Two distinct sets of Ca\textsuperscript{2+} and K\textsuperscript{+} channels are activated at different membrane potentials by the climbing fibre synaptic potential in Purkinje neuron dendrites

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Two distinct sets of Ca\(^{2+}\) and K\(^{+}\) channels are activated at different membrane potentials by the climbing fibre synaptic potential in Purkinje neuron dendrites

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Abstract

In cerebellar Purkinje neuron (PN) dendrites, the transient depolarisation associated with a climbing fibre (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 fibre 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 analysed 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 hyperpolarised, CF-EPSPs mainly activate T-type VGCCs, SK channels and A-type VGKCs that limit the transient V\(_{m}\) below ~0 mV. In contrast, when the dendrite is depolarised, 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 PN dendrites.

Significance statement

The climbing fibre synaptic input transiently depolarises 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 modelling. We found that, according to the initial membrane potential, the climbing fibre depolarising 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 fibre (CF) synaptic input from the brainstem inferior olive to the cerebellar Purkinje neuron (PN) governs short-term (Brenowitz and Regehr, 2005) and long-term (Safo and Regehr, 2005) synaptic depression of parallel fibre (PF) synaptic 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 depolarisation 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 depolarisation spreads in the dendritic arborisation (Canepari and Vogt, 2008) where it activates voltage-gated \( \text{Ca}^{2+} \) channels (VGCCs) and voltage-gated \( \text{K}^{+} \) channels (VGKCs). The transient elevation of intracellular \( \text{Ca}^{2+} \) activates both \( \text{SK} \) \( \text{K}^{+} \) channels and \( \text{BK} \) \( \text{K}^{+} \) channels (Edgerton and Reinhart, 2003). Thus, the ensemble of activated \( \text{Ca}^{2+} \) and \( \text{K}^{+} \) channels, including VGKCs, shapes the waveform of the dendritic membrane potential (\( V_m \)) and of the \( \text{Ca}^{2+} \) 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 arborisation. To tackle this problem, a detailed compartmental model of PNs, including several ion channels, was proposed in the early 90s 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; Anwar et al., 2014). Yet, few -if any- of these predictions have been tested experimentally. Importantly, recent advancements in voltage-sensitive dye (VSD) imaging combined with \( \text{Ca}^{2+} \) imaging with a fast sampling rate, using low-affinity indicators to track the kinetics of VGCCs, allow the direct measurement of \( V_m \) and \( \text{Ca}^{2+} \) signals in the dendrites with a spatial resolution of a few microns (Jaafari et al., 2014; Jaafari et al., 2015; Jaafari and Canepari, 2016).

In this article, we report the first measurements of dendritic \( V_m \) and \( \text{Ca}^{2+} \) 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 \( \text{Ca}^{2+} \) transient produced by the local selective inhibition of various \( \text{Ca}^{2+} \) or \( \text{K}^{+} \) 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 \( \text{Ca}^{2+} \) 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 \( \text{Ca}^{2+} \)-activated \( \text{K}^{+} \) 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 \( \text{Ca}^{2+} \) buffer (Canepari and Mammano, 1999), and the two \( \text{Ca}^{2+} \) 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 unravelling the physiological kinetics of all dendritic Ca\textsuperscript{2+} and K\textsuperscript{+} 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 hyperpolarised or depolarised 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 carried out 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 \(\mu\)m thick) were prepared from 21-35 postnatal days old mice (C57Bl6) following established procedures (Vogt et al., 2011a; Vogt et al., 2011b; Ait Ouares et al., 2016) with a Leica VT1200 (Leica, Wetzlar, Germany) and incubated at 37°C for 45 minutes before use. The extracellular solution contained (in mM): 125 NaCl, 26 NaHCO\(_3\), 1 MgSO\(_4\), 3 KCl, 1 NaH\(_2\)PO\(_4\), 2 CaCl\(_2\) and 20 glucose, bubbled with 95% \(O_2\) and 5% \(CO_2\). The intracellular solution contained (in mM): 125 KMeSO\(_4\), 5 KCl, 8 MgSO\(_4\), 5 Na\(_2\)-ATP, 0.3 Tris-GTP, 12 Tris-Phosphocreatine, 20 HEPES, adjusted to pH 7.35 with KOH. In combined \(V_m\) and Ca\textsuperscript{2+} imaging experiments, PNs were loaded with the voltage-sensitive dye (VSD) JPW1114 and with the Ca\textsuperscript{2+} indicator FuraFF (at 1 mM) using a previously described procedure (Vogt et al., 2011a). In experiments of Ca\textsuperscript{2+} imaging only, Oregon Green BAPTA-5N (OG5N) was added to the internal solution at 2 mM concentration. Patch-clamp recordings were made at 32-34°C using a Multiclamp amplifier 700A (Molecular Devices, Sunnyvale, CA) 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 \(\mu\)A amplitude and 100 \(\mu\)s 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 \(\sim 2 \mu\)m diameter. Full names of chemicals used to block L-type, N-type or T-type VGCCs were 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(\alpha,5\alpha,6-exo,6\alpha)-3-(3,3-dimethylbutyl)-3-azabicyclo[3.1.0]hex-6-yl]methyl]-benzamide-hydrochloride; NNC550396: (1S,2S)-2-[3-(1H-Benzimidazol-2-yl)propyl]methylaminomethyl]-6-fluoro-1,2,3,4-tetrahydro-1-(1-methylethyl)-2-naphthalenyl-cyclopropanecarboxylate-dihydrochloride. The VSD and the Ca\textsuperscript{2+} indicators were from Invitrogen (Carlsbad, CA). \(\omega\)-Agatoxin IVA, AmmTx3, iberiotoxin and apamin were purchased from Smartox Biotechnology (St Martin d’Hères, France). All other chemicals were purchased from Tocris (Bristol, UK), from Hello Bio (Bristol, UK) or from Sigma-Aldrich (St. Louis, MO).
OPTICAL SIGNALS RECORDING AND CALIBRATION
Sequential \( V_m \) and \( \text{Ca}^{2+} \) optical measurements (Canepari et al., 2008; Canepari and Ogden, 2008) were achieved by alternating excitation of FuraFF at 385 nm with an OptoLED (CAIRN Research Ltd., Faversham, UK) and of the VSD at 532 nm using a 300 mW solid state laser (model MLL532, CNI, Changchun, China). In experiments with \( \text{Ca}^{2+} \) imaging only, OG5N was excited at 470 nm with the OptoLED. \( \text{Ca}^{2+} \) fluorescence (either from FuraFF or from OG5N) and \( V_m \) fluorescence were recorded at 525 ± 25 nm and at >610 nm respectively using a NeuroCCD-SMQ camera (RedShirtImaging, Decatur, GA). Images, de-magnified by ~0.2X to visualise an area of ~150 μm diameter, were acquired at 5 kHz with a resolution of 26 X 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. 3-4 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 \( \text{Ca}^{2+} \) signals were initially expressed as fractional changes of fluorescence \( (\Delta F/F_0) \). To match experimental \( \text{Ca}^{2+} \) signals with NEURON simulations, \( \Delta F/F_0 \) was converted into \( \text{Ca}^{2+} \)-bound-to-dye concentration \( ([\text{Ca}^{2+} \text{Dye}] ) \) as previously described (Ait Ouares 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 \( \sigma \) is the empirical dynamic range defined as the \( \Delta F/F_0 \) for saturating \( \text{Ca}^{2+} \) (Ait Ouares et al., 2016). Measurements of \( \sigma \) were obtained by saturating the indicators in the cytosol with a 30 s step of ~500 mV (in voltage clamp) that makes the somatic membrane permeable to \( \text{Ca}^{2+} \). The value of \( \sigma \) 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 hyperpolarisations from the resting \( V_m \) spread without attenuation through PN dendrites (Roth and Häusser, 2001), a prolonged hyperpolarising step of 1 s was used in each experiment to convert the VSD \( \Delta F/F_0 \) signal into mV, as shown in the example of Fig. 1A. Since in this study signals associated with the CF-EPSP were evoked at different initial \( V_m \), depolarising steps of 1 s were also used to estimate the dendritic \( V_m \) produced by somatic depolarisation. In detail, as shown in the example of Fig.1A, the two largest depolarisation 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 depolarised 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 \( \text{Ca}^{2+} \) transients, or of blocking a \( \text{Ca}^{2+} \) or a \( K^+ \) channel on the \( \text{Ca}^{2+} \) transients, were established 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 \( p < 0.005 \). In all figures and tables, a significant change was indicated with "***". The regions of interest 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 Fig. 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.csh.html?model=138382&file=/AnwarEtAl2010/cdp5.mod#tabs-1](https://senselab.med.yale.edu/ModelDB/ShowModel.csh.html?model=138382&file=/AnwarEtAl2010/cdp5.mod#tabs-1). From this model, consisting of a cylinder of 4 μm diameter and 20 μm 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. 2008) also available in ModelDB database at [https://senselab.med.yale.edu/ModelDB/ShowModel.csh.html?model=7176#tabs-1](https://senselab.med.yale.edu/ModelDB/ShowModel.csh.html?model=7176#tabs-1) and at [https://senselab.med.yale.edu/ModelDB/ShowModel.csh.html?model=112685&file=/Golgi_cell/Golgi_Sk2.mod#tabs-2](https://senselab.med.yale.edu/ModelDB/ShowModel.csh.html?model=112685&file=/Golgi_cell/Golgi_Sk2.mod#tabs-2). From this starting point, the kinetics of ion channels were changed in order to obtain traces from simulations that matched experimental V\(_m\) and Ca\(^{2+}\) optical measurements from \(~17\times17\ μm^2\) square regions at three different initial V\(_m\): hyperpolarised (\(~-80\ mV\)), intermediate (\(~-65\ mV\)) and depolarised (\(~-50\ mV\)). 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.csh.html?model=244679](http://senselab.med.yale.edu/ModelDB/showModel.csh.html?model=244679). In detail, I is the current density (expressed in mA/cm\(^2\)), \(\Pi\) is channel permeability to Ca\(^{2+}\) (expressed in cm/s), m\(_\infty\), t\(_m\), m\(_{exp}\) and h\(_\infty\), t\(_h\) are the voltage-dependent activation and inactivation parameters respectively, z\(_\infty\), 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 mV and ms 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 below -50 mV. We also reduced the activation time by 40% to reproduce the observed Ca\(^{2+}\) spiking rate at depolarised states.

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

\[
m_{\infty} = \frac{1}{1 + e^{-(V+29.450)/0.439}} \cdot \frac{1}{1 + e^{-0.1(V+50)}}
\]
\[
t_m = \begin{cases} 
0.6 \cdot \left( 0.2702 + 1.1622 \cdot e^{\frac{(V+26.798)^2}{164.19}} \right) & \text{if } V \leq -40 \text{mV} \\
0.6 \cdot 0.6923 \cdot e^{V/1089.372} & \text{otherwise} 
\end{cases}
\]

- **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 hyperpolarised and intermediate states. The activation time was decreased by 70% while the inactivation time was doubled to fit the rise and decay of the experimental \(V_m\) trace at the hyperpolarised state.

\[
I = \Pi \cdot m^2 \cdot h \cdot GHK
\]

\[
m_{m0} = \frac{1}{1 + e^{-(V+51)/6}} \cdot \text{corr}
\]

\[
\text{corr} = \begin{cases} 
1 & \text{at hyperpolarised states} \\
\frac{1}{1 + e^{-0.3(V+43)}} & \text{at intermediate states}
\end{cases}
\]

\[
h_{\infty} = \frac{1}{1 + e^{-\frac{V+72}{7}}}
\]

\[
t_m = \begin{cases} 
1 & \text{if } V \leq -90 \text{mV} \\
0.2 & \text{otherwise}
\end{cases}
\]

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

- **SK Ca\(^{2+}\)-activated K\(^+\) channel.** We used the model in Solinas et al., 2008 with 95% of the SK channels coupled to T-type VGCCs to account for effect of blocking these channels observed exclusively at hyperpolarised 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 repolarisation at depolarised states.

\[
I = \Sigma \cdot m \cdot x^2 \cdot (V + 85)
\]

\[
m_{m0} = \frac{8.5}{7.5 + a_m}
\]

\[
x_{\infty} = \frac{1}{1 + a_x}
\]

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

\[
a_x = \frac{0.4}{[\text{Ca}^{2+}]}\]

\[
m_{exp} = 1 - e^{-0.002 \cdot (7.5 + a_m)}
\]
\[ z_{\text{exp}} = 1 - e^{-0.0005} \]

- **A-type VGKC.** Starting from the formulation of A-type VGKCs channels in De Schutter and Bower, 1994, the kinetic parameters were modified in line with modifications of T-type VGCCs to account for behaviours at hyperpolarised states. The density was corrected at intermediate states to account for partial inactivation.

\[ I = \Sigma \cdot h \cdot m^+ \cdot (V + 85) \]

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

\[ h_{\infty} = \frac{a_h}{a_h + b_h} \]

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

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

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

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

\[ t_m = \frac{1}{a_m + b_m} \]

\[ t_h = \frac{1}{a_h + b_h} \]

- **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 behaviours at depolarised 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 ~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.

\[ I = \Sigma \cdot h \cdot m^+ \cdot (V + 85) \]

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

\[ h_{\infty} = \frac{1}{1 + e^{(V+25)/4}} \]
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 equation:

\[ I_{CF} = I_{HOLD} + I_{STANDING} \cdot \left(1 - e^{-\left(1 - \text{DELAY}/\text{RISE}\right)}\right) \cdot e^{-\left(1 - \text{DELAY}/\text{DURATION}\right)} \]

The parameters in this equation were tuned to obtain the match of experimental \( V_m \) and \( \text{Ca}^{2+} \) transients within the following ranges. \( I_{HOLD} \) (holding current before CF-EPSP occurrence): between -0.03 and 0.04 mA/cm\(^2\). \( I_{STANDING} \): between -0.4 and -0.1 mA/cm\(^2\). 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 Fig. 1B, were used in the simulations.

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

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

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

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

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

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

Dendritic depolarisation and Ca\textsuperscript{2+} transients associated with the CF-EPSP

In the first series of experiments (N = 12 cells), we investigated the dendritic depolarisation and the Ca\textsuperscript{2+} transients associated with the CF-EPSP by combining $V_m$ and Ca\textsuperscript{2+} imaging as described in the Materials and Methods. Within a recording field of $\sim$150X150 $\mu m^2$ (26X26 pixels), we systematically averaged fluorescence over 1-3 dendritic regions of $\sim$17X17 $\mu m^2$ (3X3 pixels) with sufficient signal-to-noise ratio in order 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 the Materials and Methods and illustrated in Fig. 1A. In the cell reported in Fig. 2A, the initial somatic $V_m$ was set by current injection and a CF-EPSP was evoked at a state of hyperpolarisation (hyp, blue trace) around -80 mV, at an intermediate $V_m$ (int, green trace) close to the resting $V_m$, and at a state of depolarisation (dep, red trace). These three conditions corresponded, in this particular cell, to initial dendritic $V_m$ of -83 mV, -64 mV and -54 mV respectively. In the two analysed 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 dep state. Correlated with these $V_m$ transients, the Ca\textsuperscript{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 since they are characterised by a rapid rise and fall. This behaviour was consistently observed in every cell investigated. The $V_m$ and Ca\textsuperscript{2+} transients in R1 are shown again in Fig. 2C to illustrate the quantitative analysis that was performed. For both dendritic $V_m$ and Ca\textsuperscript{2+} transients, we measured the maximum (max) during the first 4 ms after the CF stimulation (1st max) and between 4 and 14 ms after the CF stimulation (2nd max). The values for R1 are reported in Fig. 2C and the statistics (mean ± SD) for 19 regions in the 12 cells analysed are reported in Fig. 2D. At hyp states (with initial $V_m$ between -87 mV and -74 mV), the 1st and 2nd $V_m$ maxima were -13 ± 8 mV and -38 ± 7 mV respectively, while the Ca\textsuperscript{2+} transient ($-\Delta F/F_0$) max were 1.83 ± 0.55 % and 3.14 ± 0.85 % respectively. At int states (with initial $V_m$ between -68 mV and -61 mV), the 1st and 2nd $V_m$ maxima were 6 ± 7 mV and -21 ± 11 mV respectively, while the Ca\textsuperscript{2+} transient max were 3.65 ± 1.27 % and 3.65 ± 1.05 % respectively. Finally, at dep states (with initial $V_m$ between -54 mV and -46 mV), the 1st and 2nd $V_m$ maxima were 9 ± 6 mV and -1 ± 9 mV respectively, while the Ca\textsuperscript{2+} transients maxima were 5.36 ± 1.17 % and 6.59 ± 1.42 % respectively. The 1st max of both $V_m$ and Ca\textsuperscript{2+} transients significantly increased from the hyp state to the int state (p < 0.005, paired t-test), while the 2nd max of both the $V_m$ and Ca\textsuperscript{2+} transients significantly increased from the int state to the dep state. These results demonstrate that CF-EPSP associated dendritic depolarisation and Ca\textsuperscript{2+} influx increase with the initial $V_m$. Furthermore, dendritic spikes (typically two in the 18 ms after the stimulation) and correlated Ca\textsuperscript{2+} transients occur when the dendrite is depolarised. The dendritic depolarisation is produced by the passive spread of the CF-EPSP from the soma and proximal dendrite and activates voltage-gate Ca\textsuperscript{2+} channels (VGCCs) and voltage-gate K\textsuperscript{+} channels (VGKCs). Ca\textsuperscript{2+}-
activated K⁺ channels contribute to the repolarisation. To resolve the channels underlying the behaviours observed in the experiments reported above, we analysed pharmacologically in detail the Ca²⁺ transient associated with the CF-EPSP.

Dendritic Ca²⁺ channels activated by the CF-EPSP

In PNs, the dendritic Ca²⁺ transients associated with the CF-EPSP transient depolarisation are mediated by VGCCs, in particular P/Q-type HVA-VGCCs (Usowitz et al., 1992) and T-type LVA-VGCCs (Isope et al., 2012). Thus, we investigated the changes in the Ca²⁺ transients from OG5N fluorescence produced by the selective block of one or more VGCCs. In the representative example of Fig. 3A, the CF-EPSP was evoked at hyp (blue traces), int (green traces) and dep (red traces) states and the associated OG5N-ΔF/F₀ signal was recorded in the control condition and after local application of the P/Q-type VGCC blocker ω-agatoxin-IVA (AgaIVA, 1 μM). Importantly, to assess the postsynaptic effect while excluding any possible presynaptic effect, the changes in the Ca²⁺ transient were analysed and compared in the region next to the application pipette (R1) and in another region at more than 50 μm from the application pipette (R2). In R1 only, AgaIVA reduced the Ca²⁺ transient during the first few milliseconds after the CF-EPSP at the hyp and int states, and blocked the Ca²⁺ transient at the dep state.

In the representative example of Fig. 3B, the same protocol was used to assess the effects of local block of T-type VGCCs, using the inhibitors ML218 (ML, 5 μM) and NNC550396 (NNC, 30 μM). In this case, the blockers inhibited the late component of the Ca²⁺ transient at the hyp and int states, but had no effect on the Ca²⁺ transient at the dep state. Finally, in the representative example of Fig. 3C, local block of P/Q-type and T-type VGCCs together inhibited Ca²⁺ transient in all states. To rule out any additional component from other VGCC types, in the three representative examples of Fig. 3D, we also tested the blockers Isradipine (20 μM, L-type), PD173212 (5 μM, N-type) and SNX482 (1 μM, R-type). The Ca²⁺ transient remained unaffected under all states and cases tested. The results reported in Fig. 3 were consistently observed in all cells tested with each VGCC blocker. To quantify these results, we measured again the 1st max (during the first 4 ms after the stimulation) and the 2nd max (between 4 ms 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 Fig. 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 1st max of the Ca²⁺ transient at all initial Vₘ states (p < 0.005, paired t-test), while the 2nd max was reduced only in the int and dep states. In contrast, the block of T channels significantly reduced the 1st and 2nd maxima of the Ca²⁺ transient at hyp states and the 2nd max only and int states, while 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 (Lsr), 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 Ca²⁺ influx, associated with the CF-EPSP and mediated by P/Q-type and T-type VGCCs respectively. At hyp
states, where the depolarisation transient is typically below -10 mV (see 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 depolarisation 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 depolarisation transients associated with CF-EPSPs, possibly due to steady inactivation of A-type VGKCs that are expressed in PN dendrites (Otsu et al., 2014). To test this hypothesis, we investigated the change in the OG5N Ca²⁺ transient produced by local application of the A-type VGKC inhibitor AmmTx3 (Zoukimian et al., in press). As shown in the representative example of Fig. 5A, the block of A-type channels strongly enhanced the Ca²⁺ 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 1st and 2nd maxima of the Ca²⁺ transient at hyp and int states, also at sites located at $>50 \mu m$ from the pipette delivering the toxin, while it did not modify the Ca²⁺ transient at dep states indicating that A-type VGKCs are fully inactivated at initial $V_m > -55 mV$ (Fig. 5B). In contrast A-type VGKCs are activated at more negative initial $V_m$ and this result suggests that their activation limits the activation of P/Q-type VGCCs.

Since Ca²⁺ transients activate Ca²⁺-activated K⁺ channels, we finally investigated the change in the OG5N Ca²⁺ transient produced by local application of the BK channel inhibitor iberiotoxin (1 μM, Fig. 6A) and of the SK channel inhibitor apamin (1μM, Fig. 6B). Neither iberiotoxin nor apamin produced any change in the 1st and 2nd maxima of the Ca²⁺ 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 5/5 cells where the block of BK channels was tested, the number of Ca²⁺ spikes increased from 2 to 3 at dep states. In addition, in 4/6 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 modelling**

To extrapolate individual dendritic ion currents underlying $V_m$ transients and Ca²⁺ signals associated with CF-EPSPs at different $V_m$ initial states, we selected 4 dendritic sites from 4 different PNs within those analysed in Fig. 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, in order to assume the same conditions for the $V_m$ and Ca²⁺ recordings. In these 4 compartments, we matched the experimental $V_m$ and Ca²⁺ transients with traces obtained by computer simulations of the NEURON model illustrated in Fig. 1B, as described in the Materials and Methods. In particular, we established a unique kinetic model for each of the six types of channels, in order to consistently reproduce the behaviours 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.cshtml?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 VGKCs, and of BK and SK Ca$^{2+}$ activated K$^+$ channels was adjusted in order to match experimental data in each cell, as shown in Fig. 7. To validate the consistency of the four variants of the model, we run computer simulations by modelling the replacement of 1 mM Fura-FF with 2 mM OGG5N and analysed 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 Fig. 7 are reported in Fig. 8, showing that 90% reduction in the density of each channel qualitatively reproduced the experimental behaviour observed after toxin or drug inhibition.

In order to compare experiments and simulations, we calculated the percentage of the 1$^{st}$ max and the 2$^{nd}$ 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 Fig. 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 depolarisation transient carried by the CF-EPSP activates a robust Ca$^{2+}$ current, mediated by T-type channels, and a K$^+$ 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 VGKSs are poorly activated in 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 depolarisation transient carried by the CF-EPSP activates a Ca$^{2+}$ current, mediated by P/Q-type Ca$^{2+}$ channels, and a K$^+$ current, mediated by HVAK 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 HVAK VGKCs is prevented at hyp states by the K$^+$ current mediated by A-type VGKCs. 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 VGKCs in controlling activation of P/Q-type channels, HVAK channels 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 modelling, we unravelled 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 depolarisation 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 hyperpolarised (\( V_m \sim -80 \text{ mV} \)), the transient depolarisation produced by the CF-EPSP invading the dendritic branch activates T-type VGCCs (Isope et al., 2012) that enhance the distal dendritic \( V_m \) depolarisation produced by the spread of the EPSP. The dendritic \( V_m \) is however capped below \( \sim 10 \text{ mV} \) by the \( K^+ \) current via A-type VGKCs, 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 \( V_m \) repolarisation and that appear selectively linked to T-type VGCCs, presumably by molecular coupling (Stocke, 2004). When in contrast the dendrite is depolarised (\( V_m \sim -50 \text{ mV} \)), T-type VGCCs and A-type VGKCs are inactivated and the CF-EPSP can drive the dendrite to more positive \( V_m \) values that activate first P/Q-type VGCCs (Usowicz et al., 1992) and then HVAks leading to \( Ca^{2+} \) spikes (see the channels model available at http://senselab.med.yale.edu/ModelDB/showModel.cshml?model=244679)). In this case, \( Ca^{2+} \) influx activates BK channels (Rancz and Häusser, 2006) that fasten \( V_m \) repolarisation limiting the number of \( Ca^{2+} \) spikes. HVAKs include Kv3.3 that 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 didn’t permit the experimental assessment of the kinetics of this channel and we cannot exclude a contribution of other VGKCs 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 intermediate initial \( V_m \) (\( \sim -65 \text{ mV} \)). 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 Fig. 10.

A potential role of A-type VGKCs in synaptic plasticity
The scheme of Fig.10 also shows the result of blocking or inactivating A-type VGKCs. As these channels prevent the activation of the second set of channels when the dendrite is hyperpolarised, the modulation of this channel by synaptic transmission may provide a mechanism for triggering a CF nonlinear behaviour playing a role in associative plasticity. Indeed, it has been shown that A-type VGKCs 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 VGKCs can also be rapidly inactivated by depolarisation produced by excitatory postsynaptic potentials, driving the dendrite to a depolarised state. This mechanism occurs in the dendrites of CA1 hippocampal pyramidal neurons where inactivation of A-type VGKCs by Schaffer collateral EPSPs leads to boosting of backpropagating action potentials, a mechanism playing a role in hebbian plasticity at these synapses (Magee and Johnston, 1997). While A-type VGKCs can act as functional trigger of synaptic plasticity, these channels can be potentially the target of meta-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 spatio-temporal correlation of synaptic inputs (Losonczy et al., 2008). In the cerebellum, this phenomenon occurs in the case of long-term potentiation of mossy fibre inputs to granule cells (Rizwan et al., 2016).

**Relevance of our approach in the understanding of synergistic behaviours 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 behaviours associated with channelopathies, such as the reported cases in Fragile X syndrome (Zhang et al., 2014; Brager and Johnston, 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; Jaffari et al., 2015; Jaffari and Canepari, 2016) and of PNs (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 VGKCs 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 multi-compartment model with a few compartments, are not a realistic neuronal model of a PN since they don’t account neither for the precise morphology of the cell nor for the mechanisms present in small protrusions like 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 multi-compartmental model based on thousands of compartments (De Schutter, 1998) can be used to predict general cell integrative behaviours 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 optimised 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 over 1100 published models covering more than 130 research topics (McDougal et al., 2017) that can be used as a starting framework to produce more simplified models with realistic channel kinetics matching the complexity of Vᵦ and Ca²⁺ 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.
References


Figure Legend

Figure 1. V_m calibration protocol and illustration of NEURON model. A, On the left, fluorescence reconstruction of a PN with three regions of interest (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. On the right, somatic V_m and the VSD-ΔF/F_0 signals in R1-R3 associated with the current pulses. The VSD-ΔF/F_0 signal in each regions is converted into mV to quantify the V_m transient associated with the CF-EPSP assuming that the resting V_m is uniform (int state) and that the hyperpolarising pulse spreads into the dendrites without attenuation (hyp state). The protocol also allows determining the dendritic V_m associated with the strongest depolarising pulse (dep state). B, A dendritic region of ~17X17 μm^2 is approximated with a cylinder of 4 μm diameter and 20 μm 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 HVAK. 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.

Figure 2. Combined V_m and Ca^{2+} transients associated with the CF-EPSP. A, On the bottom, fluorescence reconstruction of a representative PN with two regions of interest indicated (R1 and R2). On the top, somatic V_m associated with a CF-EPSP at three different initial V_m: hyperpolarised (hyp blue trace); intermediate (int green trace); depolarised (dep red trace). B, On the top, dendritic V_m in R1 and R2 calibrated as illustrated in Fig. 1A corresponding to the somatic CF-EPSPs in panel A. On the bottom, corresponding FuraFF ΔF/F_0 signals. C, Analysis of the V_m and Ca^{2+} maxima (max) associated with signals in R1 reported in panel B; the 1st max of the V_m and Ca^{2+} transients is calculated within the first 4 ms after the CF stimulation; the 2nd max of the V_m and Ca^{2+} transients is calculated between 4 and 14 ms after the CF stimulation; blue traces and characters are for the hyp state; green traces and characters are for the int state; red traces and characters are for the dep state. D, Mean ± SD for 19 regions in 12 cells analysed as illustrated in panel 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 mV and -46 mV; the symbol ‘***’ indicates a significant increase in the max (p < 0.005, paired t-test).

Figure 3. Dendritic Ca^{2+} channels activated by the CF-EPSP. A, On the left, fluorescence reconstruction of a PN with two regions of interest indicated (R1 and R2); R1 is next to a pipette delivering 1 μM of the P/Q-type VGCC inhibitor AgaIVA; R2 is >50 μm from the application pipette. On the 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); dep (red trace). On the right (bottom) the corresponding OG5N ΔF/F_0 signals. B, In another PN, same as A, but with the pipette delivering the T-type VGCC inhibitors ML (5 μM) and NNC (30 μM). C, In another PN, same as A, but with the pipette delivering both the P/Q VGCC inhibitor AgaIVA (1 μM) and the T-type VGCC inhibitors ML (5 μM) and
NNC (30 μM). D, In three other PNs, from a region next to a pipette delivering a VGCC blocker, the
OG5N ΔF/F₀ signals associated with CF-EPSPs in control conditions and after local application of 20 μM
of the L-type VGCC inhibitor Isr, of 5 μM of the N-type VGCC inhibitor PD or 1 μM of the R-type VGCC
inhibitor SNX at the three different initial Vₘ.

Figure 4. Quantitative analysis of dendritic Ca²⁺ channels activated by the CF-EPSP. On the left, from
two representative cells, OG5N ΔF/F₀ signals associated with CF-EPSPs in control conditions at three
different initial Vₘ: hyp (blue trace); int (green trace); dep (red trace); superimposed (gray traces) are the
OG5N ΔF/F₀ signals after local application of either 1 μM of the P/Q-type VGCC inhibitor AgaIVA or of
the T-type VGCC blockers ML (5 μM) and NNC (30 μM); the 1ˢᵗ max of the Ca²⁺ transient is calculated
within the first 4 ms after the CF stimulation; the 2ⁿᵈ max of the Ca²⁺ transient is calculated between 4
and 14 ms after the CF stimulation; the percentages from control ΔF/F₀ maxima after application of the
VGCC blockers are reported above or below the arrows. On the right, mean ± SD of the percentages
from control ΔF/F₀ 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 are the statistics of the 1ˢᵗ max; white columns are the statistics of the 2ⁿᵈ max; the symbol **
indicates a significant change in the max (p < 0.005, paired t-test).

Figure 5. Dendritic A-type VGKC activated by the CF-EPSP. A, On the left, fluorescence reconstruction
of a PN with a region of interest indicated next to a pipette delivering 1 μM of the A-type VGKC inhibitor
AmmTx3. On the right (top), somatic Vₘ associated with CF-EPSPs in control conditions and after local
application of AmmTx3 at three different initial Vₘ: hyp (blue trace); int (green trace); dep (red trace). On
the right (bottom) the corresponding OG5N ΔF/F₀ signals. B, On the left, from the cell in A, OG5N ΔF/F₀
signals associated with CF-EPSPs in control conditions at the three different initial Vₘ; superimposed
(gray traces) are the OG5N ΔF/F₀ signals after local application of either 1 μM AmmTx3; the 1ˢᵗ max of
the Ca²⁺ transient is calculated within the first 4 ms after the CF stimulation; the percentages from control
ΔF/F₀ maxima after application of the VGCC blockers are reported above the arrows. On the right, mean
± SD of the percentages from control ΔF/F₀ maxima after application of AmmTx3 (N = 6 cells); gray
columns are the statistics of the 1ˢᵗ max; white columns are the statistics of the 2ⁿᵈ max; the symbol **
indicates a significant change in the max (p < 0.005, paired t-test).

Figure 6. Dendritic Ca²⁺-activated K⁺ channels activated by the CF-EPSP. A, On the left, fluorescence
reconstruction of a PN with a region of interest indicated next to a pipette delivering 1 μM of the BK
channel inhibitor iberiotoxin. On the right (top), somatic Vₘ associated with CF-EPSPs in control
conditions and after local application of iberiotoxin at three different initial Vₘ: hyp (blue trace); int (green
trace); dep (red trace). On the right (bottom) the corresponding OG5N ΔF/F₀ signals. B, In another PN,
same as A but with the pipette delivering 1 μM of the SK channel inhibitor apamin. C, Mean ± SD of the
percentages from control ΔF/F₀ maxima after application of iberiotoxin (N = 5 cells) or apamin (N = 6

22
cells); gray columns are the statistics of the 1st max; white columns are the statistics of the 2nd max. C, On the left, from the cell in A, OG5N ΔF/F₀ signal associated with the CF-EPSP at dep state in control condition and after addition of iberiotoxin (gray trace). On the right, from the cell in B, OG5N ΔF/F₀ signal associated with the CF-EPSP at hyp state in control condition and after addition of apamin (gray trace).

**Figure 7.** NEURON model of 4 PN dendritic compartments reproducing Vₘ and Ca²⁺ transients associated with the CF-EPSP. On the left, experimental dendritic Vₘ and Ca²⁺ transients associated with CF-EPSPs from 4 selected cells at three different initial Vₘ: hyperpolarised (hyp, blue trace); intermediate (int, green trace); depolarised (dep, red trace). On the right, simulations of dendritic Vₘ and Ca²⁺ transients associated with CF-EPSPs reproducing experimental data (gray traces).

**Figure 8.** Simulations of block of P/Q-type VGCCs, T-type VGCCs, A-type VGKC, BK and SK Ca²⁺ activated K⁺ channels from a NEURON model. Simulated Ca²⁺ transients (OG5N) associated with CF-EPSPs at three different initial Vₘ in control condition and after reduction of 90% of one individual channels from Cell 1 model variant reported in Fig. 7. For each case of 90% channel reduction, traces under control conditions are reported in gray.

**Figure 9.** Individual currents extracted from NEURON model. Ca²⁺ 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 Fig. 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 VGKC, superimposed to currents in control conditions (gray traces).

**Figure 10.** Channel activation following CF-EPSPs at hyperpolarised and depolarised states. A, In control conditions, at hyp state, the CF-EPSP activates T-type channels, that activate SK channels, and A channels that limit activation P/Q and HVAK channels; at dep state, state, the CF-EPSP activates P/Q-type channels, that 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, that activate BK channels, and HVAK channels.
Table 1. Comparison of the percentage of the control Ca\textsuperscript{2+} transient (mean ± SD of the 1\textsuperscript{st} and 2\textsuperscript{nd} maxima) produced by the pharmacological block of a channel type, in the case of experimental data, with the percentage of the control Ca\textsuperscript{2+} transient produced by the elimination of 90% of the channel in the case of simulations. The number of cells and the number of simulations used for the statistics is indicated. Simulations were performed by using 2 mM OG5N in the four variants of the model reported in Fig. 7.

<table>
<thead>
<tr>
<th>Channel block</th>
<th>Hyp</th>
<th>Int</th>
<th>Dep</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1\textsuperscript{st} max</td>
<td>2\textsuperscript{nd} max</td>
<td>1\textsuperscript{st} max</td>
</tr>
<tr>
<td>P/Q +1 μM AgaIVA (N = 6 cells)</td>
<td>50 ± 24*</td>
<td>90 ± 30</td>
<td>25 ± 12*</td>
</tr>
<tr>
<td>-90% P/Q channels (N = 4 simulations)</td>
<td>74 ± 2</td>
<td>92 ± 3</td>
<td>13 ± 2</td>
</tr>
<tr>
<td>T +5 μM ML+30 μM NNC (N = 6 cells)</td>
<td>52 ± 24*</td>
<td>21 ± 9*</td>
<td>94 ± 8</td>
</tr>
<tr>
<td>-90% T channels (N = 4 simulations)</td>
<td>19 ± 3</td>
<td>12 ± 2</td>
<td>89 ± 3</td>
</tr>
<tr>
<td>A +1 μM AmmTx3 (N = 6 cells)</td>
<td>151 ± 34*</td>
<td>187 ± 44*</td>
<td>114 ± 8*</td>
</tr>
<tr>
<td>-90% A channels (N = 4 simulations)</td>
<td>247 ± 39</td>
<td>281 ± 34</td>
<td>104 ± 2</td>
</tr>
<tr>
<td>BK +1 μM iberiotoxin (N = 5 cells)</td>
<td>97 ± 5</td>
<td>99 ± 5</td>
<td>98 ± 3</td>
</tr>
<tr>
<td>-90% BK channels (N = 4 simulations)</td>
<td>100 ± 1</td>
<td>101 ± 1</td>
<td>100 ± 1</td>
</tr>
<tr>
<td>SK +1 μM apamin (N = 6 cells)</td>
<td>101 ± 9</td>
<td>101 ± 10</td>
<td>100 ± 8</td>
</tr>
<tr>
<td>-90% SK channels (N = 4 simulations)</td>
<td>100 ± 1</td>
<td>101 ± 1</td>
<td>100 ± 1</td>
</tr>
</tbody>
</table>
A

initial $V_m (mV)$

-50
-60
-70
-80

CF-EPSP (dep) → P/Q → BK

CF-EPSP (hyp) → A → T → SK

Control

B

initial $V_m (mV)$

-50
-60
-70
-80

CF-EPSP (dep) → P/Q → BK → HVA-VGKC

CF-EPSP (hyp) → T → SK

Block or inactivation of A-type VGKC
Table 1

| Signal (% control) |  
|-------------------|---|
| Channel block     | Hyp | Int | Dep |
|                   | 1<sup>st</sup> max | 2<sup>nd</sup> max | 1<sup>st</sup> max | 2<sup>nd</sup> max | 1<sup>st</sup> max | 2<sup>nd</sup> max |
| P/Q               |     |     |     |     |     |     |
| +1 μM AgaIVA      | 50 ± 24* | 90 ± 30 | 25 ± 12* | 61 ± 31 | 10 ± 5* | 6 ± 4 |
| (N = 6 cells)     |     |     |     |     |     |     |
| -90% P/Q channels | 74 ± 2 | 92 ± 3 | 13 ± 2 | 22 ± 6 | 6 ± 2 | 4 ± 1 |
| (N = 4 simulations)|     |     |     |     |     |     |
| T                 |     |     |     |     |     |     |
| +5 μM ML+30 μM NNC| 52 ± 24* | 21 ± 9* | 94 ± 8 | 45 ± 15* | 100 ± 4 | 99 ± 11 |
| (N = 6 cells)     |     |     |     |     |     |     |
| -90% T channels   | 19 ± 3 | 12 ± 2 | 89 ± 3 | 90 ± 3 | 100 | 100 |
| (N = 4 simulations)|     |     |     |     |     |     |
| A                 |     |     |     |     |     |     |
| +1 μM AmmTx3      | 151 ± 34* | 187 ± 44* | 114 ± 8* | 160 ± 33* | 102 ± 4 | 102 ± 5 |
| (N = 6 cells)     |     |     |     |     |     |     |
| -90% A channels   | 247 ± 39 | 281 ± 34 | 104 ± 2 | 180 ± 68 | 100 | 100 |
| (N = 4 simulations)|     |     |     |     |     |     |
| BK                |     |     |     |     |     |     |
| +1 μM iberiotoxin | 97 ± 5 | 99 ± 5 | 98 ± 3 | 98 ± 4 | 98 ± 9 | 106 ± 5 |
| (N = 5 cells)     |     |     |     |     |     |     |
| -90% BK channels  | 100 ± 1 | 101 ± 1 | 100 ± 1 | 101 ± 1 | 101 ± 1 | 117 ± 18 |
| (N = 4 simulations)|     |     |     |     |     |     |
| SK                |     |     |     |     |     |     |
| +1 μM apamin      | 101 ± 9 | 101 ± 10 | 100 ± 8 | 99 ± 6 | 103 ± 7 | 97 ± 8 |
| (N = 6 cells)     |     |     |     |     |     |     |
| -90% SK channels  | 100 ± 1 | 101 ± 1 | 100 ± 1 | 100 ± 1 | 100 ± 1 | 103 ± 5 |
| (N = 4 simulations)|     |     |     |     |     |     |

**Table 1.** Comparison of the percentage of the control Ca$^{2+}$ transient (mean ± SD of the 1<sup>st</sup> and 2<sup>nd</sup> maxima) 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. The number of cells and the number of simulations used for the statistics is indicated. Simulations were performed by using 2 mM OG5N in the four variants of the model reported in Fig. 7.