Caffeine-induced $\text{Ca}^{2+}$ oscillations in type I horizontal cell of carp retina: A mathematical model

Ting Lv, Pu-Ming Zhang, Hai-Qing Gong, and Pei-Ji Liang

School of Biomedical Engineering; Shanghai Jiao Tong University; Shanghai, China

Keywords: $\text{Ca}^{2+}$ oscillations, caffeine, computational model, retinal horizontal cell, ryanodine receptor, store-operated channel

Abbreviations: 2-APB, 2-Aminoethoxydiphenyl borate; $[\text{Ca}^{2+}]_{\text{ER}}$, Free $\text{Ca}^{2+}$ concentration inside the lumen of the ER; $[\text{Ca}^{2+}]_{\text{i}}$, Intracellular free $\text{Ca}^{2+}$ concentration; ANOVA, Analysis of variance; CICR, $\text{Ca}^{2+}$-induced $\text{Ca}^{2+}$ release; ER, Endoplasmic reticulum; H1 HC, Type I horizontal cell; HC, Horizontal cell; L-VGCC, $\text{Ca}^{2+}$ channel; NCX, Na$^+$/Ca$^{2+}$ exchanger; PM, Plasma membrane; PMCA, Plasma membrane $\text{Ca}^{2+}$-ATPase; RyR, Ryanodine receptor; SERCA, Sarco/endoplasmic reticulum $\text{Ca}^{2+}$-ATPase; SNK, Student-Newman-Keuls; SOC, Store-operated channel; SOCE, Store-operated $\text{Ca}^{2+}$ entry; STIM, Stromal interaction molecule; TRP, Transient receptor potential.

Introduction

$[\text{Ca}^{2+}]_{\text{i}}$ oscillations have been observed in a variety of cell types. The present study is focused on caffeine-induced $[\text{Ca}^{2+}]_{\text{i}}$ oscillations in type I horizontal cell (H1 HC) of carp retina. Horizontal cells are interneurons in vertebrate retinas, which receive glutamate input from photoreceptors. In carp H1 HC, $[\text{Ca}^{2+}]_{\text{i}}$ increase is caused primarily by $\text{Ca}^{2+}$ influx from extracellular environment. $\text{Ca}^{2+}$ influx interacts with each other to give rise to $[\text{Ca}^{2+}]_{\text{i}}$ oscillations. However, how these components interact with each other to give rise to $[\text{Ca}^{2+}]_{\text{i}}$ oscillations is unclear.

In the present study, a computational model based on biophysical properties of H1 HC was constructed to explore the underlying mechanism(s) of caffeine-induced $[\text{Ca}^{2+}]_{\text{i}}$ oscillations observed in H1 HC of carp retina. Using this model, caffeine-induced $[\text{Ca}^{2+}]_{\text{i}}$ oscillations were simulated. The concentration-dependent caffeine effect on the pattern of $[\text{Ca}^{2+}]_{\text{i}}$ oscillations was explored, relevant contributions of RyR, SOC, L-type voltage-gated $\text{Ca}^{2+}$ channel (L-VGCC), NCX, PMCA and SERCA for caffeine-induced $[\text{Ca}^{2+}]_{\text{i}}$ oscillations were suggested, and the importance of SOC in caffeine-induced $[\text{Ca}^{2+}]_{\text{i}}$ oscillations was evaluated.

Results

Simulating caffeine-induced $[\text{Ca}^{2+}]_{\text{i}}$ oscillations in isolated H1 HC

Figure 1A–C shows the experimental observations of caffeine-induced $[\text{Ca}^{2+}]_{\text{i}}$ oscillations of 3 representative H1 HCs in
response to 3, 6, 10 mM caffeine with identical caffeine application time of 90 s, respectively.6 Statistical results of the experimental data show that, when the concentration of caffeine was increased from 3 to 10 mM, the time interval between the first and the second Ca2⁺ transients (ΔT₁-₂; Fig. 1D) was decreased from 46.08 ± 3.41 s to 28.37 ± 2.30 s (P < 0.05, ANOVA, post hoc SNK test; Fig. 1E). The amplitude decrement during the Ca2⁺ oscillations was also concentration dependent. To quantify such amplitude decrement, the amplitude of the second transient was normalized against that of the first one (A₂/A₁; Fig. 1D). When caffeine concentration was increased from 3 to 10 mM, the normalized amplitude of the second transient (A₂/A₁) was decreased from 0.82 ± 0.05 to 0.58 ± 0.03 (P < 0.05, ANOVA, post hoc SNK test; Fig. 1F).6

Caffeine-induced [Ca²⁺]i oscillations can be attributed to a number of factors (Fig. 6). Using the model cell, [Ca²⁺] oscillations induced by 3, 6, 10 mM caffeine were simulated (Fig. 2A–C) with parameter values listed in Table 3 and 4. The positive currents reflect Ca²⁺ efflux from cytoplasm to external environment or Ca²⁺ entering the ER.

Caffeine induces ER Ca²⁺ release by increasing the open probability of RyR (Pᵣᵣᵣ), and Pᵣᵣᵣ is increased when the concentration of caffeine is increased.7,8 To simulate the caffeine effect on [Ca²⁺]i, the RyR activation constant (Kₐ₉, in Eq. 10), which reflects the RyR’s sensitivity to Ca²⁺, was adjusted. [Ca²⁺] responses induced by 3, 6, 10 mM caffeine were simulated with Kₐ₉ of 0.16, 0.14, 0.13 μM, respectively. The simulated [Ca²⁺]i curves closely resemble the experimental observations (Fig. 2A–C).

Model output simulating [Ca²⁺]i oscillations induced by 3 mM caffeine is shown in Figure 2A. Upon caffeine (3 mM) application, RyR current is increased immediately, leading to an increase in [Ca²⁺], and a decrease in free Ca²⁺ concentration inside the lumen of the ER ([Ca²⁺]ER). Currents carried by PMCA, NCX, and SERCA are increased accordingly, which bring [Ca²⁺] back to the basal level, forming the first [Ca²⁺] transient (the relative contributions of PMCA and NCX are illustrated in the insets of Fig. 2A). Meanwhile, SOC current is increased slowly upon ER depletion, which leads to a gradual ER refilling. RyR current is increased slowly with the increasing [Ca²⁺]ER, results in an increase in [Ca²⁺], which further potentiates the RyR current. Once sufficient amount of RyRs are activated, Ca²⁺ recycled by ER is again released, forming the subsequent [Ca²⁺] transient. During caffeine application, periodical changes in [Ca²⁺], is concomitant with the periodical activities of the RyR, PMCA, NCX, SERCA, SOC, and L-VGCC. After caffeine removal, each Ca²⁺ process returns to the resting state.

Figure 2B and C show model output induced by 6 and 10 mM caffeine, respectively. During 6 mM and 10 mM

![Figure 1. Concentration-dependent caffeine effects on the caffeine-induced [Ca²⁺]i oscillations. (A–C) [Ca²⁺]i oscillations induced by the application of 3, 6, and 10 mM caffeine (90 s), respectively. Horizontal bars below the traces indicate the periods of caffeine applications. (D) Definition of time interval between the first and the second Ca²⁺ transients (ΔT₁-₂) and the amplitude of the first and the second Ca²⁺ transients. (E) ΔT₁-₂ was decreased with caffeine concentration. (F) A₂/A₁ ratio was decreased with caffeine concentration. Data are presented as mean ± SEM (with sample size in parentheses). *denotes statistical significance of P < 0.05 by one-way ANOVA followed by post hoc SNK test, NS: not significant.6]
caffeine applications, the overall patterns of dynamic changes in \([\text{Ca}^{2+}]_i\), \([\text{Ca}^{2+}]_{\text{ER}}\) and \(\text{Ca}^{2+}\) currents are similar to that during 3 mM caffeine application.

By comparing simulated \([\text{Ca}^{2+}]_i\) curves with 3, 6, and 10 mM caffeine, it is shown that \([\text{Ca}^{2+}]_i\) signal is caffeine concentration dependent. We also calculated the values of \(\Delta T_{1-2}\) and \(A_2/A_1\) with each caffeine concentration (Table 1). Results show that when the concentration of caffeine is increased from 3 mM to 10 mM, the \(\Delta T_{1-2}\) interval is decreased from 56.72 s to 38.46 s, and the \(A_2/A_1\) ratio is decreased from 0.81 to 0.54, both in accordance with the experimental observations.

By examining the simulated \([\text{Ca}^{2+}]_{\text{ER}}\) curves, it is found that following the initial ER \(\text{Ca}^{2+}\) release, ER \(\text{Ca}^{2+}\) release occurs again after \([\text{Ca}^{2+}]_{\text{ER}}\) reaches a certain level. The \([\text{Ca}^{2+}]_{\text{ER}}\) value at which the \(\text{Ca}^{2+}\) release is initiated is denoted by \(\theta_h\), indicating the \([\text{Ca}^{2+}]_{\text{ER}}\) threshold at which ER \(\text{Ca}^{2+}\) release occurs (Fig. 2). By comparing \([\text{Ca}^{2+}]_{\text{ER}}\) curves in Figure 2A–C, it can be noticed that \(\theta_h\) is decreased from 85.43 \(\mu\text{M}\) to 66.74 \(\mu\text{M}\) when caffeine concentration is increased from 3 mM to 10 mM (Table 1).

Caffeine effect at different concentrations is represented by using different \(K_{d,\text{Ca}}\) values, which results in RyR current modulation by influencing \(P_{\text{RyR}}\) (Eq. 10). To further explore the effect of caffeine concentration on caffeine-induced \([\text{Ca}^{2+}]_i\) oscillations, dynamic changes of \(P_{\text{RyR}}\) during caffeine application are plotted together with the corresponding \([\text{Ca}^{2+}]_i\) curves (Fig. 3A–C). It can be observed that after the first \([\text{Ca}^{2+}]_i\) transient, \(P_{\text{RyR}}\) is decreased to a trough (\(P_{\text{trough}}\)) when \([\text{Ca}^{2+}]_i\),

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**Figure 2.** Simulation of caffeine-induced \([\text{Ca}^{2+}]_i\) oscillations using the biophysical model of H1 HC. (A–C) \([\text{Ca}^{2+}]_i\) oscillations induced by 3, 6, 10 mM caffeine (90 s), respectively. Traces shown are the model output of \([\text{Ca}^{2+}]_i\), \([\text{Ca}^{2+}]_{\text{ER}}\) and \(\text{Ca}^{2+}\) currents mediated by RyR, PMCA, NCX, SERCA, SOC, and L-VGCC, respectively. \(\theta_h\) indicates the \([\text{Ca}^{2+}]_{\text{ER}}\) threshold at which ER \(\text{Ca}^{2+}\) release occurs. The insets show dynamic changes of \(I_{\text{NCX}}\) and \(I_{\text{PMCA}}\) during the first \([\text{Ca}^{2+}]_i\) transient induced by caffeine.
returns to the basal level, and a high caffeine concentration corresponds to a large \( P_{\text{trough}} \) value and a quick \( P_{\text{RyR}} \) increase (Fig. 3D). With a large \( P_{\text{RyR}} \), the second \( [\text{Ca}^{2+}]_{i} \) transient can be induce quickly. Therefore, when the concentration of caffeine is increased, the \( \Delta T_{1-2} \) interval is decreased (Table 1). After the initial release, \( [\text{Ca}^{2+}]_{\text{ER}} \) is increased gradually with time, and a fast second \( [\text{Ca}^{2+}]_{i} \) transient is related to a small corresponding \( \theta_{\text{th}} \) value, while a small \( \theta_{\text{th}} \) indicates that only a small amount of ER \( \text{Ca}^{2+} \) can be released, which results in a small \( A_{2} \). Hence the value of \( A_{2} \) is decreased when the caffeine concentration is increased. Simulation results show that \( A_{2} \) is decreased from 1.45 \( \mu \text{M} \) to 0.98 \( \mu \text{M} \) as the concentration of caffeine is increased from 3 mM to 10 mM, while the values of \( A_{1} \) is kept around 1.80 \( \mu \text{M} \). Therefore, the \( A_{2}/A_{1} \) ratio is decreased when caffeine concentration is increased (Table 1). The above results explain the caffeine concentration dependency of \( [\text{Ca}^{2+}]_{i} \) oscillations.

Effect of SOC inhibition on caffeine-induced \( [\text{Ca}^{2+}]_{i} \) oscillations

Experimental data from isolated H1 HCs showed that prolonged application of 3 mM caffeine (7 min) induced sustained \( [\text{Ca}^{2+}]_{i} \) oscillations in H1 HCs (Fig. 4A), and such \( [\text{Ca}^{2+}]_{i} \) oscillations were abolished when 2-APB (SOC inhibitor) was co-applied (Fig. 4B), suggesting that SOCE is necessary for ER refilling.

Using our model, the above experimental results (Fig. 4) are simulated. The effect of 3 mM caffeine is simulated with \( K_{d,Ca} = 0.16 \mu \text{M} \), the effect of 2-APB was simulated by setting \( V_{\text{SOC}} \) (the maximum SOC flux, Eq. 13) to 0. As shown in Figure 5A, prolonged application of caffeine (3 mM, 7 min) induces sustained \( [\text{Ca}^{2+}]_{i} \) oscillations in the model cell, and the oscillation in \( [\text{Ca}^{2+}]_{i} \) is concomitant with the periodical release and uptake of \( \text{Ca}^{2+} \), and SOC current follows the fluctuations of \( [\text{Ca}^{2+}]_{\text{ER}} \). A \( [\text{Ca}^{2+}]_{i} \) transient is induced whenever \( [\text{Ca}^{2+}]_{\text{ER}} \) reaches \( \theta_{\text{th}} \).

### Table 1. The effect of caffeine concentration on caffeine-induced \( [\text{Ca}^{2+}]_{i} \) oscillations

| Caffeine (mM) | \( \Delta T_{1-2} \) (s) | \( A_{1} \) (\( \mu \text{M} \)) | \( A_{2} \) (\( \mu \text{M} \)) | \( A_{2}/A_{1} \) | \( \theta_{\text{th}} \) (\( \mu \text{M} \)) |
|---------------|----------------|----------------|----------------|----------------|----------------|
| 3 mM          | 46.08 ± 3.41 | 56.72          | 1.79           | 1.45           | 0.82 ± 0.05    | 85.43          |
| 6 mM          | 35.40 ± 1.68 | 43.41          | 1.81           | 1.14           | 0.65 ± 0.04    | 73.34          |
| 10 mM         | 28.37 ± 2.30 | 38.46          | 1.82           | 0.98           | 0.58 ± 0.03    | 66.74          |

E: experimental results, data are presented as mean ± SEM; M: model simulation results.
Although $[\text{Ca}^{2+}]_i$ oscillations can be induced by caffeine (3 mM), $[\text{Ca}^{2+}]_\text{ER}$ increase can be immediately stopped when 2-APB is applied. In the presence of 2-APB, SOC is constantly blocked, which abolishes the SOC current (Fig. 5B). Without SOCE, $[\text{Ca}^{2+}]_\text{ER}$ is gradually decreased, unable to reach $\theta_{th}$, hence no further $\text{Ca}^{2+}$-induced $\text{Ca}^{2+}$ release (CICR) is induced and no $[\text{Ca}^{2+}]_i$ transient is generated. This result verifies that SOCE is the main $\text{Ca}^{2+}$ source for ER refilling, and the activation of SOC is required for the maintenance of sustained $[\text{Ca}^{2+}]_i$ oscillations.

**Discussion**

In the present study, we constructed a mathematical model to simulate the caffeine-induced $[\text{Ca}^{2+}]_i$ oscillations observed in type I horizontal cell of carp retina. Simulated $[\text{Ca}^{2+}]_i$ changes closely resemble the experimental observations, the concentration dependence of $\Delta T_{1,2}$ and $A_2/A_1$ of the caffeine-induced $[\text{Ca}^{2+}]_i$ oscillations can be reproduced. Relevant contributions of RyR, SOC, L-VGCC, NCX, PMCA and SERCA for the caffeine-induced $[\text{Ca}^{2+}]_i$ oscillations are suggested. The oscillations in $[\text{Ca}^{2+}]_i$ are concomitant with the periodical changes of RyR, PMCA, NCX, SERCA, SOC, and L-VGCC activities.

Simulated $[\text{Ca}^{2+}]_\text{ER}$ curves in Figures 2 and 5A show that, continuous application of caffeine leads to repeated release/uptake of ER $\text{Ca}^{2+}$. After each ER $\text{Ca}^{2+}$ release, ER is gradually refilled, and subsequent $\text{Ca}^{2+}$ release can be induced only when the $[\text{Ca}^{2+}]_\text{ER}$ level is recovered and reaches a certain threshold $\theta_{th}$. When the concentration of caffeine is increased, the value of $\theta_{th}$ is decreased (Fig. 2, Table 1). The concentration-dependent caffeine effect on $\theta_{th}$ is consistent with the experimental results reported by Kong et al.9 In their study, by recording $[\text{Ca}^{2+}]_\text{ER}$ of HEK-293 cells expressing RyR, it was found that the threshold for $\text{Ca}^{2+}$ release ($\theta_{th}$) was decreased when the concentration of caffeine was increased.

In carp retinal horizontal cell, $\text{Ca}^{2+}$ entering the cytoplasmic is extruded into the extracellular space by both PMCA and NCX.5 From the simulation output (Fig. 2, insets), it can be observed that during caffeine-induced $[\text{Ca}^{2+}]_i$ oscillations, $\text{Ca}^{2+}$ is extruded mainly by PMCA when $[\text{Ca}^{2+}]_i$ is at lower levels; while at higher $[\text{Ca}^{2+}]_i$ levels, NCX-mediated $\text{Ca}^{2+}$ current is larger than the PMCA $\text{Ca}^{2+}$ current. These results confirm the previously reported basic characteristics of PMCA and NCX, i.e., PMCA has higher affinity for $\text{Ca}^{2+}$ than NCX, while NCX has higher turnover rate,10,11 suggesting that PMCA and NCX cooperate to regulate $\text{Ca}^{2+}$ signals over a wide $[\text{Ca}^{2+}]_i$ range in H1 HC.

In addition to regulating the dynamic changes in $[\text{Ca}^{2+}]_i$, PMCA has also been shown to participate in the regulation of resting $\text{Ca}^{2+}$ homeostasis. It was reported that in rod-driven bipolar cell of mouse retina, PMCA was involved in the maintenance of the resting $[\text{Ca}^{2+}]_i$ levels.12,13 For our model cell, after

![Figure 4](https://example.com/f4.png)  **Figure 4.** The 2-APB effect on caffeine-induced $[\text{Ca}^{2+}]_i$ oscillations. (A) $[\text{Ca}^{2+}]_i$ oscillations induced by prolonged application of caffeine (3 mM, 7 min). (B) After $[\text{Ca}^{2+}]_i$ oscillations were induced by caffeine (3 mM), co-application of 2-APB (100 µM) abolished the oscillations immediately.6

![Figure 5](https://example.com/f5.png)  **Figure 5.** Model output simulating the 2-APB effect on caffeine-induced $[\text{Ca}^{2+}]_i$ oscillations. (A) Sustained $[\text{Ca}^{2+}]_i$ oscillations induced by prolonged caffeine (3 mM, 7 min) application. (B) Caffeine (3 mM, 7 min)-induced $[\text{Ca}^{2+}]_i$ oscillations are abolished when 2-APB (100 µM) is applied. Traces shown are simulation output of $[\text{Ca}^{2+}]_i$, $[\text{Ca}^{2+}]_\text{ER}$ and $\text{Ca}^{2+}$ currents mediated by RyR and SOC, respectively. $\theta_{th}$ indicates the $[\text{Ca}^{2+}]_\text{ER}$ threshold at which ER $\text{Ca}^{2+}$ release occurs.
the extracellular stimulus is removed, [Ca^{2+}]_{i} and [Ca^{2+}]_{ER} gradually return to their resting levels, which is independent on the intensity of the previously given stimulation. The levels of [Ca^{2+}]_{i} and [Ca^{2+}]_{ER}, as well as currents carried by different Ca^{2+} components in the resting state are kept within limited ranges (Table 2) until a subsequent stimulus is applied. The simulation results suggest that in the resting state, extrusion of cytoplasmic Ca^{2+} depends mainly on PMCA in H1 HC. It can also be noticed that the SOCE complex is activated at rest (I_{REST}^{SOCE} ≈ 0.4 μA/cm², Table 2). On the plasma membrane, Ca^{2+} extruded from the cell via PMCA is counterbalanced by Ca^{2+} entering the cell through SOC. On the ER membrane, the passive leakage of Ca^{2+} from the ER (I_{REST}^{leak} ≈ -0.5 μA/cm², Table 2) is compensated by SERCA (I_{REST}^{SERCA} ≈ 0.1 μA/cm², Table 2) and SOCE complex-mediated Ca^{2+} uptake. Ca^{2+} homeostasis of the model cell is maintained by the activities of PMCA, SOCE complex, ER passive leak, and SERCA, keeping resting [Ca^{2+}]_{i} and [Ca^{2+}]_{ER} levels at 28 nM and 94 μM, respectively (Table 2). The involvement of the SOCE complex in resting Ca^{2+} homeostasis has been reported in cells such as rat primary cortical neurons, mouse mammary epithelial cells, and HEK293 cells. In these cells, 2 types of STIM proteins, i.e., STIM1 and STIM2, are expressed on the ER membrane. STIM1 triggers SOCE in response to store-depletion, while STIM2 activates Orai1 upon small fluctuations in [Ca^{2+}]_{ER}, keeping resting [Ca^{2+}]_{i} and [Ca^{2+}]_{ER} stable. Therefore, our simulation results suggest that in addition to ER refilling upon stimuli-induced store depletion (Fig. 5), SOC activation in response to minor [Ca^{2+}]_{ER} fluctuation may play a crucial role in maintaining [Ca^{2+}]_{i} and [Ca^{2+}]_{ER} levels in H1 HC at the resting state.

Experimental results from carp H1 HC show that, L-VGCC was activated during caffeine-induced [Ca^{2+}] oscillations. The involvement of L-VGCC suggests that V_{m} of H1 HC should be depolarized upon caffeine application. Our simulation results show that changes in V_{m} are concomitant with changes in

| Symbol   | Value   | Unit  |
|----------|---------|-------|
| I_{REST}^{NCX} | 0.0     | μA/cm²|
| I_{REST}^{PMCA} | 0.4     | μA/cm²|
| I_{REST}^{SOC} | 0.4     | μA/cm²|
| I_{REST}^{leak} | -0.5   | μA/cm²|
| I_{REST}^{SERCA} | 0.1    | μA/cm²|
| I_{REST}^{VGCC} | 0.0     | μA/cm²|
| I_{REST}^{RyR} | 0.0     | μA/cm²|
| I_{REST}^{VGCC} | 0.0     | μA/cm²|
| [Ca^{2+}]_{i}^{REST} | 28     | nM    |
| [Ca^{2+}]_{ER}^{REST} | 94     | μM    |

**Figure 6.** Schematic illustration of Ca^{2+} pathways relevant to caffeine-induced [Ca^{2+}] oscillations in carp retinal HC. L-VGCC: L-type voltage-gated Ca^{2+} channel; NCX: Na^{+}/Ca^{2+} exchanger; PMCA: plasma membrane Ca^{2+}-ATPase; RyR: ryanodine receptor; leak: passive Ca^{2+} leak; SERCA: sarco/endoplasmic reticulum Ca^{2+}-ATPase; ER: endoplasmic reticulum; SOC: store-operated channels; PM: plasma membrane.
[Ca\(^{2+}\)]_i, and L-VGCC current is activated periodically by membrane depolarization. When the L-VGCC current is blocked by setting \(g_{\text{Ca}}\) to zero, [Ca\(^{2+}\)]_i oscillations can still be induced by caffeine (10 mM, 60 s), at the meantime, the A\(_2\)/A\(_1\) ratio and \(\Delta T\) interval are slightly increased as compared with control, showing good agreement with experimental data (data not show). Both the simulation and experimental results show that L-VGCC is involved in the caffeine-induced [Ca\(^{2+}\)]_i oscillations. However, L-VGCC is not the major source of Ca\(^{2+}\) influx during caffeine-induced [Ca\(^{2+}\)]_i oscillations, [Ca\(^{2+}\)]_i oscillations can be induced when L-VGCC is completely blocked.

Our present study provides fundamental insight into the mechanisms of caffeine-induced [Ca\(^{2+}\)]_i oscillations in carp H1 HC, and reveals possible roles of SOC in H1 HC [Ca\(^{2+}\)]_i signaling. Further studies are needed to elucidate the molecular identities and functions of SOC in H1 HC physiology.

**Methods**

Ca\(^{2+}\) imaging data

Experimental data were obtained from isolated type I horizontal cell of carp retina using fluo-3-based Ca\(^{2+}\) imaging technique. All the materials and methods have been previously reported.

Model description

All simulations were carried out using NEURON software. A cylindrical neuron was constructed, with the [Ca\(^{2+}\)]_i dynamics being modeled at the whole-cell level.

The model system is schematically presented in Figure 6, which includes Ca\(^{2+}\) processes on the plasma membrane (PM), the ER, and the store-operated Ca\(^{2+}\) entry (SOCE) complex. Biophysical properties of the neuron membrane are spatially uniform.

The PM processes include the L-VGCC, NCX, and PMCA. L-VGCC mediates Ca\(^{2+}\) entry during membrane depolarization, while NCX and PMCA extrude Ca\(^{2+}\) from the cytosol into the extracellular space.

The ER processes involve 3 parallel components: Ca\(^{2+}\) release from the ER into the cytosol mediated by RyR, passive Ca\(^{2+}\) leakage from the ER into the cytosol, and Ca\(^{2+}\) uptake from the cytosol into the ER through SERCA.

SOCE is a process in which plasma membrane Ca\(^{2+}\) channel (SOC) is activated upon Ca\(^{2+}\) store depletion. The SOCE complex is composed of proteins including STIM, SOC, and SERCA. STIM which resides on the ER membrane senses the ER Ca\(^{2+}\) content, while Orai channels and/or TRP channels form the SOC on the PM. Upon store depletion, STIM and SOC translocate and cluster at the ER-PM junctions, leading to the formation of the STIM-SOC complexes and SOC activation. Activated SOC mediates Ca\(^{2+}\) influx, and SERCA in the ER-PM junctions uptakes these Ca\(^{2+}\) to refill the depleted ER.

Besides, voltage-gated K\(^+\) and Na\(^+\) channel conductance also contribute to the regulation of [Ca\(^{2+}\)]_i, by affecting the neuronal membrane potential, therefore, these processes are also included in our model.

Hence, in our model, Ca\(^{2+}\) dynamics can be described as:

\[
d[\text{Ca}^{2+}]_i = \frac{dh_{\text{Ca}}}{dt} = \frac{f_{\text{Ca}^{2+}}}{2} \cdot \frac{I_{\text{Ca}^{2+}}}{v_{\text{Ca}^{2+}}} + J_{\text{RyR}} + J_{\text{leak}} - J_{\text{SERCA}}
\]

\[
d[\text{Ca}^{2+}]_e = f_{\text{ER}} \cdot (v_{\text{Ca}^{2+}}/v_{\text{ER}}) \cdot (J_{\text{SOC}} + J_{\text{SERCA}} - J_{\text{RyR}} - J_{\text{leak}})
\]

where [Ca\(^{2+}\)]_i is the free intracellular Ca\(^{2+}\) concentration, [Ca\(^{2+}\)]_e is the free Ca\(^{2+}\) concentration inside the ER lumen; \(f_{\text{Ca}^{2+}}\) and \(f_{\text{ER}}\) are Ca\(^{2+}\) buffering coefficients for cytoplasm and ER, respectively; \(v_{\text{Ca}^{2+}}\) and \(v_{\text{ER}}\) are the volumes of the cell (excluding ER) and the ER, respectively; \(F\) is the Faraday constant; \(I_{\text{Ca}^{2+}}\) represents the total Ca\(^{2+}\) currents across the cell membrane; \(J_{\text{RyR}}\) represents ER Ca\(^{2+}\) release via RyR; \(J_{\text{leak}}\) represents the passive Ca\(^{2+}\) leakage from the ER into the cytosol; \(J_{\text{SERCA}}\) represents the Ca\(^{2+}\) uptake from the cytosol into the ER through SERCA; \(J_{\text{SOC}}\) represents Ca\(^{2+}\) entering the ER through the SOCE complex.

The total Ca\(^{2+}\) currents across the plasma membrane are given by:

\[
I_{\text{Ca}^{2+}} = I_{\text{L-VGCC}} + I_{\text{NCX}} + I_{\text{PMCA}}
\]
Current carried by voltage-gated Na\(^+\) channel \((I_{Na})\) can be described as:
\[
I_{Na} = g_{Na} \cdot m_{Na}^3 \cdot h_{Na} \cdot (V_m - E_{Na})
\]
\[
dm_{Na} \over dt = \alpha_{m_{Na}} \cdot (1 - m_{Na}) - \beta_{m_{Na}} \cdot m_{Na}
\]
\[
\alpha_{m_{Na}} = 40 \cdot \exp((80 - V_m)/15) - 1
\]
\[
\beta_{m_{Na}} = 10 \cdot \exp(- (V_m/20))
\]
where \(g_{Na}\) is the maximum conductance of the voltage-gated Na\(^+\) channel; \(m_{Na}\) and \(h_{Na}\) are the activation and inactivation variables, respectively; \(E_{Na}\) is the reversal potential of Na\(^+\) current.

Current carried by delay time rectifying K\(^+\) channel \((I_{k,d})\) can be described as:
\[
I_{k,d} = g_{k,d} \cdot m_{k,d}^3 \cdot h_{k,d} \cdot V_K
\]
\[
V_K = V_m - E_K
\]
\[
dm_{k,d} \over dt = \alpha_{m_{k,d}} \cdot (1 - m_{k,d}) - \beta_{m_{k,d}} \cdot m_{k,d}
\]
\[
\alpha_{m_{k,d}} = 10 \cdot \exp((120 - V_K)/20) - 1
\]
\[
\beta_{m_{k,d}} = 5 \cdot \exp(- (V_K/20))
\]
where \(g_{k,d}\) is the maximum conductance of the delay time rectifying K\(^+\) channel; \(m_{k,d}\) represents the activation variable; \(E_K\) is the reversal potential of K\(^+\) current.

Current carried by outward rectifying K\(^+\) channel \((I_{k,o})\) can be described as:
\[
I_{k,o} = g_{k,o} \cdot m_{k,o}^3 \cdot h_{k,o} \cdot V_K
\]
\[
V_K = V_m - E_K
\]
\[
m_{k,o} = \alpha_{m_{k,o}} \cdot h_{k,o}
\]
\[
\alpha_{m_{k,o}} = 40 \cdot \exp((140 - V_K)/15) - 1
\]
\[
\beta_{m_{k,o}} = 8 \cdot \exp(- (V_K/20))
\]
\[
\alpha_{h_{k,o}} = 0.01 \cdot \exp(- V_K/20)
\]
\[
\beta_{h_{k,o}} = 2 \cdot \exp((30 - V_K)/10) + 1
\]
where \(g_{k,o}\) is the maximum conductance of the outward rectifying K\(^+\) channel; \(m_{k,o}\) and \(h_{k,o}\) are the activation and inactivation variables, respectively.

Current carried by anomalous rectifying K\(^+\) channel \((I_{k,a})\) can be described as:
\[
I_{k,a} = g_{k,a} \cdot m_{k,a}^3 \cdot h_{k,a} \cdot V_K
\]
\[
V_K = V_m - E_K
\]
\[
m_{k,a} = \frac{1}{1 + \exp\left(\frac{V_m - E_k}{30}\right)}
\]
where \(g_{k,a}\) is the maximum conductance of the anomalous rectifying K\(^+\) channel; \(m_{k,a}\) represents the activation variable.

On the plasma membrane, Ca\(^{2+}\) extrusion is mediated by NCX and PMCA. Current carried by NCX \((I_{NCX}^{all})\) can be described as:
\[
I_{NCX}^{all} = K_{NCX} \cdot \left\{ [Na^+]_o \cdot [Ca^{2+}]_i \cdot \exp\left( \frac{r \cdot V_m \cdot F}{RT} \right) \right\}
\]
\[
- [Na^+]_o \cdot [Ca^{2+}]_i \cdot \exp\left( - (1 - r) \cdot V_m \cdot \frac{F}{RT} \right)
\]
where \(K_{NCX}\) is a scaling coefficient; \([Na^+]_o, [Ca^{2+}]_o\) are intracellular Na\(^+\) concentration, extracellular Na\(^+\) concentration and extracellular Ca\(^{2+}\) concentration, respectively; \(R\) and \(T\) are the gas constant and the absolute temperature, respectively. The ion exchange ratio is Na\(^+:Ca^{2+}\) = 3:1, therefore the currents carried by Na\(^+\) and Ca\(^{2+}\) are equal to \(3 \times K_{NCX}^{all}\) and \(-2 \times K_{NCX}^{all}\), respectively.

Ca\(^{2+}\) extrusion mediated by PMCA can be described as:
\[
J_{PMCA} = A_{PMCA} \cdot \frac{[Ca^{2+}]_i}{K_{PMCA} + [Ca^{2+}]_i}
\]
where \(J_{PMCA}\) represents the amount of Ca\(^{2+}\) efflux; \(A_{PMCA}\) is the maximal pumping rate; \(K_{PMCA}\) is the dissociation constant.

RyR, passive ER Ca\(^{2+}\) leakage, and SERCA are involved in Ca\(^{2+}\) exchange between the cytoplasm and the ER. RyR-mediated Ca\(^{2+}\) flux from the ER into the cytosol is described as:
\[
J_{RyR} = K_{RyR} \cdot P_{RyR} \cdot (\left[Ca^{2+}\right]_ER - \left[Ca^{2+}\right]_i)
\]
\[
P_{RyR} = \frac{\left[Ca^{2+}\right]_i^3}{\left[Ca^{2+}\right]_i^3 + K_{A, Ca}^3}
\]
where \(K_{RyR}\) is the rate constant; \(P_{RyR}\) is the open probability of RyR; \(K_{A, Ca}\) is the activation constant which reflects the \([Ca^{2+}]_i\) sensitivity of ICICR.

Passive Ca\(^{2+}\) leakage from the ER into the cytosol is described as:
\[
J_{leak} = K_{leak} \cdot (\left[Ca^{2+}\right]_ER - \left[Ca^{2+}\right]_i)
\]
where \(K_{leak}\) is the rate constant.

ER Ca\(^{2+}\) uptake mediated by SERCA can be described as:
\[
J_{SERCA} = V_{SERCA} \cdot \frac{\left[Ca^{2+}\right]_i}{\left[Ca^{2+}\right]_i + K_{SERCA1}} \cdot \frac{K_{SERCA2}^6 \cdot [Ca^{2+}]_ER + K_{SERCA2}^6}{[Ca^{2+}]_ER + K_{SERCA2}^6}
\]
where $V_{\text{SERCA}}$ is the maximum SERCA flux; $K_{\text{SERCA1}}$ and $K_{\text{SERCA2}}$ are the activation and inactivation constants, respectively.

Upon store depletion, SOCE complex can be formed in the ER-PM junctions. Due to the tight coupling between the SOC and SERCA, the majority of $\text{Ca}^{2+}$ entering the cytoplasm via SOC is pumped into the ER by SERCA.\textsuperscript{29-31} $\text{Ca}^{2+}$ entering the ER through the SOCE complex can thus be described as:\textsuperscript{38}

$$J_{\text{SOC}} = V_{\text{SOC}} \cdot P_{\text{SOC}}$$

$$\frac{dP_{\text{SOC}}}{dt} = \frac{P_{\text{SOC}}^{\infty} - P_{\text{SOC}}}{\tau_{\text{SOC}}}$$

$$P_{\text{SOC}}^{\infty} = \frac{K_{\text{SOC}}^{4}}{K_{\text{SOC}}^{4} + [\text{Ca}^{2+}]_{\text{ER}}^{3}}$$

where $V_{\text{SOC}}$ is the maximum value of the SOC flux, and $P_{\text{SOC}}$ represents the fraction of STIM proteins bound to SOC, i.e., the fraction of activated SOC. $P_{\text{SOC}}^{\infty}$ is the fraction of STIM proteins dissociated from the ER $\text{Ca}^{2+}$. $K_{\text{SOC}}$ is the affinity of STIM for $[\text{Ca}^{2+}]_{\text{ER}}$. $\tau_{\text{SOC}}$ represents the time constant for the adaptation of STIM to $[\text{Ca}^{2+}]_{\text{ER}}$ changes.

| Component parameter | Symbol | Value | Unit | Reference |
|---------------------|--------|-------|------|-----------|
| L-VGCC              |        |       |      |           |
| Half inactivation parameter | $K_{\text{Ca}}$ | 0.30 | µM   |           |
| Time constant       | $\tau_{\text{Ca}}$ | 2.86 | s    |           |
| Maximum conductance | $G_{\text{Ca}}$ | 120.0 | µS/cm² |           |
| PMCA                |        |       |      |           |
| Maximum pumping rate | $A_{\text{PMCA}}$ | 0.052 | pM/cm²/ms |           |
| Dissociation constant | $K_{\text{PMCA}}$ | 7.00e⁻⁴ | mM |           |
| $\text{Na}^{+}/\text{Ca}^{2+}$ exchanger | | | | |
| Scaling coefficient | $K_{\text{NCX}}$ | 0.97 | nA/mmol⁴/cm³ | |
| $r$                 | 0.59   |       |      |           |
| RyR                 | $K_{\text{RyR}}$ | 3.50e⁻³ | ms |          |
| Activation constant | $K_{\text{d,ca}}$ | 1.00 | µM |           |
| ER $\text{Ca}^{2+}$ leak | $V_{\text{leak}}$ | 5.00e⁻⁵ | mM/m | |
| $K_{\text{SOC}}$ | 0.05e⁻⁴ | mM | | |
| Activation constant | $K_{\text{SERCA1}}$ | 0.05e⁻⁴ | mM | |
| Inactivation constant | $K_{\text{SERCA2}}$ | 0.10 | mM | |
| SOC                 | $V_{\text{SOC}}$ | 5.60e⁻⁵ | mM/m | |
| Maximum SOCE flux   | $K_{\text{SOC}}$ | 0.05 | mM | |
| STIM $[\text{Ca}^{2+}]_{\text{ER}}$ affinity | $\tau_{\text{SOC}}$ | 20.0 | s | |
| SOCE timescale      | $f_{\text{ER}}$ | 0.01 | | |
| ER $[\text{Ca}^{2+}]_{\text{ER}}$ equation | | | | |
| ER $[\text{Ca}^{2+}]_{\text{ER}}$ buffering coefficient | $f_{\text{ER}}$ | 0.01 | | |
| ER volume/cell volume (excluding ER) | $v_{\text{ER}} / v_{\text{cyt}}$ | 0.15 | | |
| $[\text{Ca}^{2+}]_{\text{ER}}$ equation | $f_{\text{ER}}$ | 0.01 | | |
| Ca$^{2+}$ buffering coefficient of cytoplasm | $f_{\text{ER}}$ | 0.01 | | |
| $[\text{Ca}^{2+}]_{\text{ER}}$ equation | | | | |
| $[\text{Ca}^{2+}]_{\text{ER}}$ initial conditions | $[\text{Ca}^{2+}]_{\text{i}}$ | 30.0 nM | | |
| $[\text{Ca}^{2+}]_{\text{ER}}$ (t = 0) | $[\text{Ca}^{2+}]_{\text{ER}}$ | 100.0 µM | | |
| $[\text{Ca}^{2+}]_{\text{i}}$ (t = 0) | $[\text{Ca}^{2+}]_{\text{i}}$ | 100.0 µM | | |
| $V_{\text{m}}$ (t = 0) | $V_{\text{m}}$ | -55.0 mV | | |
| $E_{\text{ka}}$ | $E_{\text{ka}}$ | 70.0 mV | | |
| $E_{\text{ka}}$ | $E_{\text{ka}}$ | 7.0 mV | | |
| $E_{\text{ka}}$ | $E_{\text{ka}}$ | 135.0 mV | | |
| $[\text{K}^{+}]_{\text{i}}$ | $[\text{K}^{+}]_{\text{i}}$ | 54.4 mM | | |
| $[\text{K}^{+}]_{\text{o}}$ | $[\text{K}^{+}]_{\text{o}}$ | 2.5 mM | | |

Table 3. Parameter values used in the model

Table 4. The initial conditions and geometric parameters of the model cell
Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.