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

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ABSTRACT Calcium (Ca²⁺) 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 (IP₃)-induced Ca²⁺ release. These Ca²⁺ 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 Ca²⁺, requiring stochastic single-particle methods when modeling them. We use the stochastic particle simulation program MCell to simulate Ca²⁺ transients within a three-dimensional Purkinje cell dendritic spine. The model spine includes the endoplasmic reticulum, several Ca²⁺ transporters, and endogenous buffer molecules. Our simulations successfully reproduce properties of Ca²⁺ transients in different dynamical situations. We test two different models of the IP₃ receptor (IP₃R). The model with nonlinear concentration response of binding of activating Ca²⁺ reproduces experimental results better than the model with linear response because of the filtering of noise. Our results also suggest that Ca²⁺-dependent inhibition of the IP₃R 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 Ca²⁺ and IP₃ production sensitizing IP₃R for Ca²⁺-induced Ca²⁺ 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 Ca²⁺ transients. Finally, we propose possible ways of recovering Ca²⁺ transients under ataxia.

INTRODUCTION

Ca²⁺ is a second messenger involved in many processes in eukaryotic cells. Ca²⁺ 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 Ca²⁺ release from endoplasmatic reticulum intracellular stores through inositol trisphosphate receptors (IP₃Rs). This response can be further enhanced by a well-timed additional Ca²⁺ influx triggered by climbing fiber (CF) coactivation (Fig. 1; (6,7)).

Frequently, the detection and discrimination of transient Ca²⁺ signals by molecular targets in dendritic spines happen outside mass-action equilibrium, at low particle concentrations of Ca²⁺ 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 Ca²⁺ signals can play an important role in the induction of synaptic plasticity (8–12), determining whether long-term potentiation (LTP) or depression (LTD) occurs.

SIGNIFICANCE Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ 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 Ca²⁺ signals in healthy and pathological conditions and propose a way to recover those Ca²⁺ signals in pathological conditions.
Stochastic calcium dynamics in spines

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 Ca$^{2+}$ 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 Ca$^{2+}$ transients. Rapid Ca$^{2+}$ release is achieved by opening of IP$_3$Rs, which reside on the smooth endoplasmic reticulum (ER), a Ca$^{2+}$ store inside the spine and dendrite (6,7). IP$_3$Rs require inositol-1,4,5-trisphosphate (IP$_3$) and Ca$^{2+}$ to open. Both IP$_3$ production and Ca$^{2+}$ influx are controlled by PF and CF activity (25–28). These interacting signaling pathways give the IP$_3$Rs the capacity to translate fast PF and CF inputs into longer-lasting slow-output Ca$^{2+}$ 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 IP$_3$ from PIP$_2$. IP$_3$ is free to diffuse from the PSD into the dendrite’s cytosol (30–32) before it vanishes because of degradation by IP$_3$ 3-kinase and IP$_3$ 5-phosphatase on the timescale of a few seconds (33,34). The second pathway consists of a membrane depolarization, causing Ca$^{2+}$ influx through voltage-gated Ca$^{2+}$ channels (Ca,2.1 type P/Q-voltage-gated Ca$^{2+}$ 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 Ca$^{2+}$ channels, which leads to Ca$^{2+}$ 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 10$^5$ 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 Ca$^{2+}$ 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 Ca$^{2+}$ 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 IP$_3$Rs (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 Ca$^{2+}$ transients under reduced IP$_3$ binding rates of IP$_3$Rs and then suggest ways to restore previous Ca$^{2+}$ transients.

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 Ca$^{2+}$ transient because of an opening of IP$_3$Rs on the ER (6,7). To see this figure in color, go online.
A

![Diagram showing the dendrite-neck-head volume](image)

**FIGURE 2** (A) Model geometry. The endoplasmic reticulum (ER) is visible in the head and neck. \( \text{V}_{\text{total}} = 0.512 \ \mu m^3 \), \( \text{V}_{\text{head}} = 0.100 \ \mu m^3 \), and \( \text{V}_{\text{ER}} = 0.020 \ \mu m^3 \). 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 PMCA and 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 \( \text{V}_{\text{head}} = 0.1 \ \mu m^3 \). The total volume dendrite, neck and head, is \( \sim 5 \) times as large, \( V_{\text{total}} = 0.512 \ \mu m^3 \) (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 \( V_{\text{ER}} = 0.02 \ \mu m^3 \). 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 \( 2 \times 10^5 \).

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**

SERCAs 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 [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 [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).
CaM can hold up to four Ca$^{2+}$ of Ca$^{2+}$ in karyotic cells. It is not only a buffer but also acts as a messenger target and various reaction rates (66).}

A seven-state model with various medium- and high-affinity binding sites and IP3R-related slow timescales.

FIGURE 3 Overview of buffer models for (A) Pv, (B) Cb, and (C) CaM. CaM can hold up to four Ca$^{2+}$. We used a 16-state model with individual binding sites. For ease of reading, we omitted the six states with two bound Ca$^{2+}$. Reaction rates for all can be found in Table S3.

Cb is a major Ca$^{2+}$ 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 Ca$^{2+}$.

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 Ca$^{2+}$. Once Ca$^{2+}$ is bound to CaM, it can modify the interactions of Ca$^{2+}$ with various other targets like phosphatases or kinases. One CaM protein has four Ca$^{2+}$ binding sites. We modeled the interactions with Ca$^{2+}$ using a 16-state model with various medium- and high-affinity binding sites and various reaction rates (66).

IP$_3$R models

We used 54 or 56 IP$_3$Rs (69) on the ER membrane, depending on the parameter set. A large variety of IP$_3$R 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). The model is able to reproduce 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 $T_{a2}$ and $T_{open}$ (Fig. S1). Because it is known that the binding dynamics of inhibitory Ca$^{2+}$ is slower than that of excitatory Ca$^{2+}$ by a factor of up to 100 (70,76), we decreased the inhibitory Ca$^{2+}$ binding reaction rates into and from states $T_{a3}$ and $T_{a4}$, which represent the inhibitory Ca$^{2+}$ binding sites in this model, by an additional factor $r$, (see Fig. 4 B; Table S1).

Parallel and climbing fiber stimulation

We studied the Ca$^{2+}$ 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 PPs was simulated by plasma membrane influx of Ca$^{2+}$ and IP$_3$ production close to the PSD. CF stimulation was modeled by Ca$^{2+}$ influx close to the PSD and into the dendrite end close to the spine neck (Fig. 2).

We chose different amounts of Ca$^{2+}$ per PF and CF stimuli (0–1500 Ca$^{2+}$ and 0–2000 Ca$^{2+}$, respectively) as part of our parameter scan. A single PF stimulus consists of one instantaneous injection of Ca$^{2+}$ and production of IP$_3$ (380 close to the PSD), and a PF burst was made up of five single Ca$^{2+}$ injections at 100 Hz and 1400 caged IP$_3$s, yielding an ~5 times larger IP$_3$ transient. In the latter, more relevant case, free [IP$_3$] usually peaks around 4.5–5 μM. A CF stimulus included an additional Ca$^{2+}$ 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 stimulus but was varied in optimal timing of CF stimulus.

IP$_3$ dynamics used constant production and decay rates and was able to capture biexponential IP$_3$ concentration behavior (33,34,63,77). We chose an amount of IP$_3$ in agreement with physiological concentrations (6) such that the IP$_3$Rs were saturated with IP$_3$ for the case of a PF burst. IP$_3$ production was delayed by 100 ms compared to the onset of the PF Ca$^{2+}$ influx to account for the slower process of IP$_3$ synthesis compared to instantaneous Ca$^{2+}$ influx from PF and CF stimuli. IP$_3$ diffuses freely in our model.

RESULTS

We successfully constructed a three-dimensional stochastic reaction-diffusion model of Purkinje cell dendritic spine Ca$^{2+}$ 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 Ca$^{2+}$ associated with a PF or CF stimulus.

Snapshots of the spine head including Ca$^{2+}$, IP$_3$, and IP$_3$R 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 Ca$^{2+}$ of the first PF stimulus. The particles diffuse and get absorbed by buffers immediately. The CF stimulus consists of 1700 Ca$^{2+}$, which is visible in three frames corresponding to $t = 100$ ms to $t = 100.032$ ms, showing how quickly Ca$^{2+}$ diffuses. IP$_3$ slowly enters the system at the same time at $t = 100$ ms (see also Fig. 9 A). IP$_3$Rs start to react to IP$_3$ and increased [Ca$^{2+}$] (note changing colors of IP$_3$Rs). Eventually, a global Ca$^{2+}$ transient is initiated, which leads to a prolonged increase
of \([\text{Ca}^{2+}]\), shown from \(t = 350–650\) ms (see also Fig. 6). Using many of these simulations, we studied the IP3-induced \(\text{Ca}^{2+}\) 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 \(\text{Ca}^{2+}\) 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 \(\text{Ca}^{2+}\) transients, especially on the peak values, upon a PF burst and a PF burst with a CF stimulus coactivation at \(t_{\text{CF}} = 100\) 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 \(\text{Ca}^{2+}\) transient with a spine head peak of \(\sim 2.8\) \(\mu\text{M}\) under a PF burst stimulus and a 150% increase to \(\sim 7.1\) \(\mu\text{M}\) 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 IP3Rs 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 \(\text{Ca}^{2+}\)-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 \(\text{Ca}^{2+}\) dynamics in well-mixed conditions with Doi’s model as a system of ordinary differential equations (ODEs), generating proper \(\text{Ca}^{2+}\) transients to different PF and CF stimuli conditions (see also Figs. S6 and S7).

We found with MCell simulations that the stochastic fluctuations of \(\text{Ca}^{2+}\) in the cytosol prevent any possible rise of the \(\text{Ca}^{2+}\) transient peak with CF or PF stimuli with linear \(\text{Ca}^{2+}\)-dependent IP3R channel activation. It takes only one \(\text{Ca}^{2+}\) to bind to the receptor to open if sufficient IP3 is already present. Once one or two IP3Rs are in the open state just because of basal \(\text{Ca}^{2+}\) fluctuations, they release enough \(\text{Ca}^{2+}\) to open more IP3Rs to create a global \(\text{Ca}^{2+}\) transient with a peak \(\sim 3.0\) \(\mu\text{M}\) (Fig. S5 A). Adding more \(\text{Ca}^{2+}\) 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 \(\text{Ca}^{2+}\) amplitudes left the \(\text{Ca}^{2+}\) peak values essentially unchanged. The peaks of the \(\text{Ca}^{2+}\) transients are essentially constant for \(\text{Ca}^{2+}\) PF amplitudes 0–220 (Fig. S5 F). At large PF amplitudes (>220 \(\text{Ca}^{2+}\)), the inhibitory action of \(\text{Ca}^{2+}\) on the IP3Rs decreased transient amplitudes significantly, as some of the total available IP3Rs bind inhibitory \(\text{Ca}^{2+}\) before a global transient can be initiated. Results from adding a CF stimulus with increasing CF \(\text{Ca}^{2+}\) amplitude are shown in Fig. S5 F, in which a small peak decrease is visible.
This high sensitivity to noise of IP3Rs 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 IP3R 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 ~0.4 \(\mu\)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 ~2.8 \(\mu\)M, and a peak value of ~7.1 \(\mu\)M was reached for a PF burst stimulus with CF coactivation, an increase of ~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 ~20 Ca\(^{2+}\) = 0.40 \(\mu\)M from 12 simulations, in agreement with Piochon et al. (8) (Fig. S12).

Closing of IP3Rs 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 [Ca\(^{2+}\)] during a Ca\(^{2+}\) transient (see bell-shaped open probability curve, Fig. 4) and IP3 becoming less available during IP3 degradation; see IP3R state occupation videos (Videos S1, S2, S3, and S4) with different values of \(r_s\) in the Supporting material.
B, N
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Biophysical Journal
ulations are shown.
shortly after the stimulus. The averages with SDs (Friedhoff et al.
Parameters are A, N
PFb
þ
spine head for different parameter sets to PF burst and PF burst
IP3Rs, PF
¼
1700 Ca2
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optimal time windows with a maximal peak (Fig. 8). We find
measured responses of the transient peak to a CF stimulus coactivation and beyond with even larger CF amplitudes with r
= 0.01 (circles, Fig. 7 A). We used this value throughout the study. The same applies to the response to PF stimuli with various Ca2
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amplitudes, showing larger peak values for smaller r
(Fig. 7 B). Additionally, increasing PF Ca2
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amplitude (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 Ca2
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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 Ca2
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transient elicited by the CF stimulus, and with it the induction of LTP and LTD, responds opti-

dally to a certain timing of the CF stimulus relative to the PF stimulus as Wang et al. have shown (62). They measured the Ca2
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response to different timing windows between CF and PF stimulus and used a Gaussian to fit the Ca2
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transient’s total integrated response to their re-
results, which peaked around 92 ± 37 ms and had a half-
width of 212 ± 85 ms.

We simulated these experiments by varying the CF stim-
ulus time from 0 to 400 ms after the initiation of the PF stim-
ulus. We find that the system is sensitive to the timing of the CF stimulus, as shown when determining the Ca2
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transient under such CF Ca2
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timing variation and also exhibits optima-
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 Ca2
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transients in Fig. 8 is due to
Ca2
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-induced Ca2 release (CICR) (see Fig. 4 C). The CF stimulus causes Ca2
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influx. It takes more than 200 ms for this Ca2
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rise to decay back close to prestimulus levels (Figs. 6 and S12). When IP3 production starts 100 ms after
onset of PF stimulation, CICR starts because of the presence
of IP3 and the remaining Ca2
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from the CF stimulus. The closer to IP3 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 IP3 production at 100 ms, as the blue and green curves in Fig. 6 show. We did not observe optimal time windows when we released IP3 at the onset of PF stimulation (data not shown).

The decaying phase of the Ca2
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transients in Fig. 8 toward large time windows is affected by processes terminating Ca2
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release. One of them is the decay rate of IP3 as the

amonds and fit with r
= 1.0 in Fig. 7 A). Peak values in-
crease with CF amplitude over a large range with a smaller rate of inhibitory Ca2
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binding to the IP3,R, r
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transients in Fig. 8 toward large time windows is affected by processes terminating Ca2
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release. One of them is the decay rate of IP3 as the

During a typical Ca2
þ
transient, [Ca2
þ
] 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 Ca2
þ
transients to different stimuli conditions and associated total Ca2
þ
release from IP3,Rs with single buffer species removed, showing how single buffer species shape Ca2
þ
transients and influence IP3,R dynamics, are presented in Fig. S11.

Ca2
þ
transient peak scaling with PF and CF Ca2
þ
amplitude
We find that the relation between the peak value of Ca2
þ
transients and the CF Ca2
þ
amplitude is strongly affected by the rate of Ca2
þ
-dependent inhibition of the IP3,R (Table S1). The rate value suggested in the original Moraru model entails saturation at a CF Ca2
þ
amplitude of 500 already (di-
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$_3$Rs (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$_3$Rs 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$_3$R 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 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$_3$Rs 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 1/10 $k_{on}$ (see Fig. S13). Because the decrease of $k_{on}$ might be substantial (50), the [IP$_3$] values compensating it are likely beyond the saturation values of the PLC$\beta$ pathway.

Prolonging IP$_3$ exposure

We decrease the IP$_3$R’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 $k_{on} = 83.3$ (\(\mu\)M s$^{-1}$) and $k_{decay} = 15$ 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$_3$R. The slowed IP$_3$R 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$0.47 s (red, Fig. 9 C), slowing IP$_3$ degradation down to one-sixth $k_{decay} = 2.5$ s$^{-1}$ delays the peak to $\sim$0.65 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 $k_{decay} \approx \frac{k_{on}}{6.0 \mu\text{M}}$. Simulations with the parameter value pairs of ($k_{decay}, k_{on, ax}$) 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.
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 Ca\(^{2+}\) transients and the associated cell responses in synapses of spines, eventually. In Purkinje neurons, the IP\(_3\)-induced Ca\(^{2+}\) transients are dynamical responses to outer stimuli from PFs or CFs happening at low Ca\(^{2+}\) 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 Ca\(^{2+}\) transients in response to dynamical PF and CF stimuli.

Our use of three-dimensional stochastic simulations demonstrated the necessity for IP\(_3\)R models to filter out Ca\(^{2+}\) binding noise to a sufficient degree. A linear relation between [Ca\(^{2+}\)] and the open probability at small concentrations appears not to provide that filtering and entailed Ca\(^{2+}\) dynamics insensitive to CF and PF stimulus Ca\(^{2+}\) amplitudes. However, an increase of the Ca\(^{2+}\) transient peak due to a CF stimulus provides meaning to this stimulus and is thus a necessary model requirement.

Using Moraru’s IP\(_3\)R model to provide sufficient noise filtering, we were able to reproduce the dynamic behavior of the Ca\(^{2+}\) transients from experiment with respect to the absolute and relative peak values of Ca\(^{2+}\) 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 Ca\(^{2+}\)-dependent inhibition of the IP\(_3\)R. We achieved agreement of Ca\(^{2+}\) 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 IP\(_3\)R taking the large local [Ca\(^{2+}\)] at puff sites into account (79,89,90). In summary, these simulation results suggest a graded response of the Ca\(^{2+}\) transients’ peak value to the CF Ca\(^{2+}\) amplitude.

Using our model also allowed for detailed tests on the effects of endogenous Ca\(^{2+}\) buffer molecules. We find clear indication that buffers do not only passively shape
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.

**ACKNOWLEDGMENTS**

This manuscript was developed within the scope of the IRTG 1740/TRP 2015/50122-0 and IRTG 1740/TRP2011/50151-0, funded by the DFG/FA-PESP, and the DFG grant FA 350/13-1 to M.F. and FAPESP grant 2018/06504-4 to F.M.S.d.S.

**SUPPORTING CITATIONS**

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

**REFERENCES**


