Two Distinct Sets of Ca^{2+} and K^{+} Channels Are Activated at Different Membrane Potentials by the Climbing Fiber Synaptic Potential in Purkinje Neuron Dendrites

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In cerebellar Purkinje neuron dendrites, the transient depolarization associated with a climbing fiber (CF) EPSP activates voltage-gated Ca^{2+} channels (VGCCs), voltage-gated K^{+} channels (VGKCs), and Ca^{2+} -activated SK and BK K^{+} channels. The resulting membrane potential (V_m) and Ca^{2+} transients play a fundamental role in dendritic integration and synaptic plasticity of parallel fiber inputs. Here we report a detailed investigation of the kinetics of dendritic Ca^{2+} and K^{+} channels activated by CF-EPSPs, based on optical measurements of V_m and Ca^{2+} transients and on a single-compartment NEURON model reproducing experimental data. We first measured V_m and Ca^{2+} transients associated with CF-EPSPs at different initial V_m , and we analyzed the changes in the Ca^{2+} transients produced by the block of each individual VGCCs, of A-type VGKCs and of SK and BK channels. Then, we constructed a model that includes six active ion channels to accurately match experimental signals and extract the physiological kinetics of each channel. We found that two different sets of channels are selectively activated. When the dendrite is hyperpolarized, CF-EPSPs mainly activate T-type VGCCs, SK channels, and A-type VGKCs that limit the transient $V_m \sim <0$ mV. In contrast, when the dendrite is depolarized, T-type VGCCs and A-type VGKCs are inactivated and CF-EPSPs activate P/Q-type VGCCs, high-voltage activated VGKCs, and BK channels, leading to Ca^{2+} spikes. Thus, the potentially activity-dependent regulation of A-type VGKCs, controlling the activation of this second set of channels, is likely to play a crucial role in signal integration and plasticity in Purkinje neuron dendrites.

Key words: calcium channels; cerebellar Purkinje neuron; climbing fiber; neuron modeling; neuronal dendrites; potassium channels

Significance Statement

The climbing fiber synaptic input transiently depolarizes the dendrite of cerebellar Purkinje neurons generating a signal that plays a fundamental role in dendritic integration. This signal is mediated by two types of Ca^{2+} channels and four types of K^{+} channels. Thus, understanding the kinetics of all of these channels is crucial for understanding PN function. To obtain this information, we used an innovative strategy that merges ultrafast optical membrane potential and Ca^{2+} measurements, pharmacological analysis, and computational modeling. We found that, according to the initial membrane potential, the climbing fiber depolarizing transient activates two distinct sets of channels. Moreover, A-type K^{+} channels limit the activation of P/Q-type Ca^{2+} channels and associated K^{+} channels, thus preventing the generation of Ca^{2+} spikes.

Introduction

The climbing fiber (CF) synaptic input from the brainstem inferior olive to the cerebellar Purkinje neuron (PN) governs short-

term (Brenowitz and Regehr, 2005) and long-term (Safó and Regehr, 2005) synaptic depression of parallel fiber (PF) synaptic

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inputs in the dendrites. While these important learning mechanisms eventually involve activation of metabotropic glutamate receptors at PF releasing sites and postsynaptic release of endocannabinoids (Marcaggi and Attwell, 2005), the CF signal is carried by a transient depolarization in the dendrite. The large CF-EPSP is generated in the cell body and in the proximal dendritic segment by glutamate release from hundreds of synaptic terminals (Silver et al., 1998). Then, the depolarization spreads in the dendritic arborization (Canepari and Vogt, 2008) where it activates voltage-gated Ca^{2+} channels (VGCCs) and voltage-gated K^{+} channels (VGKCs). The transient elevation of intracellular Ca^{2+} activates both SK K^{+} channels and BK K^{+} channels (Edgerton and Reinhart, 2003). Thus, the ensemble of activated Ca^{2+} and K^{+} channels, including VGKCs, shapes the waveform of the dendritic membrane potential (V_m) and of the Ca^{2+} transient (i.e., the two signals that the CF input transmits to the dendritic terminations) (Vogt and Canepari, 2010). A detailed characterization of the kinetics of each individual channel involved in the CF signal is therefore crucial for understanding how the CF transmits information to the dendritic arborization. To tackle this problem, a detailed compartmental model of PNs, including several ion channels, was proposed in the early 1990s for predicting the dendritic signals associated with synaptic responses (De Schutter and Bower, 1994). Since then, several computational models incorporating ion channel and morphology variations have been proposed to account for the emerging complex dendritic activity (Anwar et al., 2013, 2014). Yet, few, if any, of these predictions have been tested experimentally. Importantly, recent advancements in voltage-sensitive dye (VSD) imaging combined with Ca^{2+} imaging with a fast sampling rate, using low-affinity indicators to track the kinetics of VGCCs, allow the direct measurement of V_m and Ca^{2+} signals in the dendrites with a spatial resolution of a few microns (Jaafari et al., 2014, 2015; Jaafari and Canepari, 2016).

In this article, we report the first measurements of dendritic V_m and Ca^{2+} transients associated with the CF-EPSP at different initial V_m and the analysis of their correlation. Then, we report a detailed analysis of the changes in the CF-mediated dendritic Ca^{2+} transient produced by the local selective inhibition of various Ca^{2+} or K^{+} channels. Based on this rich experimental dataset, obtained from an ensemble of 59 cells, we built a NEURON model of a simplified PN dendritic compartment. The model is based on prior work (Anwar et al., 2012) and incorporates modified channel models to accurately reproduce the V_m and Ca^{2+} transients associated with the CF-EPSP at different initial V_m . The model included P/Q-type high-voltage activated (HVA) VGCCs, T-type low-voltage activated (LVA) VGCCs, A-type LVA-VGKCs, and Ca^{2+} -activated K^{+} channels (both SK and BK). The consistency of this model was assessed by quantitatively comparing the experimental results of blocking different individual channels, with the elimination of respective channels from the model. The model also included a generic HVA-VGKC (HVAK), an immobile endogenous Ca^{2+} buffer (Canepari and Mammano, 1999), and the two Ca^{2+} binding proteins Calbindin-D28k and Parvalbumin highly expressed in PNs (Schmidt et al., 2003). The systematic and extensive feedback between experiments and model simulations allowed us unraveling the physiological kinetics of all dendritic Ca^{2+} and K^{+} channels activated by the CF-mediated V_m transient, under different initial V_m conditions, providing a realistic model for the ion channels involved. In particular, we found that two distinct sets of channels are activated when the initial resting V_m is either hyperpolarized or depolarized and that the separation between these two sets is

governed by the activation of A-type VGKCs that is likely playing a crucial role in regulation and plasticity of PF inputs.

Materials and Methods

Slice preparation, electrophysiology, and pharmacology

Experiments were ethically performed in accordance with European Directives 2010/63/UE on the care, welfare, and treatment of animals. Procedures were reviewed by the ethics committee affiliated to the animal facility of the university (D3842110001). Cerebellar sagittal slices (250 μm thick) were prepared from 21 to 35 postnatal day old mice (C57BL/6) following established procedures (Vogt et al., 2011a, b; Ait Ouare et al., 2016) with a VT1200 (Leica Microsystems) and incubated at 37°C for 45 min before use. The extracellular solution contained the following (in mM): 125 NaCl, 26 NaHCO_3 , 1 MgSO_4 , 3 KCl, 1 NaH_2PO_4 , 2 CaCl_2 , and 20 glucose, bubbled with 95% O_2 and 5% CO_2 . The intracellular solution contained the following (in mM): 125 KMeSO₄, 5 KCl, 8 MgSO_4 , 5 $\text{Na}_2\text{-ATP}$, 0.3 Tris-GTP, 12 Tris-phosphocreatine, 20 HEPES, adjusted to pH 7.35 with KOH. In combined V_m and Ca^{2+} imaging experiments, PNs were loaded with the VSD JPW1114 and with the Ca^{2+} indicator FuraFF (at 1 mM) using a previously described procedure (Vogt et al., 2011a). In experiments of Ca^{2+} imaging only, Oregon Green BAPTA-5N (OG5N) was added to the internal solution at 2 mM concentration. Patch-clamp recordings were made at 32°C–34°C using a Multiclamp amplifier 700A (Molecular Devices), and signals were acquired at 20 kHz using the A/D board of the CCD camera. The measured V_m was corrected for junction potential (−11 mV) as previously estimated (Canepari et al., 2010). CF-EPSPs were elicited by current pulses of 5–20 μA amplitude and 100 μs duration delivered by a pipette. Local block of the various channels was achieved by gentle pressure application of the extracellular solution containing the specific blocker at selected effective concentration, using a pipette of $\sim 2 \mu\text{m}$ diameter. Full names of chemicals used to block L-type, N-type, or T-type VGCCs were as follows: isradipine, 4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-3,5-pyridinecarboxylic acid methyl 1-methylethyl ester; PD173212, N-[[4-(1,1-dimethylethyl)phenyl]methyl-N-methyl-L-leucyl-N-(1,1-dimethylethyl)-O-phenylmethyl]-L-tyrosinamide; ML218, 3,5-dichloro-N-[[[(1 α ,5 α ,6-exo,6 α)-3-(3,3-dimethylbutyl)-3-azabicyclo[3.1.0]hex-6-yl]methyl]-benzamide-hydrochloride; and NNC550396, (1S,2S)-2-[2-[[3-(1H-benzimidazol-2-yl)propyl]methylamino]ethyl]-6-fluoro-1,2,3,4-tetrahydro-1-(1-methylethyl)-2-naphthalenyl-cyclopropanecarboxylate-dihydrochloride. The VSD and the Ca^{2+} indicators were from Invitrogen. ω -Agatoxin IVA, AmmTx3, ibertoxin, and apamin were purchased from SmarTox Biotechnology. All other chemicals were purchased from Tocris Bioscience, Hello Bio, or Sigma-Aldrich.

Optical signal recording and calibration

Sequential V_m and Ca^{2+} optical measurements (Canepari and Vogt, 2008; Canepari et al., 2008) were achieved by alternating excitation of FuraFF at 385 nm with an OptoLED (Cairn Research) and of the VSD at 532 nm using a 300 mW solid state laser (model MLL532, CNI). In experiments with Ca^{2+} imaging only, OG5N was excited at 470 nm with the OptoLED. Ca^{2+} fluorescence (either from FuraFF or from OG5N) and V_m fluorescence was recorded at $525 \pm 25 \text{ nm}$ and at $>610 \text{ nm}$, respectively, using a NeuroCCD-SMQ camera (RedShirtImaging). Images, demagnified by $\sim 0.2\times$ to visualize an area of $\sim 150 \mu\text{m}$ diameter, were acquired at 5 kHz with a resolution of 26×26 pixels. Electrical and optical signals associated with the CF-EPSP were recorded for 20 ms (100 frames) with the CF stimulation occurring 2 ms after the beginning of trials. Three or four trials, with 20 s between two consecutive trials, were obtained to assess the consistency of the signals. Fluorescence from these trials was averaged and corrected for bleaching using a filtered trial without signal. V_m and Ca^{2+} signals were initially expressed as fractional changes of fluorescence ($\Delta F/F_0$). To match experimental Ca^{2+} signals with NEURON simulations, $\Delta F/F_0$ was converted into Ca^{2+} -bound-to-dye concentration ($[\text{Ca}^{2+}\text{Dye}]$) as previously described (Ait Ouare et al., 2016). Briefly, $[\text{Ca}^{2+}\text{Dye}] = [\text{Dye}]_{\text{TOT}} \cdot \Delta F/F_0 / \sigma$, where $[\text{Dye}]_{\text{TOT}}$ is the total dye concentration and σ is the empirical dynamic range defined as the $\Delta F/F_0$ for saturating Ca^{2+} (Ait Ouare et al., 2016). Measurements of σ were obtained by saturating the indicators in the cytosol with a 30 s

Figure 1. V_m calibration protocol and illustration of NEURON model. **A**, Left, Fluorescence reconstruction of a PN with three ROIs (R1, R2, and R3). From the resting V_m (-67 mV), negative or positive current pulses of 1 s duration were delivered from the recording electrode. Right, Somatic V_m and the VSD- $\Delta F/F_0$ signals in R1–R3 associated with the current pulses. The VSD- $\Delta F/F_0$ signal in each region is converted into millivolts to quantify the V_m transient associated with the CF-EPSP, assuming that the resting V_m is uniform (int state) and that the hyperpolarizing pulse spreads into the dendrites without attenuation (hyp state). The protocol also allows determining the dendritic V_m associated with the strongest depolarizing pulse (dep state). **B**, A dendritic region of $\sim 17 \times 17 \mu\text{m}^2$ is approximated with a cylinder of $4 \mu\text{m}$ diameter and $20 \mu\text{m}$ length in the NEURON model. The model contains P/Q-type and T-type Ca^{2+} channels; SK, BK, A-type K^+ channels; and a generic HVA. It includes four buffers: a fast immobile buffer, the Ca^{2+} indicator (either Fura-FF or OG5N), Parvalbumin, and Calbindin D-28k. It also includes Ca^{2+} extrusion and a LEAK channel.

step of -500 mV (in voltage clamp) that makes the somatic membrane permeable to Ca^{2+} . The value of σ obtained in this way for FuraFF was -0.9 . VSD $\Delta F/F_0$ was calibrated into a V_m transient using an established procedure (Canepari and Vogt, 2008). Assuming that somatic hyperpolarizations from the resting V_m spread without attenuation through PN dendrites (Roth and Häusser, 2001), a prolonged hyperpolarizing step of 1 s was used in each experiment to convert the VSD $\Delta F/F_0$ signal into millivolts, as shown in the example of Figure 1A. Because, in this study, signals associated with the CF-EPSP were evoked at different initial V_m , depolarizing steps of 1 s were also used to estimate the dendritic V_m produced by somatic depolarization. In detail, as shown in the example of Figure 1A, the two largest depolarization steps lead to the same dendritic V_m , although corresponding to two different somatic V_m . This indicates that the dendrite reaches a maximal steady V_m regardless of the current injected into the soma above certain intensity. In our experiments, V_m recordings at depolarized states were performed by injecting currents above this intensity, in this way driving the initial dendritic V_m to the maximal value that was obtained by the calibration protocol.

Experimental design and statistical analysis

The effects of changing the initial V_m on the V_m and Ca^{2+} transients, or of blocking a Ca^{2+} or a K^+ channel on the Ca^{2+} transients, were estab-

lished by performing the paired Student's t test on the signal under the two different conditions. A change in the signal was considered significant when $p < 0.005$. The ROIs used for the statistical analysis of pharmacological tests were those adjacent to the pipette delivering the channel blocker.

Procedure of matching experimental transients with a neuron model

To deduce the kinetics of each channel involved in the dendritic electrical signal associated with the CF-EPSP, we developed an original approach based on matching optical data with a simplified model, implemented in NEURON (Hines and Carnevale, 1997) as depicted in Figure 1B. To build the model, we started from a published model designed to predict activation of Ca^{2+} -activated K^+ channels in PN dendrites (Anwar et al., 2012), available in the ModelDB database at <https://senselab.med.yale.edu/ModelDB/ShowModel.cshhtml?model=138382&file=/AnwarEtAl2010/cdp5.mod#tabs-1>. From this model, consisting of a cylinder of $4 \mu\text{m}$ diameter and $20 \mu\text{m}$ length with standard passive membrane properties, several parameters and mechanisms were initially replaced with those from two earlier models (De Schutter and Bower, 1994; Solinas et al., 2007) also available in ModelDB database at (<https://senselab.med.yale.edu/ModelDB/ShowModel.cshhtml?model=7176#tabs-1>) and at (https://senselab.med.yale.edu/ModelDB/ShowModel.cshhtml?model=112685&file=/Golgi_cell/Golgi_SK2.mod#tabs-2). From this starting point, the kinetics of ion channels were changed to obtain traces from simulations that matched experimental V_m and Ca^{2+} optical measurements from $\sim 17 \times 17 \mu\text{m}^2$ square regions at three different initial V_m : hyperpolarized (~ -80 mV), intermediate (~ -65 mV), and depolarized (~ -50 mV). The specific modifications implemented for each channel are described in detail in the paragraph below. After obtaining a satisfactory set of models for each channel in one cell, the same channel models were used to match our experimental data in three more cells, only by tuning the channel densities and the current input associated with the CF-EPSP.

Ion channel mathematical functions matching experimental data

The channel functions that matched experimental V_m and Ca^{2+} transients are reported below and are available in ModelDB at <http://senselab.med.yale.edu/ModelDB/showModel.cshhtml?model=244679>. In detail, I is the current density (expressed in mA/cm^2), Π is channel permeability to Ca^{2+} (expressed in cm/s), m_∞ , t_m , m_{exp} , and h_∞ , t_h are the voltage-dependent activation and inactivation parameters, respectively, z_{∞} , z_{exp} , and t_z are the Ca^{2+} -dependent activation parameters, and GHK is the Goldman-Hodgkin-Katz factor (expressing the current per unit permeability (Anwar et al., 2012). Voltage (V) and time are expressed in millivolts and milliseconds, respectively.

P/Q-type VGCC. Starting from the formulation for P/Q-type VGCCs in Anwar et al. (2012), we multiplied the activation curve by a sigmoid function to account for the fact that we did not observe P/Q channel activation < -50 mV. We also reduced the activation time by 40% to reproduce the observed Ca^{2+} spiking rate at depolarized states.

$$I = \Pi \cdot m^3 \cdot \text{GHK}$$

$$m_\infty = \frac{1}{1 + e^{-(V+29.458)/8.439}} \cdot \frac{1}{1 + e^{-0.1 \cdot (V+50)}}$$

$$t_m = \begin{cases} 0.6 \cdot \left(0.2702 + 1.1622 \cdot e^{-\frac{(V+26.798)^2}{164.19}} \right) & \text{if } V \leq -40 \text{ mV} \\ 0.6 \cdot 0.6923 \cdot e^{V/1089.372} & \text{otherwise} \end{cases}$$

T-type VGCC. Starting from the formulation for T-type VGCCs in Anwar et al. (2012), we multiplied the activation curve by a sigmoid function to account for the observed activation at hyperpolarized and intermediate states. The activation time was decreased by 70%, whereas the inactivation time was doubled to fit the rise and decay of the experimental V_m trace at the hyperpolarized state as follows:

$$I = \Pi \cdot m^2 \cdot h \cdot \text{GHK}$$

$$m_{\infty} = \frac{1}{1 + e^{-(V+51)/6}} * corr$$

$$corr = \begin{cases} \frac{1}{1 + e^{-0.3 \cdot (V+53)}} & \text{at hyperpolarised states} \\ \frac{1}{1 + e^{-0.3 \cdot (V+45)}} & \text{at intermediate states} \end{cases}$$

$$h_{\infty} = \frac{1}{1 + e^{-\left(\frac{V+72}{7}\right)}}$$

$$t_m = \begin{cases} 1 & \text{if } V \leq -90 \text{ mV} \\ \frac{0.2}{e^{(V+40)/9} + e^{-(V+108)/18}} + 0.3 & \text{otherwise} \end{cases}$$

$$t_h = 32 + \frac{2}{e^{(V+32)/7}}$$

SK Ca^{2+} -activated K^+ channel. We used the model in Solinas et al. (2007) with 95% of the SK channels coupled to T-type VGCCs to account for effect of blocking these channels observed exclusively at hyperpolarized states.

BK Ca^{2+} -activated K^+ channel. Starting from the formulation in De Schutter and Bower (1994), we reduced the Ca^{2+} -dependent activation time to half to account for the larger slow repolarization at depolarized states as follows:

$$I = \sum \cdot m \cdot z^2 \cdot (V + 85)$$

$$m_{\infty} = \frac{8.5}{7.5 + a_m}$$

$$z_{\infty} = \frac{1}{1 + a_z}$$

$$a_m = \frac{0.11}{e^{(V-55)/14.9}}$$

$$a_z = \frac{0.4}{[Ca^{2+}]}$$

$$m_{exp} = 1 - e^{-0.002 \cdot (7.5 + a_m)}$$

$$z_{exp} = 1 - e^{-0.0005}$$

A-type VGKC. Starting from the formulation of A-type VGCCs channels in De Schutter and Bower (1994), the kinetic parameters were modified in line with modifications of T-type VGCCs to account for behaviors at hyperpolarized states. The density was corrected at intermediate states to account for partial inactivation as follows:

$$I = \sum \cdot h \cdot m^4 \cdot (V + 85)$$

$$m_{\infty} = \frac{a_m}{a_m + b_m} \cdot \frac{1}{1 + e^{-0.2 \cdot (V+50)}}$$

$$h_{\infty} = \frac{a_h}{a_h + b_h}$$

$$a_m = \frac{1.4}{1 + e^{-(V+50)/12}}$$

$$a_h = \frac{0.0175}{1 + e^{(V+85)/8}}$$

$$b_m = \frac{0.49}{1 + e^{(V+30)/4}}$$

$$b_h = \frac{1.3}{1 + e^{-(V+13)/10}}$$

$$t_m = \frac{1}{a_m + b_m}$$

$$t_h = \frac{1}{a_h + b_h}$$

HVA-VGKC (HVAK). Starting from the formulation of the “delayed rectifier channel” given in De Schutter and Bower (1994), the HVAK kinetic parameters were modified to account for the behaviors at depolarized states. Specifically, the activation curve was multiplied by a sigmoid function to track the occurrence of the first Ca^{2+} spike. Then the activation time was decreased by $\sim 95\%$ to reproduce the number and shape of the observed Ca^{2+} spikes. Notably, this is the only channel for which the experimental pharmacological block was not available. See the following equations:

$$I = \sum \cdot h \cdot m^2 \cdot (V + 85)$$

$$m_{\infty} = \frac{a_m}{a_m + b_m} \cdot \frac{1}{1 + e^{-0.4 \cdot (V+35)}}$$

$$h_{\infty} = \frac{1}{1 + e^{(V+25)/4}}$$

$$a_m = \frac{-0.0047 \cdot (V - 8)}{-0.9999 + e^{-(V-8)/12}}$$

$$b_m = e^{-(V+127)/30}$$

$$t_m = \frac{0.055}{\frac{-0.0047 \cdot (V + 12)}{-0.9999 + e^{-(V+12)/12}} + e^{\frac{V+147}{30}}}$$

$$t_h = \begin{cases} 1200 & \text{if } V < -25 \\ 10 & \text{otherwise} \end{cases}$$

Model of the CF-associated current

The model of the current associated with a CF-EPSP, designed to mimic the shape of the current reported by Llano et al. (1991), was expressed by the following equation:

$$I_{CF} = I_{HOLD} + I_{STANDING} \cdot (1 - e^{-(t-DELAY)/RISE}) \cdot e^{-(t-DELAY)/DURATION}$$

The parameters in this equation were tuned to obtain the match of experimental V_m and Ca^{2+} transients within the following ranges. I_{HOLD} (holding current before CF-EPSP occurrence): between -0.03 and 0.04 mA/cm². $I_{STANDING}$: between -0.4 and -0.1 mA/cm². DELAY: between 2 and 2.4 ms. RISE: between 0.4 and 1.8 ms. DURATION: between 2.5 and 6.

Other fixed mechanisms and parameters of the neuron model

The following standard mechanism parameters in Figure 1B were used in the simulations.

Immobile buffer (concentration and Ca^{2+} reaction kinetic parameters) is as follows (Ait Ouare et al., 2016): concentration = 1 mM; $K_{ON} = 570$ $\mu M^{-1} s^{-1}$; $K_{OFF} = 5.7 \cdot 10^3 s^{-1}$.

Parvalbumin (concentration and Ca^{2+} reaction kinetic parameters for Ca^{2+} and for Mg^{2+}), corrected from empirical values reported by Lee et al. (2000) to take into account the difference in temperature and radial diffusion: binding sites concentration (two per molecule) = 150 μM ; $K_{ON}^{Ca^{2+}} = 535$ $\mu M^{-1} s^{-1}$; $K_{OFF}^{Ca^{2+}} = 0.95 s^{-1}$; $K_{ON}^{Mg^{2+}} = 4$ $\mu M^{-1} s^{-1}$; $K_{OFF}^{Mg^{2+}} = 25 s^{-1}$.

Calbindin D28-k (concentration and Ca^{2+} reaction kinetic parameters), corrected from empirical values reported by Nägerl et al. (2000) to take into account the difference in temperature and radial diffusion: fast binding site concentration (two per molecule) = 1.2 mM; $K_{ON} = 217.5$ $\mu M^{-1} s^{-1}$; $K_{OFF} = 35.8 s^{-1}$; slow binding sites concentration (two per molecule) = 1.2 M; $K_{ON} = 27.5$ $\mu M^{-1} s^{-1}$; $K_{OFF} = 2.6 s^{-1}$.

Figure 2. Combined V_m and Ca^{2+} transients associated with the CF-EPSP. **A**, Bottom, Fluorescence reconstruction of a representative PN with two ROIs indicated ($R1$ and $R2$). Top, Somatic V_m associated with a CF-EPSP at three different initial V_m : hyperpolarized (*hyp*, blue trace); intermediate (*int*, green trace); and depolarized (*dep*, red trace). **B**, Top, Dendritic V_m in $R1$ and $R2$ calibrated as illustrated in Figure 1A corresponding to the somatic CF-EPSPs in **A**. Bottom, Corresponding FuraFF $\Delta F/F_0$ signals. **C**, Analysis of the V_m and Ca^{2+} maxima (max) associated with signals in $R1$ reported in **B**; the first max of the V_m and Ca^{2+} transients

Ca^{2+} indicator (Ca^{2+} reaction kinetic parameters): $K_{ON} = 570 \mu M^{-1} s^{-1}$; concentration FuraFF = 1 mM; $K_{OFF}(FuraFF) = 5.7 \cdot 10^3 s^{-1}$; concentration OG5N = 2 mM; $K_{OFF}(OG5N) = 19.95 \cdot 10^3 s^{-1}$.

Ca^{2+} extrusion equation was adopted from Destexhe et al. (1993), with kinetic parameters and density used in Anwar et al. (2012) as follows: $K_{ON} = 3 \cdot 10^{-3} \mu M^{-1} s^{-1}$; $K_{OFF} = 1.75 \cdot 10^{-2} s^{-1}$; $K_{EXT} = 7.255 \cdot 10^{-5} \mu M^{-1} s^{-1}$; density = $10^{-9} mol cm^{-2}$.

LEAK current: $0.002 mA/(mV \cdot cm^2) \cdot (V_{init} - V_{rest})$, where V_{init} is the initial V_m (in millivolts) and V_{rest} is the resting V_m ($-65 mV$).

Results

Dendritic depolarization and Ca^{2+} transients associated with the CF-EPSP

In the first series of experiments ($N = 12$ cells), we investigated the dendritic depolarization and the Ca^{2+} transients associated with the CF-EPSP by combining V_m and Ca^{2+} imaging as described in Materials and Methods. Within a recording field of $\sim 150 \times 150 \mu m^2$ (26×26 pixels), we systematically averaged fluorescence over 1–3 dendritic regions of $\sim 17 \times 17 \mu m^2$ (3×3 pixels) with sufficient signal-to-noise ratio to reliably measure $\Delta F/F_0$ signals with both indicators, at 5 kHz. In each cell and dendritic region, the V_m was determined from voltage-sensitive dye $\Delta F/F_0$ signals using the protocol described in Materials and Methods and illustrated in Figure 1A. In the cell reported in Figure 2A, the initial somatic V_m was set by current injection and a CF-EPSP was evoked at a state of hyperpolarization (*hyp*, blue trace) $\sim -80 mV$, at an intermediate V_m (*int*, green trace) close to the resting V_m , and at a state of depolarization (*dep*, red trace). These three conditions corresponded, in this particular cell, to initial dendritic V_m of -83 , -64 , and $-54 mV$, respectively. In the two analyzed regions ($R1$ and $R2$), the dendritic V_m transient associated with the CF-EPSP had a first peak occurring within 3 ms after the stimulation that ranged from $-17 mV$ at the *hyp* state to $10 mV$ at the *int* state (Fig. 2B). A second sharp peak was observed at the

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is calculated within the first 4 ms after the CF stimulation; the second max of the V_m and Ca^{2+} transients is calculated between 4 and 14 ms after the CF stimulation. Blue traces represent the *hyp* state. Green traces represent the *int* state. Red traces represent the *dep* state. **D**, Mean $\pm$ SD for 19 regions in 12 cells analyzed as illustrated in **C**. The *hyp* states (blue columns) were with initial V_m between $-87 mV$ and $-74 mV$. The *int* states (green columns) were with initial V_m between $-68 mV$ and $-61 mV$. The *dep* states (red columns) were with initial V_m between -54 and $-46 mV$. *Significant increase in the max ($p < 0.005$, paired t test).

Figure 3. Dendritic Ca^{2+} channels activated by the CF-EPSP. **A**, Left, Fluorescence reconstruction of a PN with two ROIs indicated ($R1$ and $R2$): $R1$ is next to a pipette delivering $1 \mu\text{M}$ of the P/Q-type VGCC inhibitor AgaIVA; $R2$ is $>50 \mu\text{m}$ from the application pipette. Right, Top, Somatic V_m associated with CF-EPSPs in control conditions and after local application of AgaIVA at three different initial V_m : *hyp* (blue trace); *int* (green trace); and *dep* (red trace). Right, Bottom, The corresponding OG5N $\Delta F/F_0$ signals. **B**, In another PN, same as **A**, but with the pipette delivering the T-type VGCC inhibitors ML ($5 \mu\text{M}$) and NNC ($30 \mu\text{M}$). **C**, In another PN, same as **A**, but with the pipette delivering both the P/Q VGCC inhibitor AgaIVA ($1 \mu\text{M}$) and the T-type VGCC inhibitors ML ($5 \mu\text{M}$) and NNC ($30 \mu\text{M}$). **D**, In three other PNs, from a region next to a pipette delivering a VGCC blocker, the OG5N $\Delta F/F_0$ signals associated with CF-EPSPs in control conditions and after local application of $20 \mu\text{M}$ of the L-type VGCC inhibitor *Isr*, of $5 \mu\text{M}$ of the N-type VGCC inhibitor PD or $1 \mu\text{M}$ of the R-type VGCC inhibitor SNX at the three different initial V_m .

dep state. Correlated with these V_m transients, the Ca^{2+} transient increased from the *hyp* state to the *int* state and exhibited two sharp peaks at the *dep* state that can be defined as spikes because they are characterized by a rapid rise and fall. This behavior was consistently observed in every cell investigated. The V_m and Ca^{2+} transients in $R1$ are shown again in Figure 2C to illustrate the quantitative analysis that was performed. For both dendritic V_m and Ca^{2+} transients, we measured the maximum (max) during the first 4 ms after the CF stimulation (first max) and between 4 and 14 ms after the CF stimulation (second max). The values for $R1$ are reported in Figure 2C, and the statistics (mean $\pm$ SD) for 19 regions in the 12 cells analyzed are reported in Figure 2D. At *hyp* states (with initial V_m between -87 and -74 mV), the first and second V_m maxima were -13 ± 8 and -38 ± 7 mV, respectively, whereas the Ca^{2+} transient ($-\Delta F/F_0$) max were $1.83 \pm 0.55\%$ and $3.14 \pm 0.85\%$, respectively. At *int* states (with initial V_m between -68 and -61 mV), the first and second V_m maxima were 6 ± 7 and -21 ± 11 mV, respectively, whereas the Ca^{2+} transient max were $3.65 \pm 1.27\%$ and $3.65 \pm 1.05\%$, respectively. Finally, at *dep* states (with initial V_m between -54 and -46 mV), the first and second V_m maxima were 9 ± 6 and -1 ± 9 mV, respectively, whereas the Ca^{2+} transients maxima were $5.36 \pm 1.17\%$ and $6.59 \pm 1.42\%$ respectively. The first max of both V_m and Ca^{2+} transients significantly increased from the *hyp* state to the *int* state ($p < 0.005$, paired t test), whereas the second max of both the V_m and Ca^{2+} transients significantly increased from the *int* state to the *dep* state. These results demonstrate that CF-EPSP-associated dendritic

depolarization and Ca^{2+} influx increase with the initial V_m . Furthermore, dendritic spikes (typically two in the 18 ms after the stimulation) and correlated Ca^{2+} transients occur when the dendrite is depolarized. The dendritic depolarization is produced by the passive spread of the CF-EPSP from the soma and proximal dendrite and activates VGCCs and VGKCs. Ca^{2+} -activated K^+ channels contribute to the repolarization. To resolve the channels underlying the behaviors observed in the experiments reported above, we analyzed pharmacologically in detail the Ca^{2+} transient associated with the CF-EPSP.

Dendritic Ca^{2+} channels activated by the CF-EPSP

In PNs, the dendritic Ca^{2+} transients associated with the CF-EPSP transient depolarization are mediated by VGCCs, in particular P/Q-type HVA-VGCCs (Usowicz et al., 1992) and T-type LVA-VGCCs (Isope et al., 2012). Thus, we investigated the changes in the Ca^{2+} transients from OG5N fluorescence produced by the selective block of one or more VGCCs. In the representative example of Figure 3A, the CF-EPSP was evoked at *hyp* (blue traces), *int* (green traces), and *dep* (red traces) states, and the associated OG5N $\Delta F/F_0$ signal was recorded in the control condition and after local application of the P/Q-type VGCC blocker ω -agatoxin-IVA (AgaIVA, $1 \mu\text{M}$). Importantly, to assess the postsynaptic effect while excluding any possible presynaptic effect, the changes in the Ca^{2+} transient were analyzed and compared in the region next to the application pipette ($R1$) and in another region at $>50 \mu\text{m}$ from the application pipette ($R2$). In $R1$ only, AgaIVA reduced the Ca^{2+} transient during the first few

Figure 4. Quantitative analysis of dendritic Ca^{2+} channels activated by the CF-EPSP. Left, From two representative cells, OGSN $\Delta F/F_0$ signals associated with CF-EPSPs in control conditions at three different initial V_m : *hyp* (blue trace); *int* (green trace); and *dep* (red trace); superimposed (gray traces) are the OGSN $\Delta F/F_0$ signals after local application of either $1 \mu\text{M}$ of the P/Q-type VGCC inhibitor AgaIVA or of the T-type VGCC blockers ML ($5 \mu\text{M}$) and NNC ($30 \mu\text{M}$); the first max of the Ca^{2+} transient is calculated within the first 4 ms after the CF stimulation; the second max of the Ca^{2+} transient is calculated between 4 and 14 ms after the CF stimulation; the percentages from control $\Delta F/F_0$ maxima after application of the VGCC blockers are reported above or below the arrows. Right, Mean $\pm$ SD of the percentages from control $\Delta F/F_0$ maxima after application of the VGCC inhibitors AgaIVA ($N = 6$ cells), ML + NNC ($N = 6$ cells), AgaIVA + ML + NNC ($N = 6$ cells), Isr ($N = 4$ cells), PD ($N = 4$ cells), or SNX ($N = 4$ cells). Gray columns represent the statistics of the first max. White columns represent the statistics of the second max. *Significant change in the max ($p < 0.005$, paired t test).

milliseconds after the CF-EPSP at the *hyp* and *int* states, and blocked the Ca^{2+} transient at the *dep* state. In the representative example of Figure 3B, the same protocol was used to assess the effects of local block of T-type VGCCs, using the inhibitors ML218 (ML, $5 \mu\text{M}$) and NNC550396 (NNC, $30 \mu\text{M}$). In this case, the blockers inhibited the late component of the Ca^{2+} transient at the *hyp* and *int* states but had no effect on the Ca^{2+} transient at

the *dep* state. Finally, in the representative example of Figure 3C, local block of P/Q-type and T-type VGCCs together inhibited Ca^{2+} transient in all states. To rule out any additional component from other VGCC types, in the three representative examples of Figure 3D, we also tested the blockers isradipine ($20 \mu\text{M}$, L-type), PD173212 ($5 \mu\text{M}$, N-type), and SNX482 ($1 \mu\text{M}$, R-type). The Ca^{2+} transient remained unaffected under all states and cases tested. The results reported in Figure 3 were consistently observed in all cells tested with each VGCC blocker. To quantify these results, we measured again the first max (during the first 4 ms after the stimulation) and the second max (between 4 and 14 ms after the stimulation), and calculated the percentage of the two maxima in the presence of the VGCC blocker, with respect to the control condition, as illustrated in the examples of Figure 4. We tested the block of P/Q-type VGCCs (AgaIVA), of T-type VGCC (ML + NNC), or of P/Q-type and T-type VGCCs together (AgaIVA + ML + NNC) in $N = 6$ cells for each case. The block of P/Q channels significantly reduced the first max of the Ca^{2+} transient at all initial V_m states ($p < 0.005$, paired t test), whereas the second max was reduced only in the *int* and *dep* states. In contrast, the block of T channels significantly reduced the first and second maxima of the Ca^{2+} transient at *hyp* states and the second max only and *int* states, whereas neither max was changed at *dep* states. The block of P/Q and T channels together significantly reduced both maxima at all initial states. Finally, we tested the block of L-type VGCCs (Isr), of N-type VGCC (PD), or of R-type VGCCs (SNX) in $N = 4$ cells for each case. No changes in the two maxima were observed in any of the initial states. In summary, these results show the distinct kinetics of two components of Ca^{2+} influx, associated with the CF-EPSP and mediated by P/Q-type and T-type VGCCs, respectively. At *hyp* states, where the depolarization transient is typically $< -10 \text{ mV}$ (Fig. 2), P/Q-type VGCCs are weakly activated, but activation of these channels increases when the initial V_m becomes more positive and the transient depolarization larger. In contrast, T-type VGCCs are strongly activated at *hyp* states, but these channels inactivate as the initial V_m increases.

Dendritic K^+ channels activated by the CF-EPSP

Activation of dendritic P/Q-type VGCCs at *int* and *dep* states is correlated with larger depolarization transients associated with CF-EPSPs, possibly due to steady inactivation of A-type VGCCs that are expressed in PN dendrites (Otsu et al., 2014). To test this

Figure 5. Dendritic A-type VGCCs activated by the CF-EPSP. **A**, Left, Fluorescence reconstruction of a PN with an ROI indicated next to a pipette delivering 1 μ M of the A-type VGCC inhibitor AmmTx3. Right, Top, Somatic V_m associated with CF-EPSPs in control conditions and after local application of AmmTx3 at three different initial V_m : hyp (blue trace); int (green trace); and dep (red trace). Right, Bottom, The corresponding OG5N $\Delta F/F_0$ signals. **B**, Left, From the cell in **A**, OG5N $\Delta F/F_0$ signals associated with CF-EPSPs in control conditions at the three different initial V_m ; superimposed (gray traces) are the OG5N $\Delta F/F_0$ signals after local application of either 1 μ M AmmTx3; the first max of the Ca^{2+} transient is calculated within the first 4 ms after the CF stimulation; the percentages from control $\Delta F/F_0$ maxima after application of the VGCC blockers are reported above the arrows. Right, Mean $\pm$ SD of the percentages from control $\Delta F/F_0$ maxima after application of AmmTx3 ($N = 6$ cells). Gray columns represent the statistics of the first max. White columns represent the statistics of the second max. *Significant change in the max ($p < 0.005$, paired t test).

hypothesis, we investigated the change in the OG5N Ca^{2+} transient produced by local application of the A-type VGCC inhibitor AmmTx3 (Zoukimian et al., 2019). As shown in the representative example of Figure 5A, the block of A-type channels strongly enhanced the Ca^{2+} transient associated with the CF-EPSP both at the hyp and int states but did not produce any effect at the dep state. In contrast to the experiments with VGCC blockers, the effect of AmmTx3 was not observed exclusively in the area adjacent to the pipette delivering the toxin (data not shown). In $N = 6$ cells tested, AmmTx3 significantly enhanced the first and second maxima of the Ca^{2+} transient at hyp and int states, also at sites located at $>50 \mu\text{m}$ from the pipette delivering the toxin, although it did not modify the Ca^{2+} transient at dep states, indicating that A-type VGCCs are fully inactivated at initial $V_m > -55 \text{ mV}$ (Fig. 5B). In contrast, A-type VGCCs are activated at more negative initial V_m , and this result suggests that their activation limits the activation of P/Q-type VGCCs.

Because Ca^{2+} transients activate Ca^{2+} -activated K^+ channels, we finally investigated the change in the OG5N Ca^{2+} transient produced by local application of the BK channel inhibitor iberiotoxin (1 μM ; Fig. 6A) and of the SK channel inhibitor apamin (1 μM ; Fig. 6B). Neither iberiotoxin nor apamin produced any change in the first and second maxima of the Ca^{2+} transient at hyp, int, and dep states, a result observed in $N = 5$ cells tested with iberiotoxin and $N = 6$ cells tested with apamin (Fig. 6C). Nevertheless, in 5 of 5 cells where the block of BK channels was tested, the number of Ca^{2+} spikes in-

Figure 6. Dendritic Ca^{2+} -activated K^+ channels activated by the CF-EPSP. **A**, Left, Fluorescence reconstruction of a PN with an ROI indicated next to a pipette delivering 1 μM of the BK channel inhibitor iberiotoxin. Right, Top, Somatic V_m associated with CF-EPSPs in control conditions and after local application of iberiotoxin at three different initial V_m : hyp (blue trace); int (green trace); and dep (red trace). Right, Bottom, The corresponding OG5N $\Delta F/F_0$ signals. **B**, In another PN, same as **A**, but with the pipette delivering 1 μM of the SK channel inhibitor apamin. **C**, Mean $\pm$ SD of the percentages from control $\Delta F/F_0$ maxima after application of iberiotoxin ($N = 5$ cells) or apamin ($N = 6$ cells). Gray columns represent the statistics of the first max. White columns represent the statistics of the second max. **D**, Left, From the cell in **A**, OG5N $\Delta F/F_0$ signal associated with the CF-EPSP at dep state in control condition and after addition of iberiotoxin (gray trace). Right, From the cell in **B**, OG5N $\Delta F/F_0$ signal associated with the CF-EPSP at hyp state in control condition and after addition of apamin (gray trace).

creased from 2 to 3 at dep states. In addition, in 4 of 6 cells where the block of SK channels was tested, a slight but observable decrease in the decay of the OG5N $\Delta F/F_0$ signal was noticed (Fig. 6D). These two results indicate that both channels are activated by the CF-EPSP at different initial V_m states.

Analysis of dendritic ion currents associated with the CF-EPSP by NEURON modeling

To extrapolate individual dendritic ion currents underlying V_m transients and Ca^{2+} signals associated with CF-EPSPs at different

Figure 7. NEURON model of 4 PN dendritic compartments reproducing V_m and Ca^{2+} transients associated with the CF-EPSP. Left, Experimental dendritic V_m and Ca^{2+} transients associated with CF-EPSPs from 4 selected cells at three different initial V_m : hyperpolarized (*hyp*, blue trace); intermediate (*int*, green trace); depolarized (*dep*, red trace). Right, Simulations of dendritic V_m and Ca^{2+} transients associated with CF-EPSPs reproducing experimental data (gray traces).

V_m initial states, we selected 4 dendritic sites from 4 different PNs within those analyzed in Figure 2. The criterion for the selection was that the somatic resting V_m and CF-EPSP at different initial V_m were stable for the entire duration of the recordings, to assume the same conditions for the V_m and Ca^{2+} recordings. In these 4 compartments, we matched the experimental V_m and Ca^{2+} transients with traces obtained by computer simulations of the NEURON model illustrated in Figure 1*B*, as described in Materials and Methods. In particular, we established a unique

current, mediated by A-type channels. A very small K^+ current mediated by SK channels is elicited by the transient Ca^{2+} elevation. P/Q-type VGCCs and HVAK VGKs are poorly activated at this state. As the initial V_m becomes more positive (*int* state, green traces), part of T-type and A-type channels become inactivated, reducing the associated currents and allowing more P/Q and HVAK channels to activate. Finally, at *dep* state (red traces), T-type and A-type channels are fully inactivated and the depolarization transient carried by the CF-EPSP activates a Ca^{2+} cur-

kinetic model for each of the six types of channels, to consistently reproduce the behaviors observed in all 4 cells. The model that matches V_m and Ca^{2+} transients is available in the ModelDB database at <http://senselab.med.yale.edu/ModelDB/showModel.cshhtml?model=244679>. While the channel kinetics were the same in all simulations, the density of P/Q-type and T-type VGCCs, of A-type and HVAK VGKs, and of BK and SK Ca^{2+} -activated K^+ channels was adjusted to match experimental data in each cell, as shown in Figure 7. To validate the consistency of the four variants of the model, we run computer simulations by modeling the replacement of 1 mM Fura-FF with 2 mM OG5N and analyzed Ca^{2+} $\Delta F/F_0$ modifications produced by the elimination of 90% of each channel, in this way mimicking the experiments in which individual channels were pharmacologically blocked. As an example, the results for the model variant of Cell 1 in Figure 7 are reported in Figure 8, showing that 90% reduction in the density of each channel qualitatively reproduced the experimental behavior observed after toxin or drug inhibition. To compare experiments and simulations, we calculated the percentage of the first max and the second max of the Ca^{2+} transient after elimination of 90% of each channel. The comparisons, reported in Table 1, indicate that the observed effects on Ca^{2+} signals in the experiments are all in line with the predictions from the simulations. In addition, in all 4 variants of the model, the 90% reduction of BK channels reproduced the appearance of a third Ca^{2+} spike that was also observed in experiments. In summary, matching experimental data with NEURON simulations generated a simplified yet biological plausible dendritic model that successfully reproduces the complexity of experimental signals.

We then used the model variant of Cell 1 of Figure 7 to extrapolate the kinetics of each channel contributing to the CF-mediated signal under different conditions (Fig. 9). At *hyp* state (blue traces), the depolarization transient carried by the CF-EPSP activates a robust Ca^{2+} current, mediated by T-type channels, and a K^+

Figure 8. Simulations of block of P/Q-type VGCCs, T-type VGCCs, A-type VGCCs, BK and SK Ca^{2+} -activated K^{+} channels from a NEURON model. Simulated Ca^{2+} transients (OG5N) associated with CF-EPSPs at three different initial V_m in control condition and after reduction of 90% of one individual channels from Cell 1 model variant reported in Figure 7. For each case of 90% channel reduction, traces under control conditions are reported in gray.

rent, mediated by P/Q-type Ca^{2+} channels, and a K^{+} current, mediated by HVA channels, which are responsible for multiple Ca^{2+} spikes. The number of spikes is limited by the K^{+} current mediated by BK channels. The strong activation of P/Q-type VGCCs and HVA channels is prevented at *hyp* states by the K^{+} current mediated by A-type VGCCs. Hence, when A-type channels are reduced by 90% (purple traces), activation of P/Q-type VGCCs is strongly enhanced, substantially increasing the dendritic Ca^{2+} transient. In summary, by extrapolating the kinetics of individual Ca^{2+} and K^{+} channels, we resolved their functional interaction establishing a specific role of A-type VGCCs in controlling activation of P/Q-type, HVA, and BK channels.

Discussion

In this article, we report an original study of the dendritic V_m and Ca^{2+} transients associated with the CF-EPSP at different initial V_m . We explored experimentally, using combined V_m and Ca^{2+} imaging, the activation of five Ca^{2+} or K^{+} channels, and we propose a simplified single-compartment model that produces simulations of the V_m and Ca^{2+} transients that accurately match experimental data. Interestingly, we used 4 stable cells for this analysis, and the matching models were obtained using the same kinetic models of the six channels with relatively small variations of conductance densities. Using this strategy, which combines fast imaging techniques, pharmacological analysis and biophysical modeling, we unraveled the precise kinetics of dendritic Ca^{2+}

and K^{+} channel activation in PNs during CF-EPSPs under different V_m conditions. In particular, we established a clear role for the A-type VGKC in limiting the membrane depolarization and the activation of P/Q-type VGCCs.

Two distinct sets of channels activated by the CF-EPSP

We found that two different sets of channels are selectively activated at different initial V_m . When the dendrite is hyperpolarized ($V_m \sim -80$ mV), the transient depolarization produced by the CF-EPSP invading the dendritic branch activates T-type VGCCs (Isope et al., 2012) that enhance the distal dendritic V_m depolarization produced by the spread of the EPSP. The dendritic V_m is, however, capped $\sim <10$ mV by the K^{+} current via A-type VGCCs, limiting the opening of HVA Ca^{2+} and K^{+} channels. Under this condition, Ca^{2+} influx activates SK channels (Hosy et al., 2011) that regulate V_m repolarization and that appear selectively linked to T-type VGCCs, presumably by molecular coupling (Stocker, 2004). When, in contrast, the dendrite is depolarized ($V_m \sim -50$ mV), T-type VGCCs and A-type VGCCs are inactivated and the CF-EPSP can drive the dendrite to more positive V_m values that activate first P/Q-type VGCCs (Usovich et al., 1992) and then HVA channels leading to Ca^{2+} spikes (see the channels model available at <http://senselab.med.yale.edu/ModelDB/showModel.cshtml?model=244679>). In this case, Ca^{2+} influx activates BK channels (Rancz and Häusser, 2006) that fasten V_m repolarization limiting the number of Ca^{2+} spikes. HVA channels include Kv3.3, which is highly expressed in PNs (Goldman-Wohl et al., 1994), regulating dendritic Ca^{2+} spikes (Zagha et al., 2010; Veys et al., 2013). However, the lack of a selective channel blocker for this VGKC did not permit the experimental assessment of the kinetics of this channel, and we cannot exclude a contribution of other VGCCs with similar biophysical properties. The role of BK channels is to dampen the generation of Ca^{2+} spikes, which are typically only two when these channels are active. Finally, significant activation of both sets of channels occurs only at the intermediate initial V_m (~ -65 mV). In summary, The CF-evoked dendritic Ca^{2+} influx is mediated by two VGCCs that exhibit two different kinetics of activation and that are presumably associated with two distinct molecular pathways. In particular, P/Q-type VGCCs are believed to trigger endocannabinoid release and short-term synaptic depression (Rancz and Häusser, 2006). The scheme of activation of the two distinct sets of functionally coupled channels is illustrated in Figure 10.

A potential role of A-type VGCCs in synaptic plasticity

The scheme of Figure 10 also shows the result of blocking or inactivating A-type VGCCs. As these channels prevent the activation of the second set of channels when the dendrite is hyperpolarized, the modulation of this channel by synaptic transmission may provide a mechanism for triggering a CF nonlinear behavior playing a role in associative plasticity. Indeed, it has been shown that A-type VGCCs are modulated by Type 1 metabotropic glutamate receptors (Otsu et al., 2014), and this mechanism can play a role when the CF-EPSP is concomitant with PF activation. A-type VGCCs can also be rapidly inactivated by depolarization produced by EPSPs, driving the dendrite to a depolarized state. This mechanism occurs in the dendrites of CA1 hippocampal pyramidal neurons where inactivation of A-type VGCCs by Schaffer collateral EPSPs leads to boosting of back-propagating action potentials, a mechanism playing a role in Hebbian plasticity at these synapses (Magee and Johnston, 1997). While A-type VGCCs can act as a functional trigger of synaptic plasticity, these channels can be potentially the target of meta-

Table 1. Comparison of the percentage of the control Ca^{2+} transient produced by the pharmacological block of a channel type, in the case of experimental data, with the percentage of the control Ca^{2+} transient produced by the elimination of 90% of the channel in the case of simulations^a

Channel block	Signal (% control)	<i>hyp</i>		<i>int</i>		<i>dep</i>	
		First max	Second max	First max	Second max	First max	Second max
P/Q	+1 μM AgalVA ($N = 6$ cells)	50 $\pm$ 24*	90 $\pm$ 30	25 $\pm$ 12*	61 $\pm$ 31	10 $\pm$ 5*	6 $\pm$ 4
	–90% P/Q channels ($N = 4$ simulations)	74 $\pm$ 2	92 $\pm$ 3	13 $\pm$ 2	22 $\pm$ 6	6 $\pm$ 2	4 $\pm$ 1
T	+5 μM ML + 30 μM NNC ($N = 6$ cells)	52 $\pm$ 24*	21 $\pm$ 9*	94 $\pm$ 8	45 $\pm$ 15*	100 $\pm$ 4	99 $\pm$ 11
	–90% T channels ($N = 4$ simulations)	19 $\pm$ 3	12 $\pm$ 2	89 $\pm$ 3	90 $\pm$ 3	100	100
A	+1 μM AmmTx3 ($N = 6$ cells)	151 $\pm$ 34*	187 $\pm$ 44*	114 $\pm$ 8*	160 $\pm$ 33*	102 $\pm$ 4	102 $\pm$ 5
	–90% A channels ($N = 4$ simulations)	247 $\pm$ 39	281 $\pm$ 34	104 $\pm$ 2	180 $\pm$ 68	100	100
BK	+1 μM iberiotoxin ($N = 5$ cells)	97 $\pm$ 5	99 $\pm$ 5	98 $\pm$ 3	98 $\pm$ 4	98 $\pm$ 9	106 $\pm$ 5
	–90% BK channels ($N = 4$ simulations)	100 $\pm$ 1	101 $\pm$ 1	100 $\pm$ 1	100 $\pm$ 1	101 $\pm$ 1	117 $\pm$ 18
SK	+1 μM apamin ($N = 6$ cells)	101 $\pm$ 9	101 $\pm$ 10	100 $\pm$ 8	99 $\pm$ 6	103 $\pm$ 7	97 $\pm$ 8
	–90% SK channels ($N = 4$ simulations)	100 $\pm$ 1	101 $\pm$ 1	100 $\pm$ 1	100 $\pm$ 1	100 $\pm$ 1	103 $\pm$ 5

^aData are mean SD of the first and second max. The number of cells and the number of simulations used for the statistics are indicated. Simulations were performed by using 2 mm OG5N in the four variants of the model reported in Figure 7.

*Significant change in the max ($p < 0.005$, paired t-test).

Figure 9. Individual currents extracted from the NEURON model. Ca^{2+} currents of P/Q and T channels and K^{+} currents of A, BK, SK, and HVAK channels from the simulations of Cell 1 model variant reported in Figure 7. Simulations were at *hyp* (blue traces), *int* (green traces), and *dep* (red traces) states in control conditions, and at *hyp* (purple traces) after blocking 90% of A-type VGKCs, superimposed to currents in control conditions (gray traces).

Figure 10. Channel activation following CF-EPSPs at hyperpolarized and depolarized states. **A**, In control conditions, at *hyp* state, the CF-EPSP activates T-type channels, which activate SK channels, and A channels, which limit activation P/Q and HVAK channels; at *dep* state, the CF-EPSP activates P/Q-type channels, which activate BK channels, and HVAK channels, while T channels and A channels are inactivated. **B**, When A channels are blocked or inactivated, at *hyp* state, the CF-EPSP also activates P/Q-type channels, which activate BK channels, and HVAK channels.

plasticity mechanisms to regulate dendritic functions, in particular with respect to membrane excitability. In CA1 hippocampal pyramidal neurons, coupling between local dendritic spikes and the soma can be modified in a branch-specific manner through regulation of dendritic A-type K^{+} channels, a phenomenon that allows spatiotemporal correlation of synaptic inputs (Losonczy et al., 2008). In the cerebellum, this phenomenon occurs in the case of LTP of mossy fiber inputs to granule cells (Rizwan et al., 2016).

Relevance of our approach in the understanding of synergistic behaviors of ion channels

Voltage-gated and Ca^{2+} -activated ion channels shape the integration of incoming inputs in dendritic compartments and determine the pattern of V_m and Ca^{2+} influx (Magee and Johnston, 2005). In particular, each ion channel contributes to the V_m transient that in turn regulates the state of the channel (open, close, or inactivated). This patterning of mutual interactions determines a global synergy with sets of distinct channels that are functionally coupled. Hence, the understanding of dendritic integration relies on the precise reconstruction of the kinetics of all principal channels underlying the response of a dendritic compartment to a

given physiological input. This concept also applies to dendritic abnormal behaviors associated with channelopathies, such as the reported cases in Fragile X syndrome (Brager and Johnston, 2014; Zhang et al., 2014). Yet, the direct measurement of diverse ionic currents, mediated by different ions, is beyond available experimental techniques. In the last few years, we developed techniques to directly measure the kinetics of Ca^{2+} currents mediated by VGCCs in the dendrites of CA1 hippocampal pyramidal neurons (Jaafari et al., 2014, 2015; Jaafari and Canepari, 2016) and of PN (Ait Ouares et al., 2016), starting from high-temporal resolution Ca^{2+} imaging. In the present work, we used an alternative strategy to extract, indirectly, all the Ca^{2+} and K^{+} currents underlying the CF-mediated signals, using the NEURON simulation environment (Hines and Carnevale, 1997) applied to the same type of recordings, combined with V_m imaging. This novel approach allowed us reconstructing the functional interaction among individual channels, demonstrating the role of A-type VGCCs in controlling the activation of P/Q-type VGCCs. Thus, it was possible, for the first time, to deduce the kinetics of several Ca^{2+} and K^{+} channels in parallel. It should be highlighted that a single compartment model or a multicompartment model with a few compartments is not a realistic neuronal model of a PN because they do not account for either the precise morphology of the cell or the mechanisms present in small protrusions, such as dendritic spines. Yet, our approach is based on the concept that fewer parameters allow better constraining their values when fitting with a set of experimental observations. A complex multicompartmental model based on thousands of compartments (De Schutter, 1998) can be used to predict general cell integrative behaviors leading to firing activity, but is not ideal for deducing the precise kinetics of dendritic channels from detailed experimental observations. Modeling a PN dendrite as a single compartment is practical because PN dendritic trees comprise only two VGCCs and no voltage-gated Na^{+} channels. It will be very interesting, however, to implement these optimized channel models into realistic PN models to predict the major physiological functions of these neurons. A single compartment approach would not be appropriate in dendrites of cortical and hippocampal pyramidal neurons because these dendrites are endowed with a more complex composition of voltage-gated channels and notably stronger action potential backpropagation. Precisely reconstructing dendritic or axonal compartments in other systems is possible, in principle, using the same approach by expanding the pharmacological analysis to all the channels involved in a signal. For this purpose, the database ModelDB for NEURON simulations already includes >1100 published models covering >130 research topics (McDougal et al., 2017), which can be used as a starting framework to produce more simplified models with realistic channel kinetics matching the complexity of V_m and Ca^{2+} imaging experiments at high temporal resolution. The application of this novel approach also concerns the study of alterations induced by mutated proteins associated with channelopathies.

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