Supplementary Materials for

Nanoscale molecular architecture controls calcium diffusion and ER replenishment in dendritic spines

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The PDF file includes:

Sections S1 to S8
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Other Supplementary Material for this manuscript includes the following:

Data S1 to S5
Section S1  Asymmetric calcium dynamics between SOCE and synaptic transients

We compare in Fig. S1 calcium transients during synaptic inputs (solid lines) and SOCE (dashed) in the head (red) versus base (blue) of a dendritic spine. The synaptic input transient peaks about 0.25s after the stimulation, while the SOCE increase is very shallow with a maximum around 0.6s. Interestingly, this difference is even more prominent in the base area, suggesting that during SOCE, only a small amount of calcium could reach the base.

Figure S1: Comparison of synaptic activity-related calcium transient to store-operated calcium entry (calcium wave). Cultured hippocampal neuron was transfected with synaptopodin-cherry (SP) plasmid at the age of 1 day after plating. At the age of 3 weeks the neuron was loaded with Fluo-2 calcium sensor and spontaneous activity was recorded using fast scan of Zeiss-880 confocal microscope. A randomly selected area contains a typical SP+ mushroom spine with post synaptic density (PSD) area (marked in red) and adjacent dendritic shaft (blue). A typical single spontaneous event in both compartments is shown using solid lines. Afterwards, calcium was removed from extracellular medium for 15 minutes in the presence of DNQX (7 µM), APV (20µM) and TTX (1 µM) which depleted calcium stores and did not allow new calcium ions to enter the spine as a result of synaptic activity. Then initial calcium concentration (1.5 mM) was recovered in the presence of blockers, following which slow and low amplitude calcium waves were observed in the PSD area, but not in the shaft, indicating the activity of store-operated calcium entry (SOCE) mechanism. A typical waves are shown with dotted lines. The timescale is twice longer and the SOCE amplitude is smaller compared to the synaptic activity.
Section S2  Time-dependent calcium refilling in SA in the presence of activity blockers

To investigate the time course of SOCE and SA refilling, we develop a timelapse protocol after depleting the SA with a caffeine application at time $t=0$. Afterwards, at different time points of $t = 1, 2, 5$ and 10 minutes, we find that for SP+, the amount of released calcium is proportional to the time we waited until the second application (Fig. S2A). This result was specific to SP+ spines. In addition, when SOCE is completely abolished in the presence of 50 µM 2-aminoethoxydiphenylborane: 2-APB (Fig. S2B-C), calcium is not released from the SA at a significant amount. These results confirm that SOCE refills the SA with a timescale of a few minutes and the refilling dominates any possible calcium leakage.
Figure S2: Synaptopodin-associated local calcium storage of dendritic spines reveals time-dependent refilling ability in the presence of activity blockers. Hippocampal neurons were transfected with synaptopodin-cherry (SP) and blue fluorescent protein (BFP) as a morphological marker. At the age of 3 weeks after plating, cultures were used in acute experiments. The channels/receptors serving a source of calcium entry into the neurons during normal cell activity were suppressed using a mixture of the following blockers: AMPAR was blocked using DNQX (7 µM), NMDAR was blocked with APV (20 µM) and voltage-gated channels were disabled using TTX (1 uM). After 10 minutes of preincubation with blockers, bath application of caffeine (5 mM) was performed to deplete SP-associated, ryanodine-dependent calcium stores and the responses were recorded form the spine head and the adjacent dendritic shaft local areas (as shown in Fig. 1). According to the presence of SP puncta, the spines were divided into SP-positive (SP+) and SP-negative (SP-). Ten neighboring spines of about the same sizes and shapes were recorded in each group (3 experiments). Following the initial caffeine application and extensive wash (during about 30 s), caffeine was applied again at the time points of 1, 2 5 and 10 minutes after the initial application, all in the presence of blockers. The results are presented in A for SP+ and C for SP- spine/dendrite pairs. Panel B represents a similar experiment with 5 SP+ spines in the presence of 2-aminoethoxydiphenylborane (2-APB, 50 µM), which is a store-operated calcium (SOC) blocker at the given high concentration. In this case, only 10 minutes time point was tested.
Section S3  Quantifying the SOCE transient signal

For the slow transients, by fitting with a difference of two exponentials $J_m(t) = \frac{A}{a-b}(e^{-at} - e^{-bt})$, we obtained the parameter values $a = 1.53s$ and $b = 1.43s$.

![Figure S3: Quantification of slow calcium transient timescales from store-operated calcium entry activation.](image)

The slow calcium fluctuations is induced by the application of neuron activity blockers in synaptopodin-positive spines (data presented in Fig. 1C bottom). Average dataset with 18 trials (mean in red) fitted by the difference of two exponentials (blue).

We also computed the autocorrelation functions of the two time series recorded in the spine head and the base shown in Fig. 1C for lags up to 10s:

![Figure S4: The auto-correlation of the calcium activity signals in spine head and base.](image)

The two signals start to de-correlate (first zeros) at 0.96s and 0.56s.
Section S4  Mean-field model to compute the probability to activate CICR through RyRs

We present here a mean-field model to compute the release probability of calcium from RyRs located at the base of a spine containing a SA, triggered by a calcium influx to the head. The model disregards the geometry by considering the spine as a homogeneous compartment, but leads to explicit formulas. The influx of calcium ions into the spine head is modeled by the function $J(t)$.

The probability $P_2$ to initiate CICR is the probability that at least one RyR is activated during a calcium transient. This activation is induced by the binding of two calcium ions to a single RyR among a total of $N_R$ receptors. We derive below an equation for the number $n_2(t)$ of RyRs containing 2 ions for the first time. In this approach, we approximate the arrival of a single ion to a single receptor by a Poissonian rate constant $\lambda$. When there are $n_1(t)$ RyRs bound to one calcium ion, the rate of arrival of single calcium ion to one of the RyRs is:

$$\lambda(t) = \lambda n_1(t). \quad (6)$$

When one of the $n_1(t)$ RyRs is bound to a second calcium ion, this event leads to RyR activation. For the first times of such activation $t_a$, the number of RyR bound to two ions $n_2(t_a)$ jumps to 1. Therefore, we have the following conservation conditions before the termination step:

$$n_1(t) + n_0(t) = n_R \quad (7)$$
$$n_2(t) = 0 \text{ for } t < t_a. \quad (8)$$

The first identity is based on the pre-activation condition where a RyR either contains zero or one ion. The second constraint ensures that before any activation events, the number of RyRs containing 2 ions is zero. To compute the probability of a RyR activation, we consider the probability $\tilde{p}_2(t) = Pr\{n_2(t) = 0\}$ that there are no RyRs bound to two calcium ions at time $t$. During an interval between $t$ and $t + \Delta t$, when we assume that that no ions out of the total $m_{ca}(t)$ calcium ions leave the spine, the probability of a second ion binding to one of the $n_1(t)$ single-bound RyRs is:

$$Pr\{n_2(t + \Delta t) = 0\} = Pr\{n_2(t) = 0\}(1 - \lambda n(t)n_1(t)\Delta t). \quad (9)$$

That is

$$\dot{\tilde{p}}_2 = -\lambda n_1 m_{ca}\tilde{p}_2. \quad (10)$$

Thus integrating with the initial condition that there are no RyRs bound to two calcium ions, we obtain for the probability $Pr\{n_2(0) = 0\} = 1$

$$\tilde{p}_2(t) = e^{-\int_0^t n_1(u)m_{ca}(u) du}. \quad (11)$$
Taking the first time that two calcium ions binding to one RyR as \( \tau_2 \), we can now compute the probability \( p_2(t) = Pr\{n_2(t) = 1\} = Pr\{\tau_2 < t\} \) that a second binding event occurs before time \( t \). Using the identity

\[
Pr\{n_2(t) = 0\} + Pr\{n_2(t) = 1\} = 1,
\]

we obtain the expression for the probability

\[
Pr\{n_2(t) = 1\} = 1 - e^{-\lambda \int_0^t n_1(u) m_{ca}(u) du}.
\]  

(13)

We shall now use a mean-field approximation to derive a coupled system of equations governing \( m_{ca}(t) \), \( n_1(t) \) and \( p_2(t) \). By using the rate \( \mu \) for the unbinding of calcium to RyRs, we obtain the relation for the number of RyRs bound to one calcium ion (prior to any RyR binds to two ions):

\[
\dot{n}_1 = -\mu n_1(t) + \lambda m_{ca}(t) n_0(t) - \lambda m_{ca}(t) n_1(t).
\]  

(14)

Here the first term measures unbinding events while the second term measures the binding events proportional to the number of available sites \( n_0(t) \) and the number of calcium \( m_{ca}(t) \). The third term corresponds to the binding of the second ion to one of the \( n_1(t) \) RyRs containing one calcium ion. By eliminating \( n_0 \) with Eq.7 we obtain:

\[
\dot{n}_1 = -\mu n_1 + \lambda m_{ca}(n_R - 2n_1).
\]  

(15)

The mass-action law for calcium is

\[
\dot{m}_{ca} = J(t) - \nu m_{ca} - \lambda m_{ca}(n_R - n_1) + \mu n_1,
\]  

(16)

where \( J(t) \) is the calcium influx rate into the spine. The extrusion rate of calcium from the spine (by calcium pumps and arrivals at the absorbing boundary in the base) is \( \nu \), thus the total extrusion (which is proportional to the mean number of calcium ions in the spine) is given by the second term \( \nu m_{ca} \). The third term accounts for the binding of calcium to one of the unoccupied RyRs. This binding also has the rate constant \( \lambda \), and is also proportional to the number \( n_0 = n_R - n_1 \) of unoccupied receptors as well as to \( m_{ca} \). The final term measures the unbinding of calcium ions from some of the \( n_1 \) RyR-calcium bindings with the rate \( \mu \). In summary, we obtain the system of equations:

\[
\begin{align*}
\dot{n}_1 &= -\mu n_1 + \lambda m_{ca}(n_R - 2n_1) \\
\dot{m}_{ca} &= J(t) - \nu m_{ca} - \lambda m_{ca}(n_R - n_1) + \mu n_1 \\
\dot{p}_2 &= \lambda n_1 m_{ca}(1 - p_2).
\end{align*}
\]  

(17)

We now explore the solution under the two conditions:

1. the ions are injected instantaneously, modeled with a Dirac’s delta function in time, or equivalently with the initial condition \( m_{ca}(0) = N_0, n_1(0) = 0, p_2(0) = 0, \)
2. a slow injection rate, modeled by $J_{inj}(t) = A(e^{-\lambda_1 t} - e^{-\lambda_2 t})$ with the initial condition $m_{ca}(0) = 0, n_1(0) = 0, p_2(0) = 0$.

Parameters $\lambda_1, \lambda_2$ and $A$ can be calibrated to account for empirical data. Our goal is to compute the probability to ever activate a RyR by two ions, which is obtained by taking the limit $t \to \infty$ in Eq. 13:

$$P_2 = Pr\{n_2(\infty) = 1\} = 1 - e^{-\lambda} \int_0^\infty n_1(u)m_{ca}(u)du.$$  \hspace{1cm} (18)

We are also interested in the relation between this probability $P_2$ and the parameters such as the flux $J_{inj}(t)$, the initial condition and the rate constants $\mu, \nu$ and $\lambda$.

**Section S4.1 Parameters of the mean-field model and the numerical analysis**

We solve system 17 numerically for the three variables $m_{ca}(t), n_1(t)$ and $p_2(t)$ under the two input conditions (Fig. S5). Here we scaled the total number of RyRs to one ($N_R=1$), as a representation of a possible cluster of receptors. We use the forward rate $\lambda = 6s^{-1}$, which is the reciprocal of the mean first passage time of a calcium ion to a target receptor at the spine base (approximated as 166ms in (64)). For the unbinding rate constant, we use the value $\mu = 38s^{-1}$ (65). Finally, we model calcium clearance with an exponential rate constant of $\nu = 1000s^{-1}$ when there are pumps, and $\nu = 100s^{-1}$ when there are no pumps. We summarize these parameters in Table S1.

The numerical values obtained for the probability $p_2$ (Fig. S5 two bottom plots) show that the approximations from the mean-field model are in excellent agreement with the stochastic simulations of the biophysical model (Fig. S8 Top plots).
Figure S5: Consequences of slow (A) and fast (B) calcium inputs in a homogeneous model of a dendritic spine. The total number of calcium $m_{\text{ca}}(t)$, the number of RyRs occupied by a single RyR $n_1(t)$ and the probability of opening one RyR $p_2(t)$ are shown in Top, Middle and Bottom plots, respectively. Slow injection rate modeling the STIM1-ORAI1 inputs is similar to Fig. 2Ci, while the fast, synaptic inputs are modeled with a Dirac’s Delta functions at $t=0$. Presence and absence of pumps are modeled with different extrusion rates $\nu$ (see Table S1 for parameter values).

Finally, SOCE-based refilling is associated to a low probability of RyR release with a small number of injected ions ($<$600), in contrast to the higher number of calcium ions ($>$600) entering with synaptic activation that almost surely results in a SA transient depletion by RyR-triggered CICR (Fig. S6). Therefore, the RyR opening probability for the slow and fast injection regimes has a clear separation with the difference in the total number of injected ions.
Figure S6: Distinct physiological separation of the two pathways through the modulation of RyR opening probability. In the conditions of slow inputs with 0-600 ions in 2s, RyR opening probability remains below 0.25, thus the refilling of SA is dominant (light blue area, solid brown curve). During fast, synaptic inputs with more than 600 ions, RyR probability guarantees CICR and SA depletion that increases calcium level at the spine base (pink area, solid blue curve).

Table S1: Parameters of the mean-field model

| Parameter name                          | Symbol | Value     |
|-----------------------------------------|--------|-----------|
| Arrival rate of calcium to a RyR       | $\lambda$ | $6 s^{-1}$ |
| Calcium unbinding rate from RyR        | $\mu$  | $38 s^{-1}$ |
| Clearance rate by calcium pumps        | $\nu$  | $1000 s^{-1}$ |
| Clearance rate (without calcium pumps) | $\nu$  | $100 s^{-1}$ |
Section S4.2 Analytical exploration of the fast injection regime $J(t) = N \delta_0(t)$

We now derive an analytical expression for the probability of having 2 ions bound to one RyR during a fast calcium transient activation

$$P_2 = 1 - e^{-\lambda \int_0^\infty n_1(u)m_{ca}(u)du}.$$  \hfill (19)

We shall now consider the limit of a large number $N \gg 1$ of calcium ions. In that case, since the fraction of bound calcium ions is small compared to the total number, we neglect this bound fraction and approximate the solution for the free calcium ion in system 17, $m_{ca}(t) \approx Ne^{-\nu t}$. Thus the approximation for the second equation is

$$\dot{n}_1 + \mu n_1 = \lambda m_{ca}N_R.$$  \hfill (20)

A direct integration leads to

$$n_1(t) \approx \lambda N R N e^{-\mu t} - e^{-\nu t}. \frac{\nu - \mu}{\nu + \mu}.$$  \hfill (21)

The probability to ever activate a single RyR is computed from

$$\int_0^\infty n_1(u)m_{ca}(u)du = \lambda N R N^2 \int_0^\infty e^{-\nu t} - e^{-\nu t} \frac{e^{-\mu t} - e^{-\nu t}}{\nu - \mu} dt = \frac{\lambda N R N^2}{2\nu(\nu + \mu)}.$$  \hfill (22)

Finally, we obtain

$$P_2 = 1 - e^{-\lambda N R N^2 \frac{2\nu(\nu + \mu)}{2\nu^2}},$$  \hfill (23)

which tends asymptotically to 1, as the number $N$ of calcium ions increases. Moreover, as the extrusion rate $\nu$ increases, $P_2 \approx \frac{\lambda N R N^2}{2\nu^2}$ tends to zero.

Section S4.3 Analysis of the slow calcium injection rate during STIM1-ORAI1 activation

We derive here an analytical formula for the probability $P_2$ during the slow calcium input from the SOCE that we approximate as the difference of two exponentials

$$J_{inj}(t) = A(e^{-at} - e^{-bt}).$$  \hfill (24)

First we consider the total number of calcium ions in the compartment that can be approximated by the equation

$$\dot{m}_{ca} = J(t) - \nu m_{ca},$$  \hfill (25)
leading to

\[ m_{ca}(t) \approx A \left( \frac{e^{-\nu t} - e^{-at}}{\nu - a} - \frac{e^{-\nu t} - e^{-bt}}{\nu - b} \right). \] (26)

We are left with estimating the number of bound RyRs given by Eq.20:

\[ \hat{n}_1 + \mu n_1 = \lambda N_R A \left( \frac{e^{-\nu t} - e^{-at}}{\nu - a} - \frac{e^{-\nu t} - e^{-bt}}{\nu - b} \right). \] (27)

Thus

\[ n_1(t) = \lambda N_R A \left( \frac{1}{a - \nu} \left( \frac{e^{-\mu t} - e^{-\nu t}}{\nu - \mu} - \frac{e^{-\mu t} - e^{-at}}{\mu - a} \right) - \frac{1}{b - \nu} \left( \frac{e^{-\mu t} - e^{-\nu t}}{\nu - \mu} - \frac{e^{-\mu t} - e^{-bt}}{\mu - b} \right) \right). \]

After integrating,

\[ \int_0^\infty n_1(u)m_{ca}(u)du = \frac{\lambda N_R A^2}{2} \frac{(b - a) ((\mu + b + 2 \nu) a^2 + (\mu + b + 2 \nu) (b + \nu + \mu) a + (\mu + \nu) (b + \nu) (b + \mu))}{a \nu (\mu + \nu) (b + \mu) (a + \nu) (a + \mu) (b + a)}. \] (28)

We find an expression for the RyR activation probability

\[ P_2 = 1 - e^{-\lambda N_R A^2 (b - a) ((\mu + b + 2 \nu) a^2 + (\mu + b + 2 \nu) (b + \nu + \mu) a + (\mu + \nu) (b + \nu) (b + \mu))} \frac{2}{a \nu (\mu + \nu) (b + \mu) (a + \nu) (a + \mu) (b + a)}. \] (29)

**Section S4.4  Conditional time \( \bar{\tau}_2 \) for RyR activation when ions can be expelled from the spine head**

We compute here formally the mean time for RyR to be activated starting from the moment when calcium ions are injected. This activation time \( \tau_2 \) can be computed from model 17. Indeed,

\[ \bar{\tau}_2 = \int_0^\infty t \frac{d}{dt} \Pr \{ \tau_2 < t | \tau_2 < \infty \} dt = \int_0^\infty t \frac{d}{dt} \Pr \{ \tau_2 < t, \tau_2 < \infty \} dt = \int_0^\infty \Pr \{ \tau_2 < \infty \} - \Pr \{ \tau_2 < t \} \frac{d}{dt} \Pr \{ \tau_2 < \infty \} dt. \] (30)

Using relation 18, we obtain

\[ \bar{\tau}_2 = \int_0^\infty \left( e^{-\lambda \int_0^t n_1(u)m_{ca}(u)du} - e^{-\lambda \int_0^\infty n_1(u)m_{ca}(u)du} \right) \frac{dt}{1 - e^{-\lambda \int_0^\infty n_1(u)m_{ca}(u)du}}. \] (32)

We approximate this mean time by rewriting relation 32 using that

\[ -\lambda \int_0^t n_1(u)m_{ca}(u)du - e^{-\lambda \int_0^\infty n_1(u)m_{ca}(u)du} = -\lambda \int_0^t n_1(u)m_{ca}(u)du \] (1 - e^{-\lambda \int_0^\infty n_1(u)m_{ca}(u)du}). \] (33)
and Taylor’s expansion, \(1 - e^{-X} = X + o(X)\) for small \(X\), thus

\[ \bar{\tau}_2 \approx \lambda \int_0^\infty \left( \int_0^\infty n_1(u)m_{ca}(u)du \right) e^{-\lambda \int_0^t n_1(u)m_{ca}(u)du}dt. \] (34)

Figure S7: Conditional RyR opening times for fast (A) and slow (B) inputs computed from formula 34 using the parameter values corresponding to the conditions described in Fig. S5.

**Section S4.5 Derivation of the mean-field calcium-SA interaction from the Master equations**

In this section, we present the Master equations (29) (66) (67) that we used to derive the mean field equation. We start with the change between time \(t\) and \(t + \Delta t\) of the probability for not having 2 ions bound to a single RyR. It is given by

\[ Pr\{n_2(t + \Delta t) = 0\} = \sum_{k,q} Pr\{n_2(t) = 0|q,k\} Pr\{n_1(t) = q, m_{ca}(t) = k\}(1 - \lambda qk\Delta t). \] (35)

Thus if we use the joint probability \(p_{q,k}(t) = Pr\{n_1(t) = q, m_{ca}(t) = k\}\) for having \(q\) RyR bound to one ions and the total of free calcium ions is \(k\) we get the different equation:

\[ \dot{p}_2 = -\lambda \sum_{k,q} Pr\{n_2(t) = 0|q,k\} qkp_{q,k}(t) \] (36)

and the conditional probability satisfies:

\[ Pr\{n_2(t + \Delta t) = 0|q,k\} = Pr\{n_2(t) = 0|q,k\}(1 - \lambda qk\Delta t), \] (37)
which leads with $a_{q,k}(t) = Pr\{n_2(t) = 0|q,k\}$ to the different equations:

$$\dot{a}_{q,k} = -\lambda qka_{q,k},$$  
$$a_{q,k} = A_{k,q} \exp\{-\lambda qkt\},$$  

where $A_{k,q}$ are constants. Finally, we obtain the Markov chain (not considering the boundary equation)

$$p_{q,k}(t + \Delta t) = p_{q,k}(t)(1 - (\nu k + \mu q + \lambda k(n_R - q))\Delta + \nu(k + 1)\Delta p_{q,k+1}(t) + \mu(q + 1)\Delta p_{q-1,k}(t) + \lambda k(n_R - q + 1)\Delta p_{q-1,k}(t).$$

Thus, using the boundary equations, we obtain the full system:

$$\dot{p}_{q,k}(t) = -(\nu k + \mu q + \lambda k(n_R - q))p_{q,k}(t) + \nu(k + 1)p_{q,k+1}(t) + \mu(q + 1)p_{q-1,k}(t) + \nu(k + 1)p_{q+1,k}(t) + \mu(q + 1)p_{q,k+1}(t) + \lambda k(p_{q-1,k}(t))$$

$$\dot{p}_{0,k}(t) = -(\nu k + \lambda kn_R)p_{0,k}(t) + \nu(k + 1)p_{0,k+1}(t) + \mu p_{1,k}(t) + \lambda kp_{n_R,k}(t) + \nu(n_R + 1)p_{n_R,k+1}(t) + \mu(n_R + 1)p_{n_R+1,k}(t) + \lambda kp_{n_R-1,k}(t)$$

with the initial condition

$$p_{q,k}(0) = \delta(q = 0)\delta(k = N_0).$$

The process starts with the condition that no ions are inside a RyR, thus $n_1(0) = 0$. 
Section S5  Impact of the distance between spine and SA membranes on CICR probabilities and initiation times

In Fig. 2Ci-Ciii, we studied the scenarios where calcium ions are not released from the SA. We now consider the cases where calcium ions could trigger an opening of at least one RyR, by the arrival of two calcium ions on the same receptor. One RyR activation leads to a transient depletion of SA calcium stores caused by the local calcium diffusion that can trigger the opening of in the neighbouring RyRs. We confirm here that SERCA-ORAI1 distance $d_{SA}$ (Fig. 2A) influences the probability $P_2$ and initiation time of such events during fast (Fig. S8A) and slow (Fig. S8B) calcium influx conditions.

We simulated the instantaneous injection of $N=100$ calcium ions to the spine head (as in Fig. 2Bi), and find that the probability $P_2$ is less than 0.1 when the distance varies from zero to 250nm (Fig. S8A Top, black curve). In addition, the conditional opening time $\tau_2$ computed over realizations where a RyR opening did occur is in the range of 15-35ms. When $N=300$ ion were injected, the probability $P_2$ increases and remains stable around 0.4-0.6 (Fig. S8A Top, red curve), while the conditional RyR activation times is slightly less than 20ms. When we repeated the same simulations after removing all calcium extrusion pumps, the probability $P_2$ increases higher than 0.75 in both $N=100$ (green curve) and $N=300$ (purple) injections as expected because more ions remain in the cytoplasm.

We confirm a non-intuitive result for RyR activation times (Fig. S8A bottom): for a low amount of calcium ($N=100$), the conditional time $\tau_2$ increases to a value larger than 25ms, while in the presence of more calcium ($N=300$), the times decreases to less than 10ms. Indeed, with 300 ions, the RyR opening event is not very rare ($P_2 \approx 0.5$) and with the presence of pumps, the fastest ions that would have otherwise activated RyRs faster can disappear. Therefore, conditional RyR activation becomes faster when extrusion pumps are removed (Fig. S8A Bottom: red to magenta). In contrast, for $N=100$, the probability of RyR activation is extremely low thus triggering RyR is a rare event that must occur very fast before calcium ions are captured by pumps. When pumps are removed in this scenario, the number of realisations triggering RyR activation increases drastically leading to a high probability $P_2 > 0.75$ (Fig. S8A Top, black to green) and an increase in the average conditional times (Fig. S8A Bottom, black to green).
Figure S8: **Opening probabilities and conditional opening times of RyRs during slow and fast calcium entry.** (A). Following an instantaneous calcium entry we estimated the open probabilities (Top) for the first RyR and their conditional opening times (Bottom). Color-coded legend is common to both top and bottom panels. The simulation framework corresponds to Fig. 4F. In the black and red curves, \(N=100\) and \(300\) ions were initially injected. For purple and green curves, identical conditions were used, except that the \(50\) extrusion pumps that were present in the spine head were removed. Probabilities, mean times and standard errors are computed with 100 trials. (B). Probability and opening times computed as in A, but during a slow calcium entry shown (same as Fig. 4G), with the numbers of ions \(N\) as indicated, and without calcium pumps with \(N=100\).

In the simulations with slow inputs (Fig. S8B), the probability \(P_2\) increases gradually with the distance \(d_{SA}\) (from 0 to 250nm) and the number of ions injected (\(N=100, 300, 500\)). Interestingly, the probability with \(N=100\) ions without pumps is equivalent to the case of \(N=500\) injected ions with pumps. Therefore, we conclude that CICR is controlled by the distance \(d_{SA}\), and also partially by the extrusion pumps.
Section S6  Spine calcium dynamics during LTP stimulations

We present here the calcium dynamics in spines during the LTP protocol simulation starting from $N=300$ ions shown in Fig. 5Di and 5Dii.

Figure S9: Number of calcium ions during the first 250ms of the LTP simulations. Each 100Hz high-frequency pulse impulse results in a strong calcium influx into the spine head. Calcium level in the head peaks with a delay of about 50ms and decays gradually with the stimulation strength (blue, averaged over 20 realizations). For each realization, calcium in the base increases due to successive arrivals and sporadic CICR events (peaks in green), while the average (orange) remains low compared to the concentration in the head.
Section S7  Calcium refilling with ectopic release events

We describe here our algorithm to simulate stochastic ectopic vesicular release, a well-known phenomenon due to releasing vesicles not directly in the active zone, but on the sides (68). Following such release events, post-synaptic currents as well as the calcium influx are much smaller compared to an evoked stimulation (69). We study the consequences of these events by adding it to the SA replenishment simulation (Fig. 5Diii). We recall that the refilling occurs with a slow calcium influx mediated by SOCE (Fig. 2Ciii), and repeated every 2s. In addition, we introduce here ectopic release events modeled as small calcium spikes occurring every 1s with amplitudes randomly alternating between \(N=25\) and \(50\) ions. We simulated the overall calcium entry by adding these two components for a prolonged duration of one minute and observed the SA calcium refilling (Fig. S10 blue curve). We found that at the elapse of 30s, calcium uptake is not significantly different compared to simulation without ectopic release events (red curve).

![Graph showing comparison of SA refilling with (blue) and without (red) ectopic refilling events.](attachment:image.png)

**Figure S10:** Comparison of SA refilling with (blue) and without (red) ectopic refilling events. In both cases slow calcium entry (similar to Fig. 2Ci with \(N=300\) injected ions) is present. Ectopic release events (only in the blue curve) are modeled as 1Hz spikes with an amplitude that randomly switches between \(N=25\) and \(50\) ions. The average and SEM values are calculated over 10 and 20 trials for the red and blue curves.

During the 30s period, 4500 ions enter the spine through the slow input. The ectopic injection contributes to about 1125 ions. However, such 25% difference in the refilling numbers is not reflected significantly in the final amount of calcium, because the ectopic release can also trigger more calcium release events from RyRs (decay phases in Fig. S10), interfering against the refilling process.
Section S8  Spine calcium dynamics during LTD stimulations

Figure S11: Calcium ions at the base of a spine during the simulated LTD protocol. For the two values of the number of injected calcium ions $N=300$ (top panel) and $N=500$ (bottom panel), we show single trials (green: left axis) and averages over 10 realizations (red: right axis).
| Parameter                                | Symbol | Value               |
|------------------------------------------|--------|---------------------|
| Time step                                | $\Delta t$ | $10^{-7}$ s     |
| Diffusion coefficient                    | $D$    | $600 \, \mu m^2 s^{-1}$ [37] |
| Spine head radius                        | $R$    | $1 \, \mu m$      |
| Spine neck radius                        | $a$    | $0.15 \, \mu m$  |
| Spine neck length                        | $L$    | $1.5 \, \mu m$  |
| SA head radius                           | $R_{SA}$ | $0.25 \, \mu m$ |
| SA neck length                           | $L_{SA}$ | $1.5 \, \mu m$  |
| SA neck radius                           | $a_{SA}$ | $0.05 \, \mu m$ |
| Radius (RyR and SERCA)                   |        | $10 \, nm$         |
| # SERCA pumps in the SA head             | $N_{SERCA}$ | $36$            |
| # RyR in the SA base                     | $N_R$  | $36$               |
| # ions absorbed by one SERCA             |        | $2$                |
| # ions to activate one RyR               |        | $2$                |
| First Ca waiting time SER                |        | $10 \, ms$        |
| First Ca waiting time RyR                |        | $10 \, ms$        |
| Refractory period SER                    |        | $100 \, ms$       |
| Refractory period RyR                    |        | $3 \, ms$         |
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