On the kinetics of calcium currents and the activation of calcium-activated potassium channels in layer-5 pyramidal neuron dendrites

Laila Ananda Blömer, Luiza Filipis and Marco Canepari

Univ. Grenoble Alpes, CNRS, LIPhy, F-38000 Grenoble, France

INTRODUCTION

Ca^{2+} influx via voltage-gated Ca^{2+} channels (VGCCs) contributes to the shaping of the AP by regulating the K^+ conductance during the decaying phase. Here we optically analysed the kinetics of the Ca^{2+} current associated with APs in the apical dendrite of the layer-5 pyramidal neuron of the somatosensory cortex of the mouse. We found that this current is mediated by L-type, P/Q-type, N-type, R-type and T-type VGCCs. The selective block of each channel decreases the calcium current, except for the N-type VGCC where inhibition has the opposite effect. We hypothesize the increase in Ca^{2+} influx is driven by Ca^{2+}-activated K^+ channels (CAKC), such as BK and SK; in the proximal apical dendrite it seems that each CAKC is specifically coupled to a calcium channel.

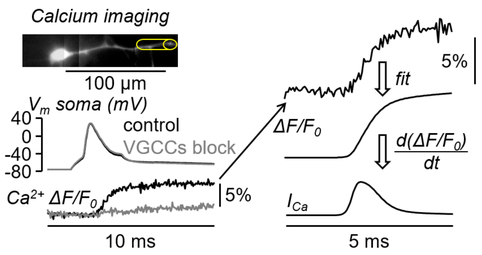
This study was developed in parallel with experiments in the axon initial segment of the same cell type (see Poster S04-169).

METHODS

Electrophysiology – sagittal 350 µm cortical brain slices from P21-35 C57Bl/6j mice. L5 somatosensory pyramidal neurons were patched in whole-cell configuration and filled with voltage sensitive dye JPW1114 or a calcium indicator (OG5N or CAL-520FF). Current injection at the soma of 3 ms and 1-2 nA amplitude.

Imaging – optical measurements at 20 kHz with 26 x 4 pixels resolution, ~100 µm from the soma. Ca^{2+} and V_m fluorescence were excited at 470 nm with an OptoLED or with a laser at 530 nm and recorded at 510 ± 42 nm or >610 nm, respectively, with a NeuroCCD-SMQ camera. Data shown is bleach corrected and an average of 6-9 or 4-5 trials for Ca^{2+} or V_m imaging, respectively.

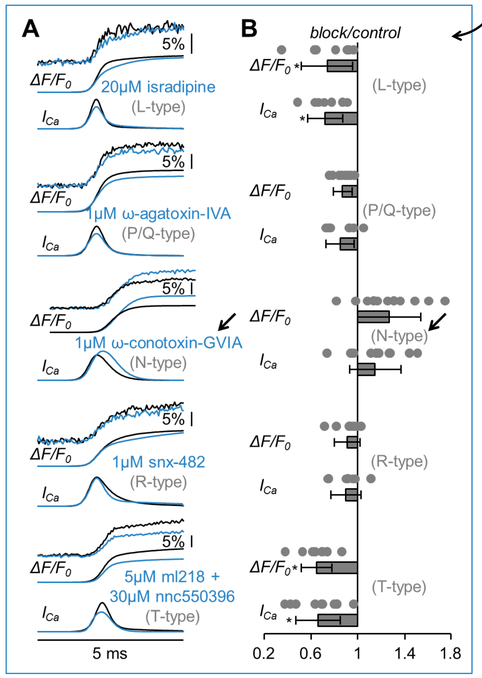
Ca^{2+} and V_m data were initially expressed as fractional changes of fluorescence ($\Delta F/F_0$ signals), calculated after subtraction of the autofluorescence background. The Ca^{2+}- $\Delta F/F_0$ signal associated with each BPAP, normalised to its asymptotic value, was initially fitted with a 4-sigmoid function. The time derivative of the fitted Ca^{2+}-$\Delta F/F_0$ signal was then calculated.



Pharmacology – Block of CAKCs: 1 µM apamin (SK) and 1 µM iberiotoxin (BK). Block of VGCCs: 20 µM Isradipine (L-type), 1 µM ω-agatoxin-IVA (P/Q-type), 1 µM ω-conotoxin-GVIA (N-type), 1 µM snx482 (R-type), 30 µM NNC550396 + 5 µM ML218 (T-type).

RESULTS

Figure 1 Calcium imaging during AP back propagation. A Examples of all VGCC channel blockers used, and the calcium current (time derivative of change in fluorescence). All blockers decrease the calcium current except conotoxin-GVIA which blocks N-type VGCCs (arrow). B Single values and percentage change of the effect of the VGCC blockers isradipine (N = 7 cells), agatoxin-IVA (N = 8), conotoxin-GVIA (N = 11), SNX-482 (N = 7) and ML218 + NCC550396 (N = 10), as the maximum change in fluorescence and the peak of the calcium current. All blockers show a decrease in calcium, except conotoxin-GVIA (arrow).



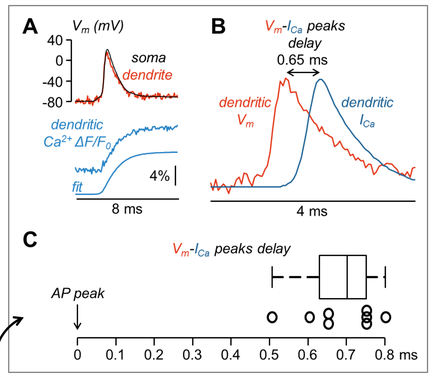
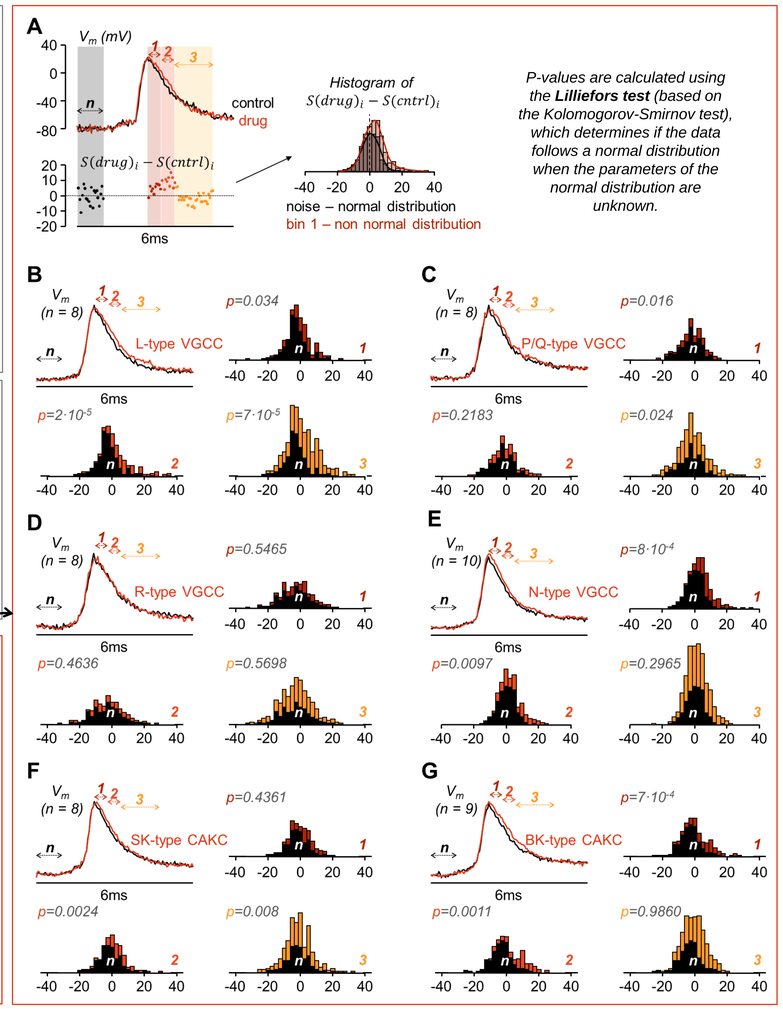


Figure 2 Delay between the calcium current and voltage peak of the backpropagating AP. A Above the AP recorded at the soma and recording (voltage sensitive dye) ~100 µm from the soma. Below the calcium trace with fit. B Same voltage trace superimposed on the calcium current calculated from the $\Delta F/F_0$. C Single values and average of the delay of the calcium current peak compared to the bAP peak (N = 8 cells).

Figure 3 Voltage imaging during AP back propagation. A Above voltage imaging (VI) in the proximal dendrite before and after blocking N-type VGCCs. Traces are compared in bins of 500 µs after the V_m peak (1), 500 µs around the Ca^{2+} peak (2) and 1.5 ms after the Ca^{2+} peak (3). Below the difference between block and control expressed as deviations from the resting potential (n). The normality of these distributions is assessed using the Lilliefors test, with the noise (n) as the estimate of the normal distribution. B-H Examples of voltage traces when applying, isradipine (B), agatoxin-IVA (C), SNX-482 (D), conotoxin-GVIA (E), apamin (F) or iberiotoxin (G), and histograms of the change in signal for each bin.



CONCLUSIONS

The present results suggest:

→ an earlier effect of N-type channels compared to other VGCCs, and promote repolarization of the dendritic AP. We concluded that calcium-activated potassium channels are activated by this early calcium influx.

→ both N-type and L-type VGCC substantially contribute to the potassium conductance associated with the AP repolarization, as showing with voltage imaging.

→ a role for N-type VGCC as calcium source for BK channels, whereas the profile of L-type channels matches more the results obtained when blocking SK channels.

To unravel the functional coupling of all these channels a computational model in NEURON will be built.

More generally, the results of this study may shed new light on functional channel-channel interactions and how the synergy of diverse channels result in the shaping of the AP.

lailablomer.eu