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.
Stochastic calcium dynamics in spines

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.

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 C\(\beta\) (PLC\(\beta\)), 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 \(\mu\)m 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.
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A  

![Diagram of model geometry](image)

B  

![Diagram of reaction scheme](image)

**FIGURE 2**  (A) Model geometry. The endoplasmic reticulum (ER) is visible in the head and neck. \( V_{\text{total}} = 0.512 \, \mu m^3 \), \( V_{\text{head}} = 0.100 \, \mu m^3 \), and \( V_{\text{ER}} = 0.020 \, \mu m^3 \). 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 PMCs, 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 \( V_{\text{head}} = 0.1 \, \mu m^3 \). 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 (63), 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 PMCs (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\(^{2+}\)] \( \approx 50 \, nM \) 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\(^{2+}\) for karyotic cells. It is not only a buffer but also acts as a messenger target and various reaction rates (66).

We picked Moraru’s model for comparison because its open probability depends nonlinearly on [Ca\(^{2+}\)], and it is eligible for easy implementation in MCell (74). This choice allows for statements on the role of IP\(_3\)R inhibition and IP\(_3\)R-related slow timescales.

**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).

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 PFs 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\(_3\), yielding an ~5 times larger IP\(_3\) transient. In the latter, more relevant case, free [IP\(_3\)] usually peaks around 4.5–5 \(\mu\)M. 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 ~100 ms after the initiation of the PF stimuli 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 in the open probability of the receptors.
of $[\text{Ca}^{2+}]$, shown from $t = 350–650$ ms (see also Fig. 6). Using many of these simulations, we studied the IP$_3$-induced 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 Ca$^{2+}$ transients in the spine head in response to a PF, CF, PF burst, and PF burst + CF stimulus when using Moraru’s IP$_3$R model, as we will see below.

We focused on the difference of Ca$^{2+}$ 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 Ca$^{2+}$ 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 IP$_3$R dynamics against Ca$^{2+}$ concentration noise: a model with linear activation characteristics

Local concentration fluctuations at IP$_3$Rs 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 Ca$^{2+}$-dependent channel activation in the IP$_3$-sensitized state as, e.g., Doi’s model uses. We and others (63) were able to reproduce dendritic spine Ca$^{2+}$ dynamics in well-mixed conditions with Doi’s model as a system of ordinary differential equations (ODEs), generating proper Ca$^{2+}$ transients to different PF and CF stimuli conditions (see also Figs. S6 and S7).

We found with MCCell simulations that the stochastic fluctuations of Ca$^{2+}$ in the cytosol prevent any possible rise of the Ca$^{2+}$ transient peak with CF or PF stimuli with linear Ca$^{2+}$-dependent IP$_3$R channel activation. It takes only one Ca$^{2+}$ to bind to the receptor to open if sufficient IP$_3$ is already present. Once one or two IP$_3$Rs are in the open state just because of basal Ca$^{2+}$ fluctuations, they release enough Ca$^{2+}$ to open more IP$_3$Rs to create a global Ca$^{2+}$ transient with a peak $\sim$3.0 $\mu$M (Fig. S5A). Adding more 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 Ca$^{2+}$ amplitudes left the Ca$^{2+}$ peak values essentially unchanged. The peaks of the Ca$^{2+}$ transients are essentially constant for Ca$^{2+}$ PF amplitudes $0–220$ (Fig. S5 F). At large PF amplitudes (>220 Ca$^{2+}$), the inhibitory action of Ca$^{2+}$ on the IP$_3$Rs decreased transient amplitudes significantly, as some of the total available IP$_3$Rs bind inhibitory Ca$^{2+}$ before a global transient can be initiated. Results from adding a CF stimulus with increasing CF Ca$^{2+}$ amplitude are shown in Fig. S5 F, in which a small peak decrease is visible.
This high sensitivity to noise of IP$_3$Rs 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$_3$R 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 $\mu$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 $\mu$M, and a peak value of $\sim$7.1 $\mu$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$20 Ca$^{2+}$ = 0.40 $\mu$M from 12 simulations, in agreement with Piochon et al. (8) (Fig. S12).

Closing of IP$_3$Rs 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 $r_x$ due to increasing [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$_3$R state occupation videos (Videos S1, S2, S3, and S4) with different values of $r_x$ in the Supporting material.
During a typical Ca\(^{2+}\) transient, [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\(_3\)Rs with single buffer species removed, showing how single buffer species shape Ca\(^{2+}\) transients and influence IP\(_3\)R 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\(_3\)R (Table S1). The rate value suggested in the original Moraru model entails saturation at a CF Ca\(^{2+}\) amplitude of 500 already (di-

**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 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 Ca2 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
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 IP₃Rs (50). Mutations associated with SCA29 were identified within or near the IP₃-binding domain. These mutations interfere with the binding of IP₃ and cause IP₃Rs of type 1 to lose any channel activity, reducing or removing IP₃-induced Ca²⁺ transients.

On that basis, we decrease the IP₃ binding rate kₗₜₗₗ of the IP₃R model to mimic ataxia, which results in lower or vanishing Ca²⁺ transients. We search to rescue the system from this pathological condition by trying to recover the original Ca²⁺ peak in two ways. First, we increase the amount of IP₃ that enters the system, representing increased activity of the PLCβ pathway, which synthesizes IP₃. In a second approach, we decrease the degradation rate of IP₃, thus increasing the IP₃ that is available to the IP₃Rs in absolute number as well as in duration.

The first method of increasing IP₃ was only able to recover the Ca²⁺ transients if we increased the amount of IP₃ like 1/kₗₜₗₗ (see Fig. S13). Because the decrease of kₗₜₗₗ might be substantial (50), the [IP₃] values compensating it are likely beyond the saturation values of the PLCβ pathway.

Prolonging IP₃ exposure

We decrease the IP₃R’s binding rate of IP₃ to values possibly representing ataxia and then reduce kₗₜₗₗ trying to recover the original Ca²⁺ transient. We start from their standard values kₗₜₗₗ = 83.3 (μM s⁻¹) and kₗₜₗₗ = 15 s⁻¹.

The control Ca²⁺ peak value can be recovered because slower IP₃ degradation increases the amount and duration of IP₃ in the system (Fig. 9 A), making up for the negative effects of slower IP₃ binding. Additionally, the decrease of kₗₜₗₗ leads to prolonged activity of the open IP₃R. The slowed IP₃ dynamics also cause some delay in reaching the Ca²⁺ peak (Fig. 9 C). Whereas the control parameters yield a Ca²⁺ transient peak at ~0.47 s (red, Fig. 9 C), slowing IP₃ degradation down to one-sixth kₗₜₗₗ = 2.5 s⁻¹ delays the peak to ~0.65 s (orange), i.e., it increases the response time by ~40% and increases the width of the Ca²⁺ transient.

We provide a more systematic analysis in Fig. 9 B. It shows the peak values in dependence on kₗₜₗₗ for five different IP₃ decay rates kₗₜₗₗ. The red curve shows results with the control value of kₗₜₗₗ. The curves with reduced kₗₜₗₗ cross the Ca²⁺ peak control value 360 (red dotted line) at specific values kₗₜₗₗ, ax, which are smaller than the kₗₜₗₗ control value. They are related to kₗₜₗₗ approximately by kₗₜₗₗ ≈ kₗₜₗₗ / 3. Simulations with the parameter value pairs of (kₗₜₗₗ, kₗₜₗₗ, ax) calculated according to this equation provide control of Ca²⁺ 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 kₗₜₗₗ with respect to the Ca²⁺ transient peak.

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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
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$_3$-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**

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