IP₃R release, respectively. The value of g_{RyR} was adjusted to yield a characteristic Ca²⁺ spark profile in the simulation condition where only RyRs are present in the dyad. g_{IP_3R} is set to be 2.85 times lower than g_{RyR} as the Ca²⁺ conductance of IP₃Rs is estimated to be lower than that of RyRs by that factor (5).

Fluxes due to SERCA uptake activity were directly adapted from (20), which takes the form

$$J_{SERCA} = 2v_{cycle}A_p$$

where v_{cycle} is the cycling rate per SERCA molecule and A_p is the cytosolic concentration of SERCA homogenously spread throughout the bulk cytosolic region. The complete expression of each term is provided in Supplementary Materials.

An SR leak flux was also introduced to maintain the cytosolic Ca^{2+} background concentration at 0.1 μ M. We use the same formulation as the SERCA model to balance J_{SERCA} such that $[Ca^{2+}]_c$ does not fall below 0.1 μ M. Therefore, the SR leak flux is expressed as

$$J_{Leak} = J_{SERCA}([Ca^{2+}]_c = 0.1)$$

All parameter values are listed in Supplementary Materials.

2.3 Calcium Channels

RyR and IP₃R Ca²⁺ channels are stochastically simulated in the model. The gating of each RyR is directly adapted from the 2-state RyR model developed in (21); IP₃Rs are modelled after the 6-state Siekmann model (22) that incorporates non-steady state kinetics developed and used in (23,24). Mathematical expressions of the IP₃R model used in (23,24) were parameterised specifically to fit the steady state intermodal transition rates of type 1 IP₃Rs (IP₃R1). IP₃R1 have different channel activities for the same range of [Ca²⁺] compared to IP₃R2, the isoform most expressed in cardiomyocytes (5). To obtain an IP₃R model specific to IP₃R2, we modified the coefficients and exponents of the IP₃R model used in (23,24) to fit the steady state intermodal transition rates of IP₃R2 instead using data obtained from (22). This modification is essential as IP₃R2 has a higher open probability at lower ranges of [Ca²⁺] relative to the IP₃R1 model used in (23,24) (compare **Figure 7**C and **Figure 7**D in Supplementary Materials), thus allowing IP₃R2 to remain active for longer in the conditions of the dyad. Full details are provided in Supplementary Materials. In simulations involving IP₃R2, its gating behaviour was computed at a fixed IP₃ concentration ([IP₃]) of 0.15 µM, similar to that used in (23).

2.4 Model Geometry

The dyad and its surrounding cytoplasmic space are represented on a 1-dimensional (1D) simulation domain of 8 µm length. The 1D simulation domain reflects the portion of a typical experimental confocal line scan taken where a dyad is located. The buffering of Ca^{2+} by mobile buffers ATP, CaM, and the Ca²⁺ indicator dye, Fluo-4 occurs throughout this domain. The domain consists of 200 elements of size 0.04 µm, with the center nine elements (0.36 µm long) representing the dyadic region where RyRs and IP₃Rs are placed (Figure 1). Elements outside the dyadic region represent the bulk cytosol where Ca^{2+} is subject to additional buffering by TnC and sequestration into the NSR compartment by SERCA. In all simulations, the number of RyRs in their specified element is fixed at 15, consistent with the average number of RyRs in a cluster as determined by super resolution microscopy techniques in healthy cardiomyocytes (25–27). Similar data on IP₃R clusters are not yet available. Therefore, the number of IP₃Rs in their specified element is varied between 0, 5, 10, and 20, corresponding to circumstances where there are no, low, intermediate, and high levels of IP₃R expression relative to the number of RyRs. The JSR compartment is designated the same location and number of elements as the dyadic region. Open RyRs and IP₃Rs thus result in a Ca²⁺ flux from the JSR into the dyadic region of the cytosol that is driven by the difference in $[Ca^{2+}]$ between these two compartments. Ca^{2+} in the JSR is subject to buffering by CSQ and refill from the NSR compartment. The non-junctional regions of the NSR compartment are homogenously distributed with SERCA that pumps Ca²⁺ from the bulk cytosol into the SR. SR leak fluxes are likewise present along non-junctional regions of the NSR compartment and leaks Ca^{2+} into the bulk cytosolic region to maintain a baseline $[Ca^{2+}]$ of 0.1 μ M in the cytosol. The aforementioned intercompartmental fluxes connect the compartments elementwise as illustrated in **Figure 1**. A no-flux condition was imposed on all boundaries of the simulation domain.



Figure 1. Schematic diagram of the compartments, fluxes, and arrangement of Ca^{2+} -handling proteins in the dyad and its 1D representation in the model. The dyad is represented on an 8-µm, 1D computational domain with three compartments: cytosol, JSR, and NSR. The center nine elements of the cytosolic compartment represent the dyadic region where RyRs and IP₃Rs are located while the remaining elements represent the bulk cytosol where Ca^{2+} is additionally subject to J_{SERCA} , J_{Leak} and J_{B_4} . Ca^{2+} from the JSR is released into the dyadic region via open RyRs and IP₃Rs and diffuses along the cytosolic compartment, reacting with Ca^{2+} buffers before eventually being sequestered back into the NSR which refills the JSR.

2.5 Calcium Spark Properties

We consider two properties of Ca^{2+} sparks in our results: amplitude and full duration at half maximum (FDHM). These two properties provide a measure of the magnitude and duration of the Ca^{2+} spark respectively. The amplitude of a Ca^{2+} spark is defined as the difference in $[Ca^{2+}]$ from zero to the peak of the Ca^{2+} trace, whereas its FDHM is defined as the duration at which the Ca^{2+} spark exceeds half of its amplitude. The amplitude and FDHM of Ca^{2+} sparks are measured from their Ca^{2+} trace which is taken from the center of the dyad. An example of such a measurement is illustrated in **Figure 2**.





2.6 Numerical Methods and Implementation

The system of PDEs were discretised using the forward time centered space finite difference scheme, similar to (28). The resulting system of ODEs was solved using the explicit Euler method with adaptive time stepping capped at a maximum of 1×10^{-4} ms and a regular spatial resolution of 0.04 µm. Stochastic IP₃R and RyR gating states were solved using a hybrid Gillespie method as described in (29). The time at which any one receptor changes state may determine the time step forward for which the system is solved (adaptive time stepping). Simulations for each IP₃R number condition were repeated 200 times. In all simulations, the model was run for 1000 ms to ensure the system achieves steady state before they were analyzed to obtain the results presented. Recording of the simulations start at \approx 950 ms, an earlier time point than the allocated 1000 ms for the system to achieve steady state. All codes and computations were implemented in MATLAB (The MathWorks Inc., Natick, Massachusetts).

3 Results

3.1 1D model reproduces calcium spark dynamics

The first column of **Figure 3**A illustrates the typical time evolution of $[Ca^{2+}]$ in different compartments of the model during a Ca²⁺ spark in RyR-only simulations i.e., no IP₃Rs. To replicate CICR during ECC that arise following the Ca²⁺ influx via LTCCs, Ca²⁺ sparks were initiated by introducing a 2-ms Ca²⁺ flux, reaching \approx 30 μ M, to elements in the dyadic region where RyRs are placed at the 1000 ms time point. This influx can be observed by the initial rise in $[Ca^{2+}]$ (with no variance) that is taken at the center element of the dyadic region. The resultant initial opening of RyRs occurs rapidly and releases a greater amount of Ca²⁺ from the JSR, thus providing a temporary positive feedback mechanism for the opening of other RyRs via CICR. RyRs open shortly after the initiating Ca²⁺ trigger and peaked at \approx 13 RyRs for \approx 9 ms before closing completely after \approx 16 ms on average, consistent with simulation results from (21) whereby RyRs terminate after \approx 20 ms of activity. During this time, dyadic [Ca²⁺] increased to \approx 300 μ M on average and declined back to \approx 0.1 μ M due to diffusion and chelation by buffers in the cytosol. Meanwhile, JSR [Ca²⁺] declines and reaches its nadir at \approx 300 μ M \approx 13 ms after the initiation trigger, during which point RyRs have already begun closing. These results reinforce the induction decay mechanism of Ca^{2+} spark termination proposed by (30), whereby the decay of the Ca^{2+} flux through RyRs due to JSR depletion retards and eventually impedes inter-RyR regenerative CICR during a Ca²⁺ spark, thereby resulting in its termination. Following spark termination, JSR [Ca²⁺] is gradually replenished by that in the NSR at a time constant of \approx 130 ms, consistent with experimental data (18,19). The JSR refill rate is adjusted to achieve this refill time constant in simulations where 10 IP₃Rs are present as we assume that to be the average number of IP₃Rs in a cluster. Together, our 1D model of the dyad is capable of reproducing Ca^{2+} spark dynamics in reasonable agreement to that reported in other modelling and experimental studies (17–19,21).



Figure 3. Ca^{2+} dynamics associated with LTCC-initiated Ca^{2+} sparks with different numbers of IP₃Rs in the dyad. A: First to fourth row: Time evolution of dyadic [Ca^{2+}] (Notice the subtle progressive decrease in Ca^{2+} spark amplitude, reflected also in the leftmost swarm plot in **B**. Insets show an average baseline dyadic [Ca^{2+}] that increases with the number of IP₃Rs.), time evolution of JSR [Ca^{2+}], number of open RyRs, and number of open IP₃Rs associated with a Ca^{2+} spark. Mean and 95% confidence intervals, illustrated as solid lines and its surrounding shaded region respectively, are obtained from 200 simulations performed for each IP₃R number condition. **B**: Swarm plots showing a decreasing average Ca^{2+} spark amplitude but unchanged FDHM with increasing number of IP₃Rs. Analysis by 1 way ANOVA with Tukey-Kramer post hoc test. Data points are obtained from the same 200 simulations as that in **A**.

3.2 Increased IP₃R2 expression decreases Ca²⁺ spark amplitude and Ca²⁺ stores

Figure 3A illustrates the effect of incorporating an increasing number of IP₃R2 in the dyad. Despite varying the number of IP₃Rs in the dyad, our model is capable of robustly simulating Ca²⁺ spark events. Due to the activity of IP₃Rs, their incorporation into the dyad essentially causes a Ca²⁺ "leak" from the JSR into the dyad. Hence, a 1000-ms wait time was allocated to allow the system to equilibrate to a steady state before simulating any Ca²⁺ release events. This amount of time was sufficient for the system to equilibrate as triggering Ca²⁺ sparks in simulations with longer wait times did not alter the resultant steady state [Ca²⁺]. After equilibration, the average baseline dyadic [Ca²⁺] rose above (insets of first row in **Figure 3**A) while that in the JSR fell below (second row of **Figure 3**A) the model's initial conditions of 0.1 μ M and 1 mM respectively. Moreover, the magnitude of these changes increases with the number of IP₃Rs present in the dyad. We thus attribute these effects to the increased average number of open IP₃Rs (fourth row of **Figure 3**A). Altogether, our results suggest that the presence of IP₃R activity elevates dyadic [Ca²⁺] at the expense of that in the JSR.

To test the effect of dyadic IP₃R activity on Ca²⁺ spark dynamics, we initiated Ca²⁺ sparks in simulations where IP₃Rs are present by similarly introducing a Ca²⁺ flux into RyR-containing elements as described earlier. Generated Ca²⁺ sparks have amplitudes that decrease with increasing number of IP₃Rs (Figure **3**B). This correlates well with a lower JSR $[Ca^{2+}]$ available for release at steady state. However, the duration of these Ca²⁺-triggered Ca²⁺ sparks, measured by its FDHM, is not significantly different (Figure 3B). This result can also be indirectly inferred from the time to complete closing of RyRs and time to nadir of JSR [Ca²⁺] that are not significantly altered with increasing number of IP₃Rs. Mechanistically, the elevated dyadic $[Ca^{2+}]$ together with the lower JSR $[Ca^{2+}]$ at steady state jointly results in RyR Ca²⁺ release fluxes that sustain inter-RyR CICR while depleting the JSR such that the Ca²⁺ spark duration remains unchanged. In all cases, the occurrence of Ca²⁺ sparks coincide with the transient opening of RyRs while the average IP₃R activity remained relatively constant throughout the simulation. This suggests that RyRs, and not IP₃Rs, are primarily responsible for the manifestation of Ca²⁺ sparks, which is consistent with experimental results that show an almost complete loss of Ca²⁺ sparks when RyRs are inhibited (15). Our model also successfully reproduced the experimental observation that JSR Ca²⁺ decreases to the same level after a Ca²⁺ spark event regardless of its initial concentration (19), further bolstering our confidence of this model in simulating Ca²⁺ sparks.

3.3 IP₃Rs increase propensity for spontaneous Ca^{2+} sparks in the dyad

By virtue of elevating dyadic $[Ca^{2+}]$, IP₃Rs may play a role in enhancing the formation of Ca²⁺ sparks (8,14,15). Indeed, cardiomyocytes treated with G_q agonists or IP₃ exhibit an increased number of spontaneous Ca²⁺ spark events, which was attributed to IICR (7,8,31). But the mechanism underlying this observation is not fully resolved. To test whether the colocalization of IP₃Rs with RyRs in the dyad is responsible for the increase in spontaneous Ca²⁺ spark events, we performed simulations in the absence of LTCC initiations such that all Ca²⁺ sparks that are generated occur spontaneously. After a 1000 ms wait time for system equilibration, the model was allowed to run for a further 2000 ms from which our results were obtained. Simulations for each IP₃R number condition were repeated 200 times.

We recorded the number of Ca²⁺ spark events generated from these simulations and their associated properties (amplitude and FDHM). We find that the percentage of simulations with at least 1 Ca²⁺ spark event increases with the number of IP₃Rs (**Figure 4**A). Consistent with triggered Ca²⁺ sparks, the average amplitudes of spontaneously generated Ca²⁺ sparks decrease (Figure 4B) with increasing number of IP₃Rs while their FDHM remain unchanged (Figure 4C). We hypothesise that the increase in spontaneously generated Ca^{2+} sparks is due to the sensitization of RyRs by an elevated dyadic [Ca^{2+}]. To verify that RyRs are more active due to their sensitization by IICR, we also recorded the number of RyR openings that did not develop into full Ca²⁺ sparks (an example of detecting these events is shown in Figure 8 of Supplementary Materials). Expectedly, the average number of RyR openings that do not lead to the formation of Ca²⁺ spark events also increased with the number of IP₃Rs in the dyad (Figure 4D), signifying that RyRs are indeed more active in the presence of more IP₃Rs. This increased number of spontaneous RyR openings raises the probability for Ca²⁺ spark formation and contributes to the decreased JSR [Ca²⁺] at steady state to some degree. Altogether, consistent with experimental data, our model predicts that the presence of dyadic IP₃R activity contributes to an increased occurrence of Ca²⁺ sparks and we attribute this increase in the number of spontaneous Ca²⁺ sparks to the increase in dyadic [Ca²⁺] brought about by IICR.



Figure 4. The number of spontaneous Ca^{2+} sparks increase with the number of IP_3Rs in the dyad. A: Percentage of simulations where at least 1 Ca^{2+} spark event spontaneously occurred. **B**: Swarm plot showing the average amplitude of spontaneous Ca^{2+} spark events decrease with increasing number of IP_3Rs . Analysis by 1 way ANOVA with Tukey-Kramer post hoc test. **C**: Swarm plot showing the FDHM of spontaneous Ca^{2+} sparks that remains unchanged with the number of IP_3Rs . Analysis by 1 way ANOVA with Tukey-Kramer post hoc test. **D**: Average number of RyR opening events that do not lead to a Ca^{2+} spark increases with the number of IP_3Rs . All results presented in this figure are obtained from 200 simulations for each IP_3R number condition.

3.4 Stochastic IP₃R gating behaviour is essential to effectively elicit spontaneous Ca²⁺ sparks

To explicitly correlate the increase in spontaneous Ca^{2+} sparks with dyadic $[Ca^{2+}]$ elevation, we set out to artificially mimic the effect of IP₃R activity in the dyad. As indicated in our previous results (**Figure 3** and **Figure 4**), we expect that a simple elevation of dyadic $[Ca^{2+}]$, consequent of an increased number of IP₃Rs, would increase the occurrence of spontaneous Ca^{2+} sparks. To this end, we first implemented a deterministic constant Ca^{2+} flux at IP₃R-containing elements in the dyadic region that continuously "leaks" Ca^{2+} from the JSR to artificially raise dyadic $[Ca^{2+}]$. The implementation of these Ca^{2+} fluxes is equivalent to specifying a number of IP₃Rs to be constitutively open throughout the time course of the simulation. To further illustrate the incremental effect of this constant dyadic $[Ca^{2+}]$ elevation on spontaneous Ca^{2+} spark events, we specified the equivalent number of constitutively open IP₃Rs to manipulate the magnitude of the constant Ca^{2+} flux such that it qualitatively reflects the average Ca^{2+} dynamics result of our default model configuration for each IP₃R number condition (compare **Figure 3** and **Figure 10** in Supplementary Materials). 200 simulations were performed with this modification in the model. Surprisingly, the number of spontaneous Ca^{2+} spark events generated in this set of simulations were significantly lower than that in **Figure 4** and were insufficient for us to confirm our proposed mechanism (**Figure 11** in Supplementary Materials).

We hypothesized that this disparity arose due to the lack of randomness of the JSR Ca²⁺ "leak" fluxes which were originally provided by the stochastic gating of IP₃Rs. The randomness is associated with larger fluctuations of dyadic [Ca²⁺] which should be more effective at opening RyRs due to its nonlinear sensitivity to [Ca²⁺] (see Section 7.2 of Supplementary Materials). To test this hypothesis, we implemented Ca²⁺ fluxes that randomly occur during the time course of the simulation for a randomly determined time interval in place of the constant deterministic Ca²⁺ fluxes previously described. Here, the incremental effect of an elevated dyadic $[Ca^{2+}]$ was manipulated by adjusting the probability of an equivalent number of IP₃Rs to be open. This probability was likewise adjusted to yield qualitatively similar Ca²⁺ dynamics as that produced by our default model configuration for each IP₃R number condition (compare Figure 3 and Figure 13 in Supplementary Materials). 200 simulations were performed with this modification to the model, keeping all else constant. Remarkably, implementing a randomly occurring Ca²⁺ flux greatly increased the number of spontaneous Ca²⁺ spark events which displayed similar characteristics as those simulated by our default model configuration (compare Figure 4 and Figure 14 in Supplementary Materials). With these two sets of simulations, we not only correlated the increase in propensity of Ca^{2+} spark formation with dyadic [Ca^{2+}] elevation, but also demonstrated the significance of the stochastic nature of IP₃R gating that sporadically elevate dyadic [Ca²⁺] to effectively elicit this outcome.

4 Discussion

While activating IP₃Rs in the cardiomyocyte influences Ca^{2+} handling and ECC (7–12), the mechanistic basis of this observation is not established. Recent evidence suggest that IICR modulates ECC through the localization of IP₃Rs to functionally important Ca^{2+} signalling sites (14), a quintessential example of which are dyads (8,15). Specifically, Ca^{2+} release via IP₃Rs expressed in the dyad is hypothesized to sensitize native RyRs, with which they colocalize, towards activation via IICR (8,14,15). Consequently,

the propensity for RyR opening and the formation of Ca^{2+} sparks (elementary Ca^{2+} release events underlying ECC-associated Ca^{2+} transients) is increased. Using a 1D model of the dyad that incorporate the behaviour of both RyRs and IP₃Rs, we set out to test this hypothesis while uncovering its underlying Ca^{2+} dynamics.

4.1 IP₃R-mediated Ca²⁺ release prime RyRs for release

A notable finding of our simulations is that the probability of spontaneous Ca^{2+} spark events increase with the number of IP₃Rs. We were also able to uncover the mechanism by which this occurs through our recording of $[Ca^{2+}]$ evolution with time at different compartments of the dyad. Our model predicts that while RyRs are almost always in their closed state at baseline $[Ca^{2+}]$ (third row of **Figure 3**A and **Figure 8**C), IP₃Rs exhibit greater activity, as evidenced by the number of open IP₃Rs throughout the simulation time course (fourth row of **Figure 3**A). This difference in behaviour is also correspondingly reflected in their open probability versus cytosolic $[Ca^{2+}]$ curves (32,33). Consequently, the activity of IP₃Rs in the dyad is akin to introducing a Ca^{2+} leak from the JSR into the dyad. Increasing the number of IP₃Rs increases the magnitude of this "leak", as can be seen from a lower average JSR $[Ca^{2+}]$ (second row of **Figure 3**A), due to an increased number of open IP₃Rs on average (fourth row of **Figure 3**A). This "eventless" and SR Ca^{2+} -modulating "leak" due to IICR is consistent with that proposed in (34). The consequent decrease in JSR $[Ca^{2+}]$ led to Ca^{2+} sparks with lower amplitudes. On the other hand, the average baseline dyadic $[Ca^{2+}]$ is increased due to this IICR (insets in first row of **Figure 3**A). This elevation in dyadic $[Ca^{2+}]$ sensitizes RyRs (as seen from an increased RyR activity in **Figure 4**C) thereby increasing the propensity for Ca^{2+} spark formation (**Figure 4**A).

In our efforts to fully elucidate the aforementioned mechanism, we find that an intermittent Ca²⁺ "leak" from the JSR into the dyad, granted by the stochasticity of IP₃R gating, is an essential feature to eliciting the spontaneous Ca²⁺ sparks observed. Our model predicts that an artificial sustained JSR [Ca²⁺] "leak", resulting in a constant dyadic [Ca²⁺] elevation, is less effective at generating spontaneous Ca²⁺ sparks compared to those that are randomly occurring (compare Figure 11 and Figure 14 in Supplementary Materials), such as that brought about by IP₃Rs. Mechanistically, the stochasticity of this "leak" permits some refilling of the JSR prior to an upcoming release, thus generating relatively larger Ca²⁺ fluxes that sporadically elevate dyadic [Ca²⁺] to levels higher than when a constant "leak" flux is present (compare Figure 12 and Figure 15 in Supplementary Materials). The presence of these larger, albeit intermittent, elevations in dyadic [Ca²⁺] increases the probability that a higher number of RyRs are simultaneously activated due to the super-linear dependence of RyR opening probability on [Ca²⁺] (see Section 7.2 of Supplementary Materials), significantly increasing the successful formation of spontaneous Ca²⁺ sparks. Increasing the magnitude of this stochastic "leak" flux expectedly increased the occurrence of spontaneous Ca²⁺ spark events. Altogether, our results support the notion that IICR via IP₃Rs expressed in dyads increases the propensity for RyR-mediated Ca²⁺ spark formation by elevating dyadic [Ca²⁺]. However, the stochasticity of IP₃R gating is key to this outcome.

Our findings have important implications about the wider role of IP₃Rs in cardiomyocytes. As we show that IICR increases the probability of Ca²⁺ spark events by raising dyadic [Ca²⁺], this mechanism may provide a means to activate RyR clusters that are usually "silent" during ECC. This recruitment of RyR clusters can potentially explain the enhanced Ca²⁺ transient amplitude observed in some studies under conditions of IP₃R stimulation (7–11). Indeed, in a recent study in which a dyadic Ca²⁺ reporter was employed, IP₃R activation was found to result in an increase in the number of dyads recruited during ECC (15). In diseased cardiomyocytes, the greater expression of IP₃Rs (8,12) may also suggest a compensatory mechanism for the increased decoupling of RyRs from LTCCs due to T-tubule degradation (35,36) to rescue Ca²⁺ spark formation. However, IICR in dyads could also contribute to increased spontaneous Ca²⁺ release events in cardiomyocytes, which can have arrhythmogenic consequences (7–11). Furthermore, our simulations showing a progressive decrease in JSR [Ca²⁺] with increasing IP₃R numbers (second row of **Figure 3**A) also supports an IP₃R function proposed by (34) where an IP₃R overexpression increase Ca²⁺ leaks that fine tune SR levels, thereby protecting against arrhythmias.

4.2 Model limitations and implications

We developed a 1D spatial model of a dyad that reproduced all major characteristics of a Ca^{2+} spark. This enabled its utilization in conducting a qualitative investigation into the influence of IP₃R activity on the dynamics of Ca^{2+} sparks in the dyad. While computationally less expensive, the reduced order of our model from 3D to 1D requires simplifying assumptions that presents several limitations which we discuss below.

4.2.1 Arbitrary RyR and IP₃R Placement

In our model, we chose to fix the number of RyRs in a cluster at 15 based on recent estimates obtained from super resolution imaging data (25–27). Since similar data on IP₃R clusters is unavailable in the literature, the number of IP₃Rs in a cluster is varied to illustrate the effect of increased IP₃R presence on the same RyR cluster. These clusters are then arbitrarily placed in elements of the dyadic region as shown in **Figure 1**. Results presented throughout this study is based on simulations of the model with this specific arrangement of RyRs and IP₃Rs. However, simulations that were performed with randomly determined placement of RyRs and IP₃Rs in the dyadic region with all else kept constant qualitatively reproduced similar results as that shown in **Figure 2** (see **Figure 16** in Supplementary Materials).

The 1D nature of our model precludes our ability to place each RyR in its own element in 3D space such that it can detect Ca^{2+} that has diffused from other RyRs in the cluster. RyRs and IP₃Rs that belong to the same cluster are placed in one element such that all Ca^{2+} channels in that element are assumed to detect the same dyadic [Ca^{2+}]. Similar assumptions have also been employed in previous modelling studies simulating Ca^{2+} sparks (20,37,38). While we acknowledge that developing models of higher dimensions permits one to incorporate the spatial arrangement of individual RyRs in the dyad, which influences Ca^{2+} spark fidelity (17,27), our reduced-order model is sufficient for our purposes of illustrating the effect of IP₃R activity on Ca^{2+} spark dynamics and derive an underlying mechanism for its increased occurrence in the dyad.

4.2.2 Visualisation of Ca²⁺ Spark Fluorescence

 Ca^{2+} spark characteristics obtained from experiments are derived from the fluorescence measurement of indicator dyes. To corroborate experimental observations with modelling results, modelling studies incorporate the reaction kinetics of the indicator dye to concurrently simulate the fluorescence of the indicator dye along with the underlying change in $[Ca^{2+}]$. Although the reaction kinetics between Ca^{2+} and the indicator dye Fluo-4 was included in our model, we could not reliably corroborate its simulated fluorescence with experimental measurements, which show that Ca^{2+} spark amplitudes (in terms of dye fluorescence) are unchanged when IP₃Rs are stimulated (15).

We find that the rise in dyadic $[Ca^{2+}]$ during a Ca^{2+} spark saturates the indicator dye, resulting in a plateau of the fluorescence trace (see **Figure 9**A in Supplementary Materials). Previous modelling and experimental studies have established that $[Ca^{2+}]$ in microdomains such as dyads can be elevated to levels exceeding 20 times of that in the bulk cytosol during a cell-wide Ca^{2+} release (17,20,21,28,39), which is substantially in excess of the $[Ca^{2+}]$ levels accurately reported by Fluo-4. This potentially explains the plateau of the fluorescence trace during a Ca^{2+} spark. Consequently, any change in $[Ca^{2+}]$ elicited by IP₃ would thus be obscured – our model's prediction of a decreasing Ca^{2+} spark amplitude with increasing IICR may even be experimentally undetectable by dye fluorescence. However, we also partly attribute this saturation to the 1D geometry of our model – restriction of species' diffusion to

one dimension. Hence, our 1D model precludes a realistic visualisation of Ca²⁺ sparks as they would be experimentally observed. We acknowledge this as a limitation of our model.

Nevertheless, the Ca^{2+} dynamics associated with Ca^{2+} sparks simulated by our model (**Figure 3**A) agree with previous experimental and model findings. It is hence well suited for our purposes of investigating the functional interactions between IP₃Rs and RyRs, where knowing the concentrations and dynamics of Ca^{2+} within the dyad are required.

5 Conclusions

By incorporating the behaviour of both RyRs and IP₃Rs in our 1D model of the dyad, we show that the stochastic activity of IP₃Rs elevate dyadic [Ca²⁺], which sensitizes proximal RyRs toward activation. The colocalization of IP₃Rs with RyRs in the dyad thus increases the propensity for RyR-mediated Ca²⁺ sparks which potentially underlies the ECC-modulating effects seen in ventricular cardiomyocytes treated with G_q agonists. In this regard, further work (experimental and modelling) is needed to link our findings of IP₃R-influenced Ca²⁺ spark formation to multiscale whole-cell cardiomyocyte models incorporating IP₃ signalling (40) and Ca²⁺ cycling (41,42) to elucidate its overall impact on global cytosolic Ca²⁺ transient dynamics and ECC (43,44).

Acknowledgements

This research was supported in part by the Australian Government through the Australian Research Council Discovery Projects funding scheme (project DP170101358) to EJC and VR, the Australian Research Council Centre of Excellence in Convergent Bio-Nano Science and Technology (project CE140100036) to EJC, the KU Leuven Global PhD Partnerships with The University of Melbourne Grant (GPUM/21/036) to VR and HLR, and The University of Melbourne's Research Computing Services and the Petascale Campus Initiative. HLR wishes to acknowledge financial support from the Research Foundation Flanders (FWO) through Project Grant G08861N and Odysseus programme Grant 90663. JC would like to thank Dr Pengxing Cao for the useful discussions on IP₃R modelling. S.T.J. is supported by the Australian Research Council (Project No. DE200100988).

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7 Supplementary Materials

7.1 Parameter Values

Values for every parameter used to simulate the Ca²⁺ reaction diffusion in our model are shown in Table 1 and Table 2. All values are adapted directly from (17) except where otherwise indicated.

Species	Concentration (µM)	Diffusivity, <i>D</i> (μm²/ms)	Forward Reaction Rate, k_{on} (μ M ⁻¹ ms ⁻¹)	Backward Reaction Rate, k _{off} (ms ⁻¹)
$[Ca^{2+}]_{c}$	0.1 (initial)	0.22	-	- 7
$[Ca^{2+}]_{ISR}$	1000 (initial)	0.35 ¹	-	-
$[Ca^{2+}]_{NSR}$	1000 (initial)	0.06	-	-
ATP	455 (total)	0.14	0.225	45
CaM	24 (total)	0.025	0.025	0.238
Fluo-4	100 (total)	0.042	0.0488 ¹	0.0439 ¹
TnC	70 (total)	0	0.039	0.02
CSQ	30000 (total)	0	0.1	63.8



Table 2. Parameter values involved in calculating Ca²⁺-handling protein fluxes and JSR refill.

Ca ²⁺ -Handling Protein	Parameter	Description	Value
RyR	g_{RyR}	RyR Ca ²⁺ release flux rate	2.8 ms ^{-1 2}
IP₃R	g_{IP_3R}	IP ₃ R Ca ²⁺ release flux rate	0.982 ms ^{-1 3}
SERCA	A_p°	SERCA concentration	75 μM ⁴
	K_{D_c}	SERCA sensitivity to $[Ca^{2+}]_c$	910 μM
	$K_{D_{SR}}$	SERCA sensitivity to $[Ca^{2+}]_{NSR}$	2240 μM
JSR	g_{refill}	JSR refill flux rate	0.20 ms ^{-1 5}

7.2 RyR Model

The RyR model used in our simulations is directly adapted from that developed by (21) of the rat. The gating of each RyR is modelled as a 2-state Markov process (**Figure 5**).

$$CLOSE \xleftarrow{k_{open}}{k_{close}} OPEN$$

Figure 5. State diagram of RyR model. Developed by (21), this model of the RyR consists of 2 states, denoted by OPEN and CLOSE, that the RyR transitions between at transition rates k_{open} and k_{close} .

Where the Ca²⁺-dependent transition rates, in ms, between the states, are expressed as,

$$k_{open} = \min(3.17 \times 10^2 \times [Ca^{2+}]_c^{2.8}, 0.7)$$

¹ Value taken from (21)

² Adjusted to give a realistic Ca²⁺ spark profile

³ Value calculated as 2.85 times lower than g_{RyR} as Ca²⁺ conductance of IP₃Rs is estimated to be ~2.85 lower than RyRs (5)

⁴ Value taken from (45)

⁵ Adjusted to give a $[Ca^{2+}]_{ISR}$ exponential recovery time constant of ~130 ms as in (17–19)

$k_{close} = \max(0.25 \times [Ca^{2+}]_c^{-0.5}, 0.9)$

7.3 IP₃R Model

IP₃Rs in our simulations are modelled after that developed by (24) who modified the park-drive model (22) to account for unsteady state kinetics of IP₃Rs when subject to constantly changing concentrations of regulatory ligands (in this case, Ca^{2+}). The gating of each IP₃R is modelled as a 6-state Markov process (**Figure 6**).



Figure 6. State diagram of IP₃R model. Developed by (22), this model of the IP₃R consists of six states that are categorised into two modes of activity: Park and Drive. Park mode is when the channel is at the closed state C_4 or open state O_5 . Drive mode is when the channel is at closed states C_1 , C_2 , C_3 or open state O_6 . State transition rates are denoted by q. Intramodal transition rates are constants whereas intermodal transition rates are dependent on ligand concentration.

Intramodal transition rates are constants whose values are shown in Table 3 below,

Table 3. Constant IP₃R2 transition rates. Values obtained from (22).

IP ₃ R2 State Transition Rate	Value (ms ⁻¹)
q ₁₂	1.14
q_{21}	0.0958
<i>q</i> ₂₃	0.0047
<i>q</i> ₃₂	0.0119
q_{26}	10.100
q_{62}	3.270
q_{45}	0.0041
q_{54}	3.420

Intermodal transition rates q_{24} and q_{42} are ligand-dependent whose expressions are given by,

$$q_{24} = a_{24} + V_{24}(1 - m_{24}h_{24})$$
$$q_{42} = a_{42} + V_{42}m_{42}h_{42}$$

Where variables a, V, m, and h are functions of concentrations of ligands IP₃, $[IP_3]$ and Ca²⁺, $[Ca^{2+}]$ and are given by the following expressions. These expressions take a similar form to that in (23,24).

$$\begin{aligned} a_{24} &= \frac{100}{[IP_3]^{54.5} + 0.923^{54.5}} \\ a_{42} &= 1.0 + \frac{24.5}{[IP_3]^{2.8} + 3.4^{2.8}} \\ V_{24} &= 200.3 + \frac{24.1[IP_3]^{54.9} + 46.8^{54.9}}{[IP_3]^{54.9} + 46.8^{54.9}} \\ V_{42} &= 60.0 + \frac{745.0}{[IP_3]^{8.6} + 1.0^{8.6}} \\ m_{24} &= \frac{[Ca^{2+}]^{n_{24}}}{k_{24}^{n_{24}} + [Ca^{2+}]^{n_{24}}} \\ m_{42} &= \frac{[Ca^{2+}]^{n_{42}}}{k_{-24}^{n_{-24}} + [Ca^{2+}]^{n_{42}}} \\ h_{24} &= \frac{k_{-24}^{n_{-24}}}{k_{-42}^{n_{-24}} + [Ca^{2+}]^{n_{42}}} \\ h_{42} &= \frac{k_{-42}^{n_{-42}}}{k_{-42}^{n_{-42}} + [Ca^{2+}]^{n_{42}}} \\ h_{42} &= \frac{k_{-42}^{n_{-42}}}{k_{-42}^{n_{-42}} + [Ca^{2+}]^{n_{42}}} \\ k_{24} &= 0.0358 \\ k_{42} &= 0.15 \\ n_{24} &= 9.5 \\ n_{42} &= 5.5 \\ k_{-24} &= 15.9 + \frac{774.2}{[IP_3]^{2.7} + 33.0^{2.7}} \\ k_{-42} &= 0.8 + \frac{19000}{[IP_3]^{11.6} + 86.8^{11.6}} \\ n_{-24} &= 1.14 + \frac{1.19[IP_3]^{1.25}}{[IP_3]^{1.25} + 20.7^{1.25}} \\ n_{-42} &= 1.7 + \frac{37.8}{[IP_2]^{15.1} + 1.2^{15.1}} \end{aligned}$$

0

Coefficients and exponents in expressions of the gating variables stated above are determined by fitting the curve of q_{24} and q_{42} to their known steady state data points. These data points (**Figure 7**A) were previously derived from experimental data by (22) and are specific to IP₃R2. We chose to fit our plots of q_{24} and q_{42} to data points obtained at 1 µM and 10 µM [IP₃] and 5 mM [ATP] as there were more data points that we could fit our curves to and also because 5 mM [ATP] was closer to the physiological [ATP] in cardiomyocytes. The resultant fitted curves of q_{24} and q_{42} as a function of [Ca²⁺] and [IP₃] are shown in **Figure 7**A. q_{24} and q_{42} at 0.15 µM [IP₃], the concentration at which IP₃ is fixed in all our simulations, were then extrapolated from these expressions and is shown in **Figure 7**B. The corresponding open probability curves calculated (**Figure 7**C) are comparable to those obtained from experiments (32).



Figure 7. Intermodal transition rates q_{24} and q_{42} vs [Ca²⁺] and open probability curves of IP₃R2. A: q_{24} and q_{42} vs [Ca²⁺] curves of IP₃R- at [IP₃] = 1 and 10 μ M. Curves were obtained by tuning coefficients and exponents in expressions for variables *a*, *V*, *m*, *h*, and *k* to give curves of best fit for experimentally obtained q_{24} and q_{42} data points from (22). **B**: q_{24} and q_{42} vs

 $[Ca^{2+}]$ curves at $[IP_3] = 0.15 \ \mu$ M. These curves were obtained by extrapolating from expressions used to plot the same curves in **A** as no experimental data points were available at this $[IP_3]$. **C**: The corresponding open probability, P_0 , vs $[Ca^{2+}]$ curve of IP_3R2 . **D**: P_0 vs $[Ca^{2+}]$ curve of the IP_3R1 model developed in (23).

To account for IP₃R2 gating behaviour in an environment where $[Ca^{2+}]$ is constantly changing, the nonsteady state kinetics of the Ca²⁺-dependent gating variables were assumed to obey the differential equation of the form below (24),

$$\frac{dG}{dt} = \lambda_G (G_\infty - G)$$

Where, *G* represent the current value of gating variables m_{24} , h_{24} , m_{42} , and h_{42} and G_{∞} represents the value of the same variables at steady state. λ_G is the equilibrium approach rate whose values are given in Table 4.

Table 4. The equilibrium approach rate for all Ca²⁺-dependent gating variables. Values are obtained from (23,24).

Equilibrium Approach Rate	Value (ms ⁻¹)
$\lambda_{m_{24}}$	0.1
$\lambda_{h_{24}}$	0.04
$\lambda_{m_{4,2}}$	0.1
$\lambda_{h_{42}}$	0.1 when IP ₃ R is open, 5×10^{-4} when closed

7.4 SERCA Model

The SERCA model implemented and its parameters were directly adapted from (20) which based it on the simplified thermodynamically realistic model developed by (46). The Ca²⁺ uptake flux by SERCA, J_{SERCA} , is given by

$$J_{SERCA} = 2\nu_{cycle}A_p$$

Where each term is defined as:

$$\begin{aligned} v_{cycle} &= \frac{3.24873 \times 10^{12} K_c^2 + K_c (9.17846 \times 10^6 - 11478.2 K_{SR}) - 0.329904 K_{SR}}{D_{cycle}} \\ D_{cycle} &= 0.104217 + 17.293 K_{SR} + K_c (1.75583 \times 10^6 + 7.61673 \times 10^6 K_{SR}) \\ &+ K_c^2 (6.08462 \times 10^{11} + 4.50544 \times 10^{11} K_{SR}) \end{aligned}$$

$$K_c &= \left(\frac{[Ca^{2+}]_c}{K_{D_c}}\right)^2 \\ K_{SR} &= \left(\frac{[Ca^{2+}]_{NSR}}{K_{D_{SR}}}\right)^2 \end{aligned}$$

 v_{cycle} corresponds to the cycling rate per SERCA molecule while A_p corresponds to the cytosolic concentration of SERCA molecules. K_{D_c} and $K_{D_{SR}}$ are constants quantifying the sensitivity of SERCA activity to $[Ca^{2+}]_c$ and $[Ca^{2+}]_{NSR}$ respectively. Their values are given in Table 2.

7.5 Ca²⁺ Spark Analysis

 Ca^{2+} releases at the dyad are identified as Ca^{2+} sparks when it involves the opening of > 5 RyRs in the dyad. This classification is justified as Ca^{2+} sparks that occur in our simulations typically involve the opening of 12 – 20 RyRs in the dyad. The amplitude and FDHM of Ca^{2+} sparks were then obtained from Ca^{2+} trace of Ca^{2+} spark events using the *findpeaks* function in MATLAB (**Figure 8**B and **Figure 8**C).



Figure 8. Detection and analysis of Ca²⁺ spark events. A: The $[Ca^{2+}]$ equivalent of a line scan image of a Ca²⁺ spark. **B:** Ca²⁺ spark detection and measurement of its amplitude and FDHM. Ca²⁺ sparks detected are denoted by an inverted triangle at its peak $[Ca^{2+}]$. Inset shows how the amplitude and FDHM of a detected Ca²⁺ spark is measured. **C:** Detection of spontaneous RyR openings that do not develop into a full Ca²⁺ spark. Full Ca²⁺ spark events were excluded from this detection.

7.6 Ca²⁺ Spark Fluorescence

Figure 9A shows the simulated fluorescence line scan images and traces from the center of the dyad together with their equivalent $[Ca^{2+}]$ counterpart **Figure 9**B. Due to the 1D nature of our model, we convolved the simulated fluorescence with a 1D Gaussian PSF with a FWHM of 0.41 µm. Notice the plateau in the fluorescence trace of a Ca^{2+} spark that indicates the saturation of the indicator dye (**Figure 9**A). Our simulated fluorescence result shows a similar Ca^{2+} spark amplitude independent of IP₃R activity, which is consistent with experimental data (15). However, we are unable to reliably conclude this as it may be biased by the saturation of the indicator dye.



Figure 9. Ca^{2+} spark fluorescence and its underlying [Ca^{2+}]. A: Line scan images of a Ca^{2+} spark and its fluorescence trace taken at the center of the line scan. B: The [Ca^{2+}] equivalent of a line scan image and its [Ca^{2+}] trace taken at the center of the line scan.

7.7 Mechanism Verification

To verify that the mechanism of the increased propensity of Ca^{2+} spark formation is indeed due to the leak-like function of dyad-localized IP₃Rs that elevates dyadic [Ca^{2+}], we performed simulations with hypothetical Ca^{2+} "leak" fluxes from the JSR to the dyad in place of the IP₃R model. To demonstrate the incremental effect of this "leak", we adjusted its magnitude such that the resulting Ca^{2+} dynamics associated with an LTCC-triggered Ca^{2+} spark is representative of that by our default model (compare **Figure 3** with **Figure 10**A and **Figure 13**A). With this modification to the model, we then performed simulations with no LTCC triggers to show that an increased occurrence of spontaneous Ca^{2+} sparks is correlated to an elevated dyadic [Ca^{2+}], thus verifying this mechanism.

We first performed simulations with a constant Ca^{2+} "leak" flux with the expectation that the number of spontaneous Ca^{2+} spark events would be increased with the magnitude of this "leak". However, the number of spontaneous Ca^{2+} sparks generated was not sufficient for us to draw this conclusion (**Figure 11**). Hence, we repeated the steps detailed above in a subsequent set of simulation with Ca^{2+} fluxes that randomly occur for randomly determined time intervals. This modification recovered the result of our default model configuration (**Figure 14**), demonstrating the importance of stochastic IP₃R gating.



7.7.1 Constant Ca²⁺ Flux



Figure 10. Ca^{2+} dynamics associated with LTCC-initiated Ca^{2+} sparks for simulations with a constant Ca^{2+} "leak" flux at IP₃Rcontaining elements. A: From first to fourth row: Time evolution of dyadic [Ca^{2+}] (Insets show an average baseline dyadic [Ca^{2+}] that increases with the equivalent number of IP₃Rs), time evolution of JSR [Ca^{2+}], the number of open RyRs, and the equivalent number of open IP₃Rs associated with a Ca^{2+} spark. The mean and 95% confidence intervals, shown as solid lines and the surrounding shaded region respectively, are obtained from 200 simulations performed for each IP₃R number condition. B: Swarm plots showing a decreasing average Ca^{2+} spark amplitude but unchanged FDHM with increasing equivalent number of IP₃Rs. Analysis by 1 way ANOVA with Tukey-Kramer post hoc test. Data points were obtained from the same 200 simulations as that in A.





Figure 11. Simulations with constant Ca^{2+} "leak" fluxes from the JSR into the dyad elicited a significantly lower number of spontaneous Ca^{2+} spark events. A: Percentage of simulations where at least 1 Ca^{2+} spark event spontaneously occurred. B: Swarm plot showing the amplitude of spontaneous Ca^{2+} spark events. C: Swarm plot showing the FDHM of spontaneous Ca^{2+} sparks. D: Average number of RyR opening events that do not lead to a Ca^{2+} spark increases with the equivalent number of IP₃Rs. All results presented in this figure were obtained from 200 simulations for each IP₃R number condition. Insufficient Ca^{2+} spark events were generated to reliably perform a statistical analysis.



Figure 12. Dyadic $[Ca^{2+}]$ trace of a simulation that has no Ca^{2+} spark events. Replacing IP₃Rs with a constant JSR Ca^{2+} leak flux leads to a constant dyadic $[Ca^{2+}]$ elevation. Spikes are correlated with eventless RyR openings shown in the lower panel.

7.7.2 Random Ca²⁺ Flux



Figure 13. Ca^{2+} dynamics associated with LTCC-initiated Ca^{2+} sparks for simulations with randomly occurring Ca^{2+} "leak" fluxes at IP₃R-containing elements. A: From first to fourth row: Time evolution of dyadic [Ca^{2+}] (Insets show average baseline

dyadic $[Ca^{2+}]$ that increases with the equivalent number of IP_3Rs), time evolution of JSR $[Ca^{2+}]$, the number of open RyRs, and the equivalent number of open IP_3Rs associated with a Ca^{2+} spark. The mean and 95% confidence intervals, shown as solid lines and the surrounding shaded region respectively, are obtained from 200 simulations performed for each IP_3R number condition. **B**: Swarm plots showing a decreasing average Ca^{2+} spark amplitude but unchanged FDHM with increasing equivalent number of IP_3Rs . Analysis by 1 way ANOVA with Tukey-Kramer post hoc test. Data points were obtained from the same 200 simulations as that in **A**.



Figure 14. Simulations with randomly occurring Ca^{2+} "leak" fluxes from the JSR into the dyad qualitatively reproduced similar results as that with IP₃Rs. A: Percentage of simulations where at least 1 spontaneous Ca^{2+} spark event occurred. B: Swarm plot showing the amplitude of spontaneous Ca^{2+} sparks that decreases with increasing equivalent number of IP₃Rs. Analysis by 1 way ANOVA with Tukey-Kramer post hoc test. C: Swarm plot showing the FDHM of spontaneous Ca^{2+} sparks that remains unchanged with the equivalent number of IP₃Rs. Analysis by 1 way ANOVA with Tukey-Kramer post hoc test. D: Average number of RyR opening events that do not lead to a Ca^{2+} spark increases with the equivalent number of IP₃Rs. All results presented in this figure were obtained from 200 simulations for each IP₃R number condition.



Figure 15. Dyadic $[Ca^{2+}]$ trace of a simulation that has no Ca^{2+} spark events. Replacing IP₃Rs with a randomly occurring JSR Ca^{2+} leak flux leads to sporadic elevation of dyadic $[Ca^{2+}]$ that reaches higher $[Ca^{2+}]$ than that with a constant Ca^{2+} leak flux and is more successful at sensitizing RyRs.

7.8 Model Robustness to Receptor Placement

The main results presented in this study were based on simulations of the model with RyRs and IP₃Rs placed at elements in the dyadic region as shown in **Figure 1**. To test the robustness of these results to changes in receptor placement, we ran similar simulations of the model whereby Ca²⁺ sparks are triggered. But this time the placement of RyRs and IP₃Rs in the dyadic region are randomly determined for each simulation. Results of these simulations are shown in **Figure 16** and are qualitatively similar to that shown in **Figure 3**. Therefore, the results obtained from our model is robust to changes in RyR and IP₃R placement.





Figure 16. Ca^{2+} dynamics associated with LTCC-initiated Ca^{2+} sparks for simulations with randomly determined placement of RyRs and IP₃Rs. A: From first to fourth row: Time evolution of dyadic $[Ca^{2+}]$ (Insets show an average baseline dyadic $[Ca^{2+}]$ that increases with the number of IP₃Rs), time evolution of JSR $[Ca^{2+}]$, the number of open RyRs, and the number of open IP₃Rs associated with a Ca^{2+} spark. The mean and 95% confidence intervals, shown as solid lines and the surrounding shaded region respectively, are obtained from 200 simulations performed for each IP₃R number condition. **B:** Swarm plots showing a decreasing average Ca^{2+} spark amplitude but unchanged FDHM with increasing number of IP₃Rs. Analysis by 1 way ANOVA with Tukey-Kramer post hoc test. Data points were obtained from the same 200 simulations as that in **A**.

Highlights

- IP₃R activity in dyads increases propensity for RyR-mediated Ca²⁺ spark formation.
- IP_3R activity raises dyadic $[Ca^{2+}]$ in the vicinity of RyRs, leading to their sensitization.
- IP₃R-influenced sparks have lower amplitudes but similar duration.

Conflict of Interest

Declarations of interest: none