

Stochastic reaction-diffusion modeling of calcium dynamics in 3D dendritic spines of Purkinje cells

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ABSTRACT Calcium (Ca^{2+}) is a second messenger assumed to control changes in synaptic strength in the form of both long-term depression and long-term potentiation at Purkinje cell dendritic spine synapses via inositol trisphosphate (IP_3)-induced Ca^{2+} release. These Ca^{2+} transients happen in response to stimuli from parallel fibers (PFs) from granule cells and climbing fibers (CFs) from the inferior olivary nucleus. These events occur at low numbers of free Ca^{2+} , requiring stochastic single-particle methods when modeling them. We use the stochastic particle simulation program MCell to simulate Ca^{2+} transients within a three-dimensional Purkinje cell dendritic spine. The model spine includes the endoplasmic reticulum, several Ca^{2+} transporters, and endogenous buffer molecules. Our simulations successfully reproduce properties of Ca^{2+} transients in different dynamical situations. We test two different models of the IP_3 receptor (IP_3R). The model with nonlinear concentration response of binding of activating Ca^{2+} reproduces experimental results better than the model with linear response because of the filtering of noise. Our results also suggest that Ca^{2+} -dependent inhibition of the IP_3R needs to be slow to reproduce experimental results. Simulations suggest the experimentally observed optimal timing window of CF stimuli arises from the relative timing of CF influx of Ca^{2+} and IP_3 production sensitizing IP_3R for Ca^{2+} -induced Ca^{2+} release. We also model ataxia, a loss of fine motor control assumed to be the result of malfunctioning information transmission at the granule to Purkinje cell synapse, resulting in a decrease or loss of Ca^{2+} transients. Finally, we propose possible ways of recovering Ca^{2+} transients under ataxia.

SIGNIFICANCE Ca^{2+} is a second messenger that can trigger synaptic plasticity in dendritic spines of Purkinje cells, associated among other things with motor learning and motor fine control. Disrupted Ca^{2+} signals in those spines could lead to pathological conditions such as cerebellar ataxia, a lack of coordination of muscle movements. The molecular reaction mechanisms defining the spatiotemporal aspects of such Ca^{2+} signals in the noisy environment of dendritic spines in health and disease are not fully understood. Here, we develop a stochastic reaction-diffusion model in MCell to study how synaptic inputs from parallel fibers and climbing fibers reaching spines of Purkinje cells are shaping Ca^{2+} signals in healthy and pathological conditions and propose a way to recover those Ca^{2+} signals in pathological conditions.

INTRODUCTION

Ca^{2+} is a second messenger involved in many processes in eukaryotic cells. Ca^{2+} signals activate several enzymatic targets involved in the induction of synaptic plasticity in dendritic spines of Purkinje cells in the cerebellum and cause an increase or decrease of synaptic strength. Glutamate released at parallel fiber (PF) synapses binds to metabotropic glutamate receptors (mGluRs) on the Purkinje dendritic spines that activate signaling pathways associated

with Ca^{2+} release from endoplasmic reticulum intracellular stores through inositol trisphosphate receptors (IP_3Rs). This response can be further enhanced by a well-timed additional Ca^{2+} influx triggered by climbing fiber (CF) coactivation (Fig. 1; (6,7)).

Frequently, the detection and discrimination of transient Ca^{2+} signals by molecular targets in dendritic spines happen outside mass-action equilibrium, at low particle concentrations of Ca^{2+} with endogenous buffers involved, and within the rather small three-dimensional structure of the spine which entails a very noisy signaling environment. Therefore, the spatiotemporal characteristics of Ca^{2+} signals can play an important role in the induction of synaptic plasticity (8–12), determining whether long-term potentiation (LTP) or depression (LTD) occurs.

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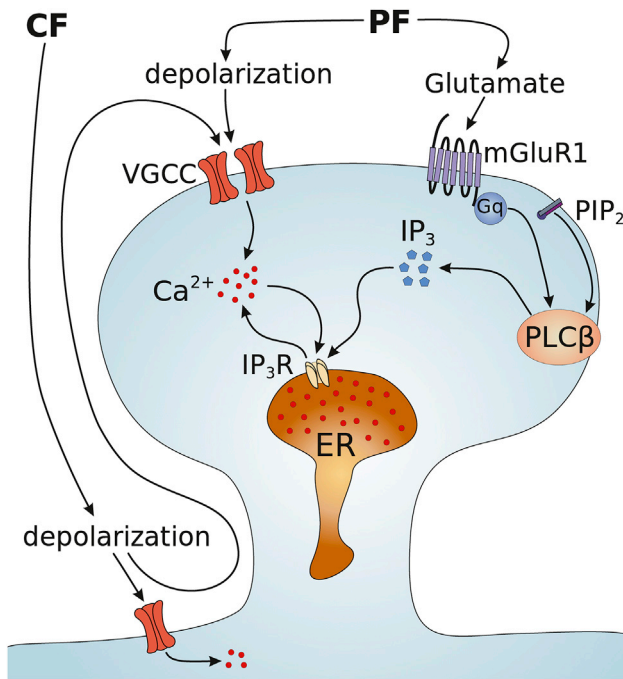


FIGURE 1 Illustration of a spine segment of a Purkinje cell showing the spine head at the top, neck in the middle, and beginning of the dendrite at the bottom. Visible are the signaling pathways of parallel and climbing fiber stimulation (1–5), which can trigger a cytosolic Ca²⁺ transient because of an opening of IP₃Rs on the ER (6,7). To see this figure in color, go online.

Computational models of the three-dimensional structure of dendritic spines and their kinetic mechanisms in their noisy environment can be very helpful in understanding the biophysical constraints shaping the Ca²⁺ dynamics that control synaptic plasticity in neurons (13). We model the system using stochastic single-particle simulations to account for the noise and low concentration properties of particles involved in the signaling processes (14–16).

Spines are dynamic extensions of neuronal dendrites and play an important role in cell signaling, neuronal excitability, information processing at the cellular level, and synaptic plasticity (17). They receive synaptic inputs from axons that release neurotransmitters, which bind to postsynaptic receptors on the spines (18,19). Because spines can react to outer and inner stimuli by changes in synaptic efficiency and in their morphological structure, their global topological arrangement becomes a mirror of sensory history and experience. Studying the dynamics of spine behavior is crucial for understanding learning, memory, motor functions, and other large-scale cognitive processes (20–24).

Spines are targets of signaling and contain molecular signaling mechanisms that regulate and are regulated by intracellular Ca²⁺ transients. Rapid Ca²⁺ release is achieved by opening of IP₃Rs, which reside on the smooth endoplasmic reticulum (ER), a Ca²⁺ store inside the spine and dendrite (6,7). IP₃Rs require inositol-1,4,5-trisphosphate (IP₃) and Ca²⁺ to open. Both IP₃ production and Ca²⁺ influx

are controlled by PF and CF activity (25–28). These interacting signaling pathways give the IP₃Rs the capacity to translate fast PF and CF inputs into longer-lasting slow-output Ca²⁺ signals (29).

PF stimulation activates two signaling pathways. It triggers glutamate release at the synapse, which leads to an activation of type-1 metabotropic glutamate receptors (mGluR1) located at the postsynaptic density (PSD) at the top of the spine head. The activated mGluRs activate G-protein-coupled receptors (G_q) that cause the activation of phospholipase Cβ (PLCβ), which synthesizes IP₃ from PIP₂. IP₃ is free to diffuse from the PSD into the dendrite's cytosol (30–32) before it vanishes because of degradation by IP₃ 3-kinase and IP₃ 5-phosphatase on the timescale of a few seconds (33,34). The second pathway consists of a membrane depolarization, causing Ca²⁺ influx through voltage-gated Ca²⁺ channels (Ca_v2.1 type P/Q-voltage-gated Ca²⁺ channels), which are highly expressed in Purkinje dendrites (35,36).

CF stimulation also generates a membrane depolarization opening the same type of P/Q-voltage-gated Ca²⁺ channels, which leads to Ca²⁺ influx not only into the spine but also into the dendrite (27), summarized in Fig. 1 (1–5).

Whereas PF synapses are located at the head of a dendritic spine coupled to the PSD, CFs attach to the dendrite itself, creating synapses at the dendritic arbor in ~2–3 μm intervals (37). One Purkinje cell is connected to up to 10⁵ parallel fibers but to only a single climbing fiber (17).

It is assumed that the activity patterns of PFs attached to Purkinje cells in the cerebellum mediate fine control of movement and promote an increase in synaptic strength (LTP), whereas the activity patterns of CFs encode information about failure of such movement and can trigger a decrease in synaptic strength (LTD) when succeeding PF stimuli (38,39).

The endogenous Ca²⁺ binding proteins (buffers) calbindin D28k (Cb), parvalbumin (Pv), and calmodulin (CaM) are highly expressed in Purkinje cells (17,40,41). Their role is to shape Ca²⁺ transients occurring in the cytosol by setting their spatiotemporal parameters such as amplitude and decay time, which are crucial for successful information transmission on cellular level via signaling pathways (42–46).

Various brain disorders are associated with malfunctioning neuronal information processing which can be related to atypically functioning dendritic spines and IP₃Rs (46–50). Among them, cerebellar ataxia is a serious and heterogeneous neurological condition involving a loss of coordination of muscle movement (51). Most forms of cerebellar ataxia have no cure to this day (52). Thus, it is important to develop computational models to study the role of dendritic spines with respect to ataxia (53). To model ataxia in our approach, we look at Ca²⁺ transients under reduced IP₃ binding rates of IP₃Rs and then suggest ways to restore previous Ca²⁺ transients.

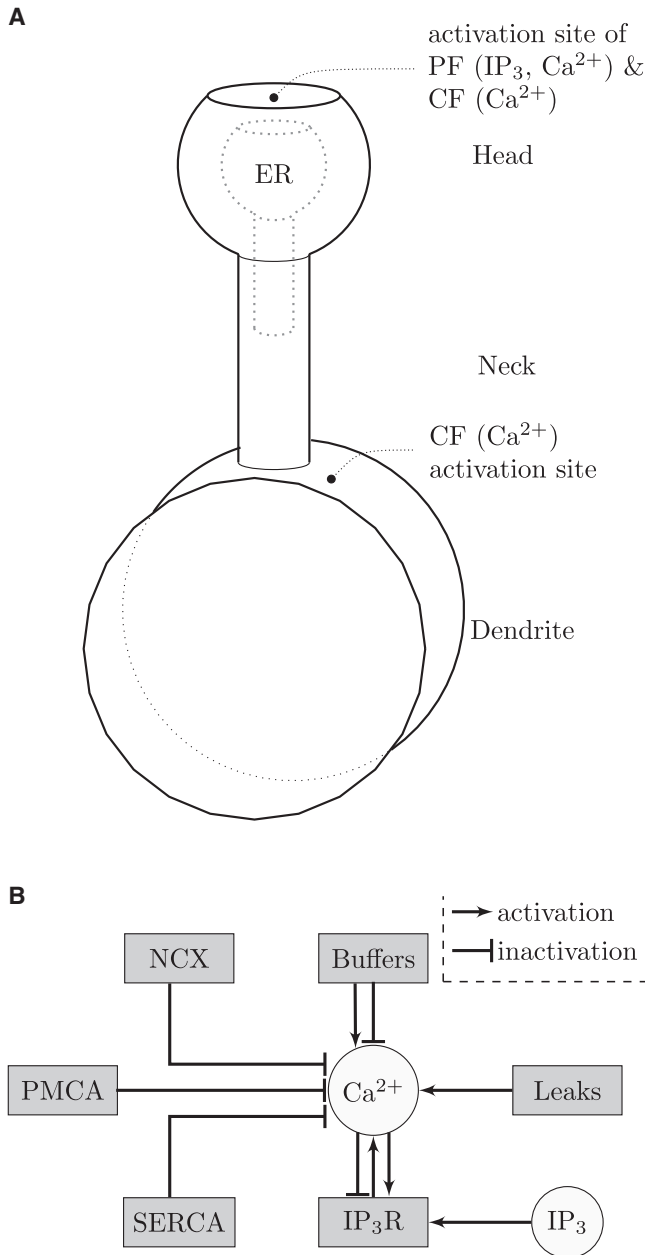


FIGURE 2 (A) Model geometry. The endoplasmic reticulum (ER) is visible in the head and neck. $V_{total} = 0.512 \mu\text{m}^3$, $V_{head} = 0.100 \mu\text{m}^3$, and $V_{ER} = 0.020 \mu\text{m}^3$. Release sites of Ca^{2+} and IP_3 for PF and Ca^{2+} for CF activation are marked by dots. Exact sizes of the geometry can be found in Table S4. (B) Interaction scheme of particle species used in the simulations from a cytosolic perspective. SERCAs, leak channels, and IP_3 Rs are located on the ER membrane, and PMCAs, NCXs, and more leak channels are located on the outer plasma membrane. Ca^{2+} , IP_3 , and the buffers are free to diffuse in the cytosol, the volume within the plasma membrane, and outside the ER.

METHODS

Model description

The model studies the Ca^{2+} response to outer stimuli from active PFs and CFs. Simulation methods are explained in the Supporting materials and

methods. Stochastic reaction-diffusion, particle-based simulations use MCell (54–56), and deterministic simulations use Copasi (57). Whereas MCell offers a biophysically realistic approach to a biological problem, accounting for low particle concentrations, inherent stochasticity, and complex three-dimensional geometries (58), Copasi describes the kinetic reactions in a well-mixed volume efficiently, without the influence of diffusion or complex geometry. In this way, the dynamics of the model can be tested in a computationally fast environment before going into more expensive reaction-diffusion simulations in complex geometries. Copasi was, for instance, used to approximately find concentrations of each species at equilibrium, i.e., the initial state then used for simulations in MCell. We focus on explaining model components here. All parameter values not mentioned explicitly in the text are listed in the Supporting materials and methods.

Geometry

We created the simple three-dimensional dendrite-neck-head volume shown in Fig. 2 A for our MCell simulations. The head consists of a sphere with the top and bottom being cut off. The top area models the PSD, and the bottom of the sphere connects to the spine's cylindrical neck. A cylindrical dendrite is attached to the other side of the neck. The head volume is $V_{head} = 0.1 \mu\text{m}^3$. The total volume dendrite, neck and head, is ~ 5 times as large, $V_{total} = 0.512 \mu\text{m}^3$ (59).

We created another volume inside the head and neck to represent the smooth ER. It is a scaled copy of the head and neck, just smaller in size, with $V_{ER} = 0.02 \mu\text{m}^3$. Although the surface of the ER is home to IP_3 Rs, ER Ca^{2+} -ATPases (SERCAs), and leak channels, we did not model the inside of it. For Ca^{2+} release by IP_3 Rs, we assume a constant Ca^{2+} efflux (60) on the timescale of interest not affected by ER depletion. We are aware of this being an approximation because ER depletion is suggested by the results of Okubo et al. (61), with intraluminal diffusion as the major flux of replenishment. However, luminal concentrations, together with the ER size required to account for luminal diffusion on the timescale of release, would render our study unfeasible because of particle numbers above 2×10^5 .

Additionally, we used no-flux boundary conditions at the dendrite sites for all particle species, modeling a situation in which neighboring spines also receive stimuli (see (62), their Fig. 1 b).

Molecular components of Ca^{2+} dynamics

General and steady-state particle concentrations, number of particles, and diffusion coefficients can be found in Tables S5 and S6.

Ca^{2+} transporters

SERCA pumps are described by a three-state model (63,64), Fig. S3 and Table S4, subsequently binding two Ca^{2+} before decaying back into the rest state, removing two Ca^{2+} from the cytosol. We put 68 SERCAs onto the ER membrane (64).

We included five sodium-calcium exchangers (NCXs) (63,64) on the outer plasma membrane without modeling sodium dynamics. Our model assumes constant intracellular and extracellular $[\text{Na}^+]$ as a simplification. We used a simple two-state model (64), i.e., one NCX receptor can bind one Ca^{2+} and then either release it back into the cytosol or decay back into the rest state, removing one Ca^{2+} .

Plasma membrane Ca^{2+} -ATPase (PMCA) is another Ca^{2+} pump that helps to maintain a low Ca^{2+} concentration in the cytosol of all eukaryotic cells. We used 13 PMCAs (63,64) and also a two-state model similar to the NCX model, but with different reaction rates (65,66).

We include 10 leak channels on each the ER and plasma membrane of the dendrite that yield a small constant influx of Ca^{2+} into the cytosol. The leak fluxes fix free $[\text{Ca}^{2+}] \approx 50 \text{ nM}$ in steady state.

Buffers. The buffer species in the model are Pv, Cb, and CaM (Fig. 3). We describe Pv by a three-state model. It binds either one Ca^{2+} or one magnesium (Mg^{2+}) (42,67).

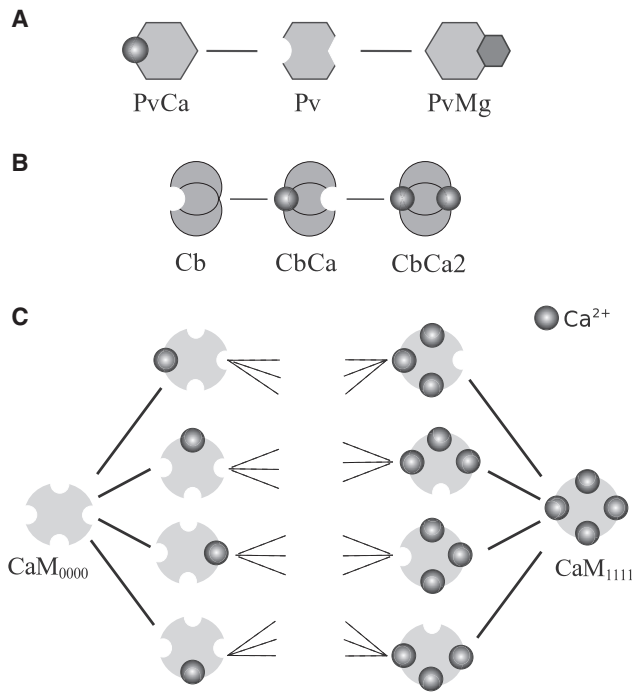


FIGURE 3 Overview of buffer models for (A) Pv, (B) Cb, and (C) CaM. CaM can hold up to four Ca²⁺. We used a 16-state model with individual binding sites. For ease of reading, we omitted the six states with two bound Ca²⁺. Reaction rates for all can be found in Table S3.

Cb is a major Ca²⁺ buffer in spines of hippocampal and also Purkinje neurons. We used a three-state kinetic model with a low- and a high-affinity site (42,68), with the low-affinity site being present only if the high-affinity site is filled. One Cb protein can therefore bind up to two Ca²⁺.

CaM is a multifunctional calcium-binding protein expressed in all eukaryotic cells. It is not only a buffer but also acts as a messenger target of Ca²⁺. Once Ca²⁺ is bound to CaM, it can modify the interactions of Ca²⁺ with various other targets like phosphatases or kinases. One CaM protein has four Ca²⁺ binding sites. We modeled the interactions with Ca²⁺ using a 16-state model with various medium- and high-affinity binding sites and various reaction rates (66).

IP₃R models

We used 54 or 56 IP₃Rs (69) on the ER membrane, depending on the parameter set. A large variety of IP₃R models have been developed in the last three decades (70–73). We compare here two models. Doi's model has been chosen because it has been applied to spine dynamics before (63). We picked Moraru's model for comparison because its open probability depends nonlinearly on [Ca²⁺], and it is eligible for easy implementation in MCell (74). This choice allows for statements on the role of IP₃R inhibition and IP₃R-related slow timescales.

Doi's model. A seven-state model for the IP₃R was proposed by Doi et al. (63). The model is able to reproduce the bell-shaped open probability curve $P_0([Ca^{2+}])$ (Fig. 4 C). An IP₃R requires one IP₃ to bind before one Ca²⁺ binds to open; otherwise, it will stay closed and can only bind more Ca²⁺ if it does not previously release the bound Ca²⁺. Once the receptor opens, it releases Ca²⁺ into the cytosol with a constant Ca²⁺ release rate (Figs. 4 A and S2; Table S2).

Moraru's model. We chose a subsection of the IP₃R model of Moraru et al. (74), in which we ignored an additional slow IP₃ binding site that is irrelevant for the timescales considered in our model. An IP₃R can maximally bind four Ca²⁺ and four IP₃ molecules, and any state transition that includes binding or unbinding of one Ca²⁺ or one IP₃ is allowed. There

is an additional transition from the state T_{42} with four bound IP₃ (75) and two bound Ca²⁺ to the open state, T_{open} . Moraru's model also reproduces the bell-shaped open probability curve (Fig. 4 C). The amplitude of the open probability is easily controlled by changing the reaction rates k_{open} and k_{close} of the transitions between T_{42} and T_{open} (Fig. S1). Because it is known that the binding dynamics of inhibitory Ca²⁺ is slower than that of excitatory Ca²⁺ by a factor of up to 100 (70,76), we decreased the inhibitory Ca²⁺ binding reaction rates into and from states T_{x3} and T_{x4} , which represent the inhibitory Ca²⁺ binding sites in this model, by an additional factor r_s (see Fig. 4 B; Table S1).

Parallel and climbing fiber stimulation

We studied the Ca²⁺ response to four different types of stimuli after experimental results (8): a single PF stimulus, PF burst, CF stimulus, and PF burst + CF stimulus. Stimulation by active PFs was simulated by plasma membrane influx of Ca²⁺ and IP₃ production close to the PSD. CF stimulation was modeled by Ca²⁺ influx close to the PSD and into the dendrite end close to the spine neck (Fig. 2).

We chose different amounts of Ca²⁺ per PF and CF stimuli (0–1500 Ca²⁺ and 0–2000 Ca³⁺, respectively) as part of our parameter scan. A single PF stimulus consists of one instantaneous injection of Ca²⁺ and production of IP₃ (380 close to the PSD), and a PF burst was made up of five single Ca²⁺ injections at 100 Hz and 1400 caged IP₃, yielding an ~5 times larger IP₃ transient. In the latter, more relevant case, free [IP₃] usually peaks around 4.5–5 μM. A CF stimulus included an additional Ca²⁺ release of 200 particles in the dendrite (Fig. 1). The typical time of the CF stimulus is $t_{CF} = 100$ ms after the initiation of the PF stimuli but was varied in **Optimal timing of CF stimulus**.

IP₃ dynamics used constant production and decay rates and was able to capture biexponential IP₃ concentration behavior (33,34,63,77). We chose an amount of IP₃ in agreement with physiological concentrations (6) such that the IP₃Rs were saturated with IP₃ for the case of a PF burst. IP₃ production was delayed by 100 ms compared to the onset of the PF Ca²⁺ influx to account for the slower process of IP₃ synthesis compared to instantaneous Ca²⁺ influx from PF and CF stimuli. IP₃ diffuses freely in our model.

RESULTS

We successfully constructed a three-dimensional stochastic reaction-diffusion model of Purkinje cell dendritic spine Ca²⁺ dynamics that reproduces many aspects found in experiments (8,62). Because of the nature of computational modeling, we were able to shed light on some aspects of the system's response to stimuli that are otherwise extremely hard to control experimentally, e.g., removing certain buffer species or changing the amplitude of Ca²⁺ associated with a PF or CF stimulus.

Snapshots of the spine head including Ca²⁺, IP₃, and IP₃R states on the ER from a typical simulation are shown in Fig. 5. At $t = 0$ ms in the first frame, the red dot is the initially localized collection of 110 Ca²⁺ of the first PF stimulus. The particles diffuse and get absorbed by buffers immediately. The CF stimulus consists of 1700 Ca²⁺, which is visible in three frames corresponding to $t = 100$ ms to $t = 100.032$ ms, showing how quickly Ca²⁺ diffuses. IP₃ slowly enters the system at the same time at $t = 100$ ms (see also Fig. 9 A). IP₃Rs start to react to IP₃ and increased [Ca²⁺] (note changing colors of IP₃Rs). Eventually, a global Ca²⁺ transient is initiated, which leads to a prolonged increase

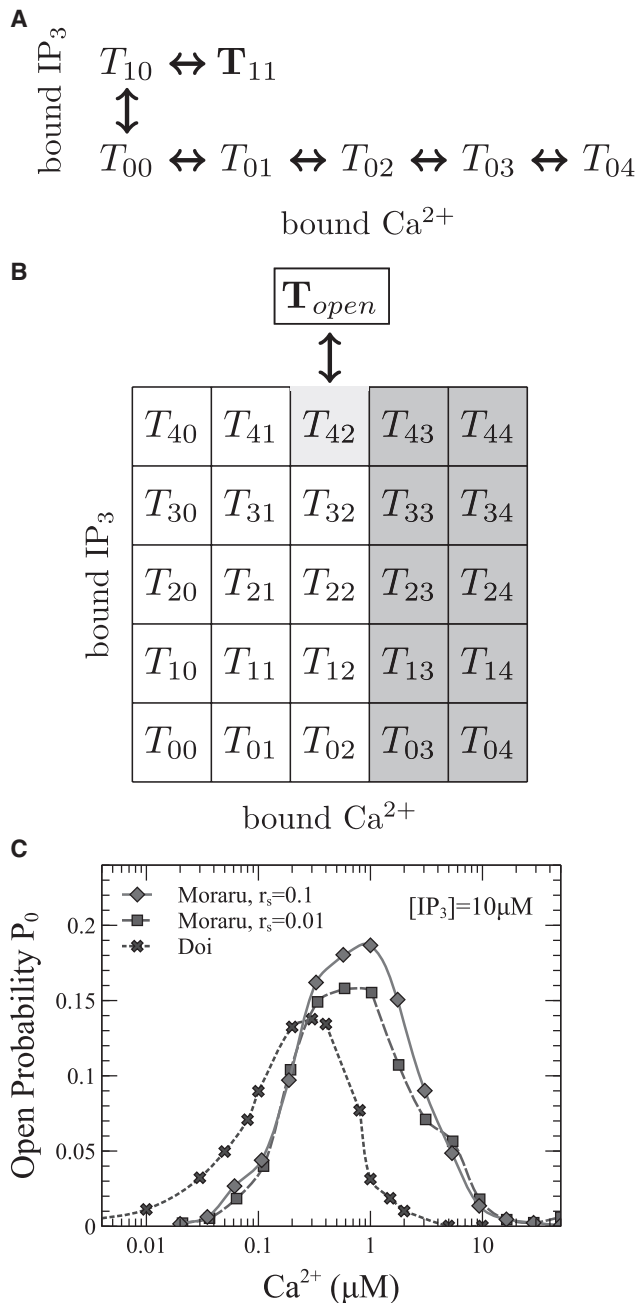


FIGURE 4 (A) Doi's model: T_{00} represents an empty IP₃R. The IP₃R acts as a coincidence detector and only opens if IP₃ binds before Ca²⁺, making T_{11} the open state. Otherwise, it buffers Ca²⁺ and becomes inactivated, preventing binding of IP₃. (B) Subsection of Moraru's model. All vertical and horizontal transitions are possible. When four IP₃ and two Ca²⁺ (activating) are bound, T_{42} (light gray), there is a probability to go into the open state T_{open} , whence additional Ca²⁺ will be released. Further Ca²⁺ binding of the IP₃R will lower the probability of opening, effectively promoting the 10 states T_{x3} and T_{x4} to inhibitory Ca²⁺ states (gray), $x \in [0, 4]$. We set the Ca²⁺ binding and dissociation rates of the inhibitory states to be slower than the rates of the activating states, expressed by ratio r_s . Parameter values can be found in Table S1. (C) IP₃R open probability at constant $[IP_3] = 10 \mu M$ for Doi's (dotted) and our version of Moraru's model with inhibitory Ca²⁺ binding scaling $r_s = \{10^{-1}, 10^{-2}\}$ (dashed and solid, respectively). Data points are results of stochastic computations with Copasi. The rise of the open probability at low Ca²⁺ causes CICR.

of $[Ca^{2+}]$, shown from $t = 350$ – 650 ms (see also Fig. 6). Using many of these simulations, we studied the IP₃-induced Ca²⁺ responses of our model spine to different stimuli. We chose these stimuli in accordance with available experimental data (8,62). We were able to approximately reproduce properties of Ca²⁺ transients in the spine head in response to a PF, CF, PF burst, and PF burst + CF stimulus when using Moraru's IP₃R model, as we will see below.

We focused on the difference of Ca²⁺ transients, especially on the peak values, upon a PF burst and a PF burst with a CF stimulus coactivation at $t_{CF} = 100$ ms after the onset of the PF burst, as these two cases are assumed to encode the induction of LTP and LTD, respectively (38,39,45). We expect the system to show a clear Ca²⁺ transient with a spine head peak of $\sim 2.8 \mu M$ under a PF burst stimulus and a 150% increase to $\sim 7.1 \mu M$ with a CF coactivation (8), showing a supralinear response to summation of stimuli, also generally found in other model approaches (29,63,78) and experiments (44,62).

Robustness of IP₃R dynamics against Ca²⁺ concentration noise: a model with linear activation characteristics

Local concentration fluctuations at IP₃R upon opening or closing are large (79,80), and therefore, channel state noise strongly affects channel state dynamics. In this section, we investigate the noise response of linear Ca²⁺-dependent channel activation in the IP₃-sensitized state as, e.g., Doi's model uses. We and others (63) were able to reproduce dendritic spine Ca²⁺ dynamics in well-mixed conditions with Doi's model as a system of ordinary differential equations (ODEs), generating proper Ca²⁺ transients to different PF and CF stimuli conditions (see also Figs. S6 and S7).

We found with MCell simulations that the stochastic fluctuations of Ca²⁺ in the cytosol prevent any possible rise of the Ca²⁺ transient peak with CF or PF stimuli with linear Ca²⁺-dependent IP₃R channel activation. It takes only one Ca²⁺ to bind to the receptor to open if sufficient IP₃ is already present. Once one or two IP₃R are in the open state just because of basal Ca²⁺ fluctuations, they release enough Ca²⁺ to open more IP₃R to create a global Ca²⁺ transient with a peak $\sim 3.0 \mu M$ (Fig. S5 A). Adding more Ca²⁺ because of PF (Fig. S5, B–D) or CF (Fig. S5 E) stimuli did not show any further peak increase because the transients arising from basal fluctuations are saturated already. Even large CF Ca²⁺ amplitudes left the Ca²⁺ peak values essentially unchanged. The peaks of the Ca²⁺ transients are essentially constant for Ca²⁺ PF amplitudes 0–220 (Fig. S5 F). At large PF amplitudes (>220 Ca²⁺), the inhibitory action of Ca²⁺ on the IP₃R decreased transient amplitudes significantly, as some of the total available IP₃R bind inhibitory Ca²⁺ before a global transient can be initiated. Results from adding a CF stimulus with increasing CF Ca²⁺ amplitude are shown in Fig. S5 F, in which a small peak decrease is visible.

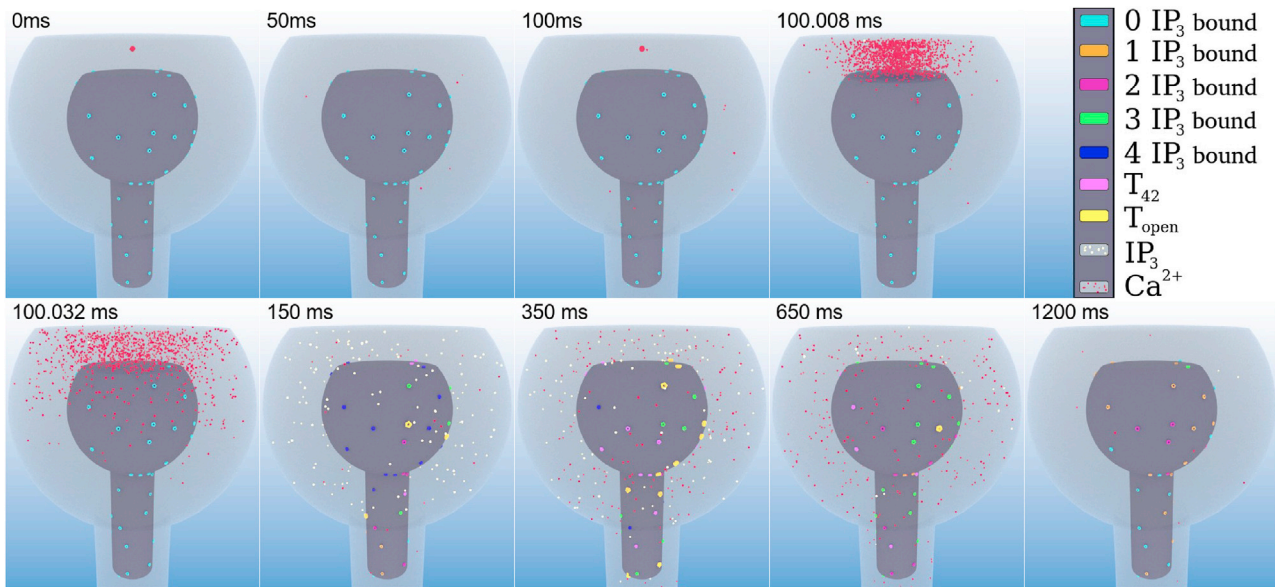


FIGURE 5 Snapshots of the spine head from a typical simulation with Ca^{2+} (red), IP_3 (beige), and IP_3Rs colored according to their state (Moraru's model) as explained by the legend. At $t = 0$ ms, the large red dot shows the 110 Ca^{2+} coming from the first PF stimulus, and at $t \approx 100$ ms, 1700 Ca^{2+} from the CF stimulus. The highly localized Ca^{2+} stimuli very rapidly spread by diffusion and are absorbed by buffers. Spatial averages of the Ca^{2+} concentration are shown in Fig. 6. To see this figure in color, go online.

This high sensitivity to noise of IP_3Rs in this model has not been observed in stochastic simulations based on molecule numbers only, i.e., in non-spatially-resolved simulations (81). Given the same molecule number amplitude, the local concentration amplitude of fluctuations in our spatially detailed simulations is larger than in the spatially lumped simulations of Koumura et al. (81). This effect of local noise most likely explains the different results with respect to noise sensitivity (14,15,82) and renders spatially resolved simulations necessary (83).

Therefore, we turn to a model with nonlinear Ca^{2+} -dependent activation characteristics in the following.

Moraru's IP_3R model

A model with nonlinear Ca^{2+} -activation characteristics like Moraru's model exhibited better robustness against basal fluctuations, and we use it from now on.

Ca^{2+} transient peak response to a PF and CF stimulus

Piochon et al. (8) estimated the peak of the Ca^{2+} transient in the spine head after a single PF stimulus paired with a CF stimulus to be $\sim 0.4 \mu\text{M}$, whereas the response to a single PF stimulus was lost in noise. More interestingly, a PF burst stimulus triggered a Ca^{2+} response with a peak value of $\sim 2.8 \mu\text{M}$, and a peak value of $\sim 7.1 \mu\text{M}$ was reached for a PF burst stimulus with CF coactivation, an increase of $\sim 150\%$. Ca^{2+} peak increase with CF coactivation is crucial for the current understanding of initiation of synaptic plasticity in the

form of long-term depression (LTD) (9,17), even though LTD has also been observed after very strong PF stimulation alone (8,26,84,85).

We were able to reproduce Ca^{2+} transients with peak values in agreement with experimental data for the cases of single PF with additional CF coactivation, PF burst, and PF burst with CF coactivation. Summarized Ca^{2+} results from our simulations for some example parameter sets are shown in Fig. 6 A, where the peak of the Ca^{2+} transients in response to a PF burst and a PF burst + CF stimulus are shown. The peak values of Ca^{2+} transients computed deterministically in Copasi increased clearly with increasing PF and CF Ca^{2+} amplitudes and showed no saturation for tested parameters. The system was very sensitive to CF coactivation (see Figs. S8 and S9).

Averages and the standard deviation (SD) of actual transients of Ca^{2+} are shown in Fig. 6 B. The SD due to the inherent randomness is large but does not blur the difference between a single PF burst and combined PF burst + CF stimulus.

We simulated a single PF stimulus with CF coactivation and obtained an average peak value of $\sim 20 \text{ Ca}^{2+} = 0.40 \mu\text{M}$ from 12 simulations, in agreement with Piochon et al. (8) (Fig. S12).

Closing of IP_3Rs was caused by a mixture of reaching the inhibitory states T_{x3} and T_{x4} with three or four Ca^{2+} bound for larger values of r_s , due to increasing $[\text{Ca}^{2+}]$ during a Ca^{2+} transient (see *bell-shaped open probability curve*, Fig. 4) and IP_3 becoming less available during IP_3 degradation; see IP_3R state occupation videos (Videos S1, S2, S3, and S4) with different values of r_s in the Supporting material.

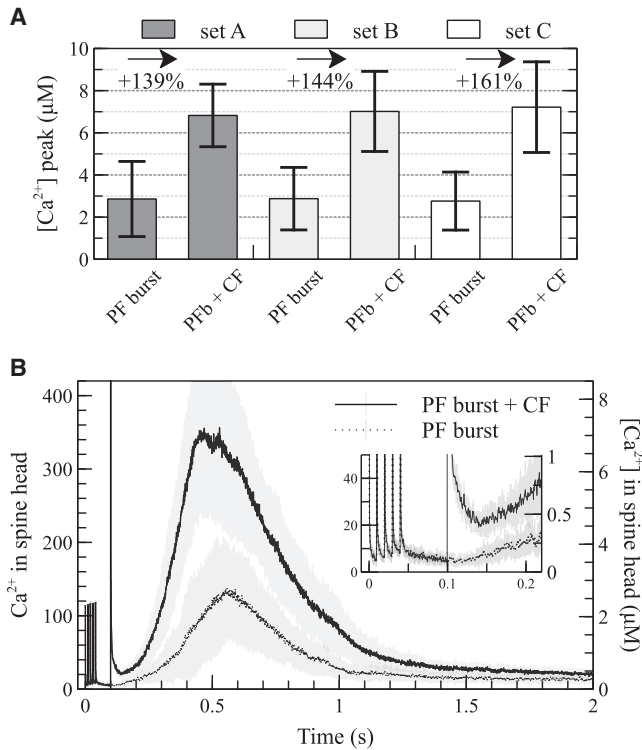


FIGURE 6 (A) Moraru's model: peak values of Ca^{2+} transients in the spine head for different parameter sets to PF burst and PF burst + CF (Pfb + CF) stimuli, averaged over 12 simulations. Error bars show SDs. Parameters are A, $N = 54$ IP_3Rs , $\text{PF} = 90$ Ca^{2+} , and $\text{CF} = 1700$ Ca^{2+} ; B, $N = 54$ IP_3Rs , $\text{PF} = 150$ Ca^{2+} , and $\text{CF} = 1700$ Ca^{2+} ; and C, $N = 56$ IP_3Rs , $\text{PF} = 110$ Ca^{2+} , and $\text{CF} = 1700$ Ca^{2+} . Set C is our control parameter set and will be used from now on. (B) Ca^{2+} in spine head after PF burst (dotted) and PF burst + CF (solid) stimuli with control set. The inset shows a magnification of the first 220 ms. At $t = 0.1$ s, the highly localized Ca^{2+} from the CF stimulus is visible as a spike. The injected Ca^{2+} gets absorbed by buffers immediately explaining the immediate return to lower $[\text{Ca}^{2+}]$ shortly after the stimulus. The averages with SDs (greyish areas) of 12 simulations are shown.

During a typical Ca^{2+} transient, $[\text{Ca}^{2+}]$ stays approximately constant in the spine head, decreases linearly down the neck and becomes constant again in the dendrite segment of the volume, see [Video S5](#).

Results of Ca^{2+} transients to different stimuli conditions and associated total Ca^{2+} release from IP_3Rs with single buffer species removed, showing how single buffer species shape Ca^{2+} transients and influence IP_3R dynamics, are presented in [Fig. S11](#).

Ca^{2+} transient peak scaling with PF and CF Ca^{2+} amplitude

We find that the relation between the peak value of Ca^{2+} transients and the CF Ca^{2+} amplitude is strongly affected by the rate of Ca^{2+} -dependent inhibition of the IP_3Rs ([Table S1](#)). The rate value suggested in the original Moraru model entails saturation at a CF Ca^{2+} amplitude of 500 already (*di-*

amonds and fit with $r_s = 1.0$ in [Fig. 7 A](#)). Peak values increase with CF amplitude over a large range with a smaller rate of inhibitory Ca^{2+} binding to the IP_3R , r_s . We find measured responses of the transient peak to a CF stimulus coactivation and beyond with even larger CF amplitudes with $r_s = 0.01$ (circles, [Fig. 7 A](#)). We used this value throughout the study. The same applies to the response to PF stimuli with various Ca^{2+} amplitudes, showing larger peak values for smaller r_s ([Fig. 7 B](#)). Additionally, increasing PF Ca^{2+} amplitudes (without CF coactivation), mimicking a situation of intense PF stimulation, increases peak values even further, reaching the same or even higher peak values than with CF coactivation and smaller PF Ca^{2+} amplitudes in [Fig. 7 B](#). This resembles the situation in which a strong PF stimulus alone, rather than PF stimulation with CF coactivation, can trigger LTD ([8,26,84,85](#)).

Optimal timing of CF stimulus

The size of the Ca^{2+} transient elicited by the CF stimulus, and with it the induction of LTP and LTD, responds optimally to a certain timing of the CF stimulus relative to the PF stimulus as Wang et al. have shown ([62](#)). They measured the Ca^{2+} response to different timing windows between CF and PF stimulus and used a Gaussian to fit the Ca^{2+} transient's total integrated response to their results, which peaked around 92 ± 37 ms and had a half-width of 212 ± 85 ms.

We simulated these experiments by varying the CF stimulus time from 0 to 400 ms after the initiation of the PF stimulus. We find that the system is sensitive to the timing of the CF stimulus, as shown when determining the Ca^{2+} transient peaks under such CF Ca^{2+} timing variation and also exhibits optimal time windows with a maximal peak ([Fig. 8](#)). We find optimal responses for different strengths of PF stimuli also including the parameter value set A in [Fig. 6](#).

We compare our results in [Fig. S10](#) to the experimental data from Wang et al. ([62](#)).

The rising phase of the Ca^{2+} peaks in [Fig. 8](#) is due to Ca^{2+} -induced Ca^{2+} release (CICR) (see [Fig. 4 C](#)). The CF stimulus causes Ca^{2+} influx. It takes more than 200 ms for this Ca^{2+} rise to decay back close to prestimulus levels ([Figs. 6](#) and [S12](#)). When IP_3 production starts 100 ms after onset of PF stimulation, CICR starts because of the presence of IP_3 and the remaining Ca^{2+} from the CF stimulus. The closer to IP_3 production the CF stimulus occurs, the stronger the CICR. Interestingly, this does not necessarily lead to an optimal response at a timing window at the time of onset of IP_3 production at 100 ms, as the blue and green curves in [Fig. 6](#) show. We did not observe optimal time windows when we released IP_3 at the onset of PF stimulation (data not shown).

The decaying phase of the Ca^{2+} peaks in [Fig. 8](#) toward large time windows is affected by processes terminating Ca^{2+} release. One of them is the decay rate of IP_3 as the

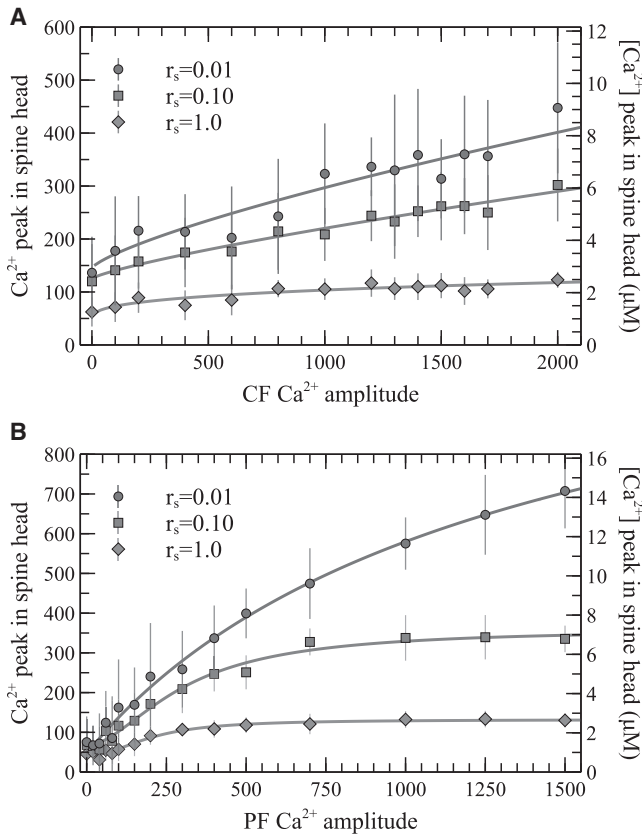


FIGURE 7 Peak scaling of Ca^{2+} transients against (A) CF Ca^{2+} amplitude for the PF burst + CF scenario and (B) against PF Ca^{2+} amplitude for the PF burst scenario for three different inhibitory Ca^{2+} binding time-scale ratios $r_s = \{1.0, 0.1, 0.01\}$; see [Table S1](#). The slower the rate of inhibition, the larger the resulting Ca^{2+} transients for a given CF or PF Ca^{2+} amplitude. The value of r_s sets the largest possible peak value when varying PF and CF Ca^{2+} amplitudes. Large enough transients triggered by CF coactivation with intermediate Ca^{2+} amplitudes are only reached when r_s is on the order of 10^{-2} . Data points are averages of 12 stochastic simulations and were fitted to Hill curves, error bars show SDs.

simulations in [Fig. 9](#) show (which we will discuss in more detail below). The slower IP_3 is removed from the spine head—either by degradation or by diffusing out of the spine—the longer the Ca^{2+} transient.

Ca^{2+} -dependent inhibition is another process contributing to the termination of Ca^{2+} release and affecting the peak dependency on the CF time window as the comparison between the purple curve and all other ones in [Fig. 8](#) shows. Additionally, the peak scaling data in [Fig. 7](#) illustrate the role of Ca^{2+} -dependent inhibition in setting peak height. Increasing the rate of Ca^{2+} -dependent inhibition leads to a longer time window providing optimal response (*purple curve* in comparison to the *blue one*).

However, increasing the inhibition rate by a factor of three does not shorten the Ca^{2+} transient by the same factor and does not abolish optimal time windows because Ca^{2+} -dependent inhibition is only one of several factors shaping the transient.

Ataxia

It has been shown that spinocerebellar ataxia type 29 (SCA29), characterized by early-onset motor delay, hypotonia, and gait ataxia, can be caused by malfunctioning type 1 IP_3Rs ([50](#)). Mutations associated with SCA29 were identified within or near the IP_3 -binding domain. These mutations interfere with the binding of IP_3 and cause IP_3Rs of type 1 to lose any channel activity, reducing or removing IP_3 -induced Ca^{2+} transients.

On that basis, we decrease the IP_3 binding rate k_{on} of the IP_3R model to mimic ataxia, which results in lower or vanishing Ca^{2+} transients. We search to rescue the system from this pathological condition by trying to recover the original Ca^{2+} peak in two ways. First, we increase the amount of IP_3 that enters the system, representing increased activity of the $\text{PLC}\beta$ pathway, which synthesizes IP_3 . In a second approach, we decrease the degradation rate of IP_3 , thus increasing the IP_3 that is available to the IP_3Rs in absolute number as well as in duration.

The first method of increasing IP_3 was only able to recover the Ca^{2+} transients if we increased the amount of IP_3 like $1/k_{on}$ (see [Fig. S13](#)). Because the decrease of k_{on} might be substantial ([50](#)), the $[\text{IP}_3]$ values compensating it are likely beyond the saturation values of the $\text{PLC}\beta$ pathway.

Prolonging IP_3 exposure

We decrease the IP_3R 's binding rate of IP_3 to values possibly representing ataxia and then reduce k_{decay} trying to recover the original Ca^{2+} transient. We start from their standard values $k_{on} = 83.3 (\mu\text{M s})^{-1}$ and $k_{decay} = 15 \text{ s}^{-1}$.

The control Ca^{2+} peak value can be recovered because slower IP_3 degradation increases the amount and duration of IP_3 in the system ([Fig. 9 A](#)), making up for the negative effects of slower IP_3 binding. Additionally, the decrease of k_{decay} leads to prolonged activity of the open IP_3R . The slowed IP_3R dynamics also cause some delay in reaching the Ca^{2+} peak ([Fig. 9 C](#)). Whereas the control parameters yield a Ca^{2+} transient peak at $\sim 0.47 \text{ s}$ (*red*, [Fig. 9 C](#)), slowing IP_3 degradation down to one-sixth $k_{decay} = 2.5 \text{ s}^{-1}$ delays the peak to $\sim 0.65 \text{ s}$ (*orange*), i.e., it increases the response time by $\sim 40\%$ and increases the width of the Ca^{2+} transient.

We provide a more systematic analysis in [Fig. 9 B](#). It shows the peak values in dependence on k_{on} for five different IP_3 decay rates k_{decay} . The red curve shows results with the control value of k_{decay} . The curves with reduced k_{decay} cross the Ca^{2+} peak control value 360 (*red dotted line*) at specific values $k_{on, ax}$, which are smaller than the k_{on} control value. They are related to k_{decay} approximately by $k_{decay} \approx \frac{k_{on, ax}}{6.0 \mu\text{M}^{-1}}$. Simulations with the parameter value pairs $(k_{decay}, k_{on, ax})$ calculated according to this equation provide control of Ca^{2+} peak values with our control parameter set for all other parameter values. A decay rate reduction calculated according to this equation compensates the pathological reduction of $k_{on, ax}$ with respect to the Ca^{2+} transient peak.

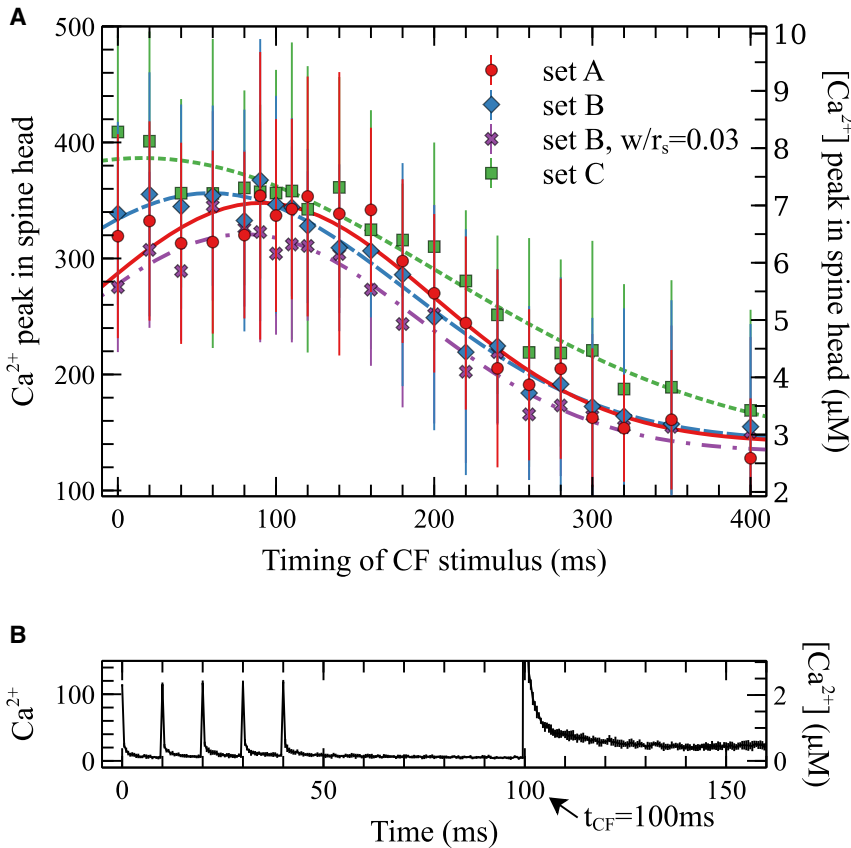


FIGURE 8 (A) Peak of Ca²⁺ transients against timing of CF stimulus for three parameter sets from Fig. 6 and an additional one with $r_s = 0.03$. 12 stochastic simulations per data point were used, and points were fitted to Gaussians with the condition to converge against peak values of PF burst stimulation alone for very large and small t_{CF} . Error Bars indicate SDs. The maxima of the Gaussian fits occur at 91 ms (red), 59 ms (blue), 16 ms (green), and 80 ms (purple), in qualitative agreement with experimental data (62) ($+92 \pm 37$ ms). The half-widths of the Gaussian fits are 257 ms (red), 294 ms (blue), 443 ms (green), and 260 ms (purple), in qualitative agreement with experimental data (62) (212 ± 85 ms). (B) Illustrating PF and CF stimulus timing by showing five Ca²⁺ spikes in spine head from a PF burst stimulus at 100 Hz from 0 to 40 ms and a Ca²⁺ spike from a CF stimulus, here $t_{CF} = 100$ ms. To see this figure in color, go online.

DISCUSSION

Cerebellar learning theories suggest that learning is expressed as a change of neuronal weights, i.e., synaptic strengths, reflecting the topological properties of a neuronal network state. Understanding learning therefore requires knowledge of the molecular mechanisms assumed to encode synaptic plasticity and information transmission at the lowest neuronal level, which are Ca²⁺ transients and the associated cell responses in synapses of spines, eventually. In Purkinje neurons, the IP₃-induced Ca²⁺ transients are dynamical responses to outer stimuli from PFs or CFs happening at low Ca²⁺ concentrations. Whereas PFs are assumed to carry information about movement and fine motor control, CFs are assumed to carry error information that gives feedback about the network state that triggered the movement (86–88). We developed a model that is based on complex single-particle stochastic reaction and diffusion processes within a small three-dimensional geometry to study Ca²⁺ transients in response to dynamical PF and CF stimuli.

Our use of three-dimensional stochastic simulations demonstrated the necessity for IP₃R models to filter out Ca²⁺ binding noise to a sufficient degree. A linear relation between [Ca²⁺] and the open probability at small concentrations appears not to provide that filtering and entailed Ca²⁺ dynamics insensitive to CF and PF stimulus Ca²⁺ ampli-

tudes. However, an increase of the Ca²⁺ transient peak due to a CF stimulus provides meaning to this stimulus and is thus a necessary model requirement.

Using Moraru’s IP₃R model to provide sufficient noise filtering, we were able to reproduce the dynamic behavior of the Ca²⁺ transients from experiment with respect to the absolute and relative peak values of Ca²⁺ transients under stimuli (8) and the behavior of peaks under variation of the timing of the CF stimulus (62).

The signal of the CF stimulus turning LTP into LTD might be binary information or graded information. If simply the presence of a stimulus entails LTD, we face binary signaling. If the strength of the stimulus encodes the strength of depression, we see a graded response. We found this characteristic of the signaling by the CF stimulus to depend on the rate of Ca²⁺-dependent inhibition of the IP₃R. We achieved agreement of Ca²⁺ transient peak values with experimental results at slow inhibition rates. Although these rates are slower than originally suggested by the authors of the model, they are still compatible with puff data of the IP₃R taking the large local [Ca²⁺] at puff sites into account (79,89,90). In summary, these simulation results suggest a graded response of the Ca²⁺ transients’ peak value to the CF Ca²⁺ amplitude.

Using our model also allowed for detailed tests on the effects of endogenous Ca²⁺ buffer molecules. We find clear indication that buffers do not only passively shape

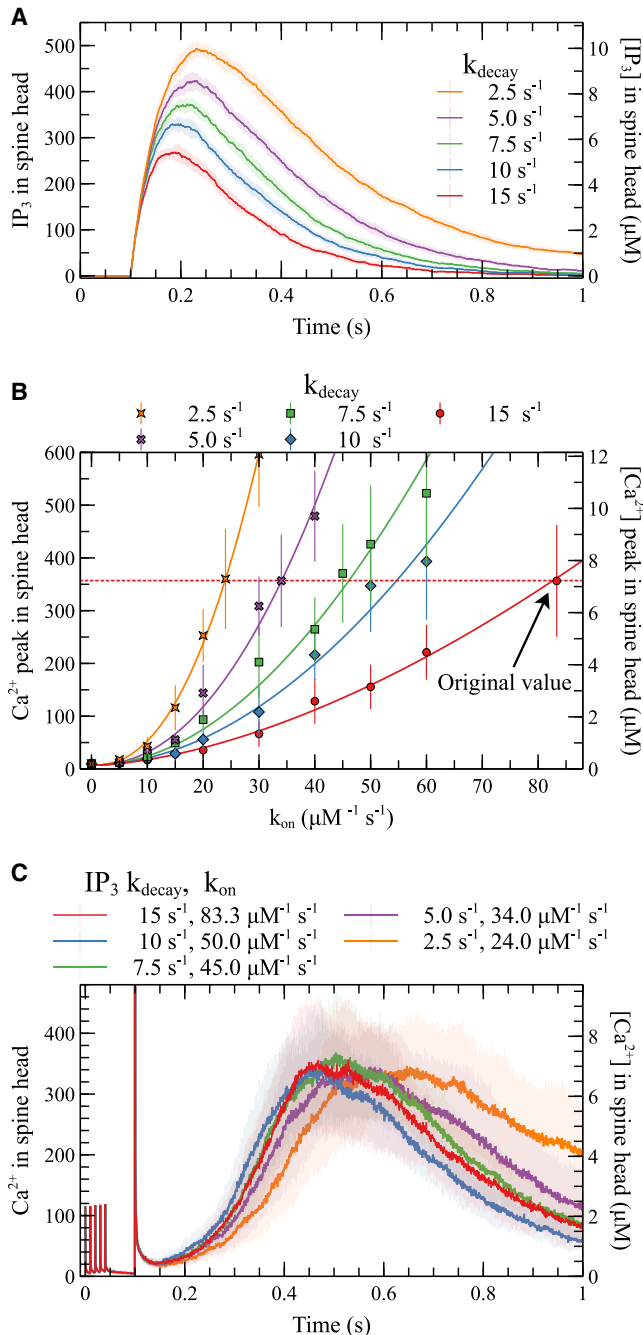


FIGURE 9 (A) Time courses of average $[\text{IP}_3](t)$ and SDs in the spine head for different IP_3 decay rates are shown. (B) Data points show averages of Ca^{2+} peak values and SDs for different values of IP_3 decay rates k_{decay} against the binding rate of IP_3 . Each color and fit represent one decay rate. Binding rates smaller than the original value possibly represent ataxia. (C) Averages of 12 Ca^{2+} transients and SDs for cases with modified IP_3 decay and binding rates, taken from (B), that approximately peak around the original peak value of 360 Ca^{2+} (dotted red line in (B)) are shown. To see this figure in color, go online.

amplitude and decay and rise times of Ca^{2+} transients but actively modulate state dynamics of IP_3 Rs, resulting in an increase or decrease of released Ca^{2+} .

It has been experimentally established that the timing window of PF and CF stimuli is critical to the induction of LTP and LTD and thereby also to the properties of the IP_3 R-induced Ca^{2+} transients (62). The mechanism our results suggest is that the relative timing of IP_3 production elicited by PF stimuli, which sensitizes IP_3 Rs for CICR to the moment of the influx of Ca^{2+} due to the CF stimulus causing CICR, sets the optimal time window.

We simulated ataxia on the basis of the assumption that it manifests itself in our model by a substantially reduced rate of binding of IP_3 to the IP_3 R. Reducing the rate of IP_3 degradation by IP_3 3-kinase and IP_3 5-phosphatase turned out to be able to compensate the reduced binding without strong stimulation of the PLC pathway. Whether the recovered peak values are enough to trigger an increase of AMPA receptors in agreement with observations by Piochon et al. (8), even with delayed peak times and decay of the Ca^{2+} transients, is an open question left for future research. Its outcome decides whether reduction of IP_3 degradation offers new ways of addressing ataxia pharmacologically.

SUPPORTING MATERIAL

Supporting material can be found online at <https://doi.org/10.1016/j.bpj.2021.03.027>.

AUTHOR CONTRIBUTIONS

G.A. and F.M.S.d.S. designed research. V.N.F. performed research, contributed analytic tools. M.F. supervised simulations. V.N.F., M.F., and F.M.S.d.S. analyzed data and wrote the manuscript.

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SUPPORTING CITATIONS

References (91–97) appear in the Supporting material.

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