Dendritic BK Channels activation by N-type voltage-gated Ca^{2+} channels in neocortical layer-5 pyramidal neurons

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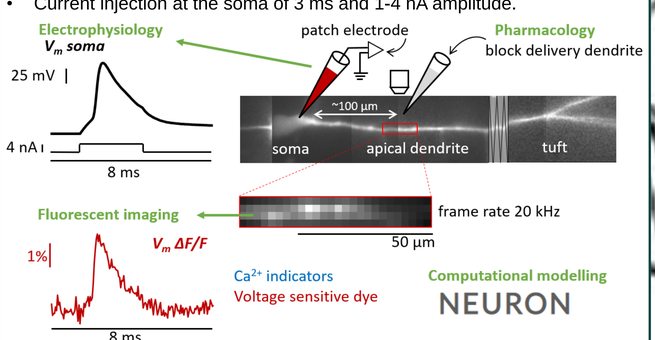
INTRODUCTION

The action potential (AP) generated in the axon actively back-propagates (bAP) into the dendrites through the sequential activation and deactivation of diverse ion channels. Here, using ultrafast membrane potential (V_m) and Ca^{2+} imaging, we show that N-type voltage-gated Ca^{2+} channels (VGCCs) in the apical dendrite of layer-5 neocortical pyramidal neurons, selectively target large-conductance Ca^{2+}-activated K^+ channels (BK CAKCs). We show that this BK CAKC activation occurs within 500 µs following the AP peak, i.e. before the peak of the Ca^{2+} current elicited by the AP. This way, when N-type VGCCs are inhibited, the early widening of the AP shape boosts the other activated VGCCs increasing the total Ca^{2+} influx. We built a realistic NEURON model showing that a strong coupling between N-type and BK channels is necessary to reproduce the experimental results.

METHODS

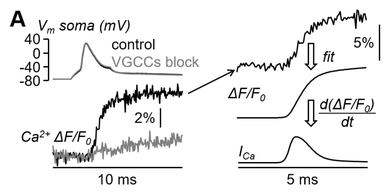
Electrophysiology

- Sagittal 350 µm cortical brain slices from P21-35 C57Bl/6j mice.
- L5 somatosensory pyramidal neurons in whole-cell patches filled with voltage sensitive dye JPW1114 or a calcium indicator (OG5N or CAL-520FF).
- Current injection at the soma of 3 ms and 1-4 nA amplitude.

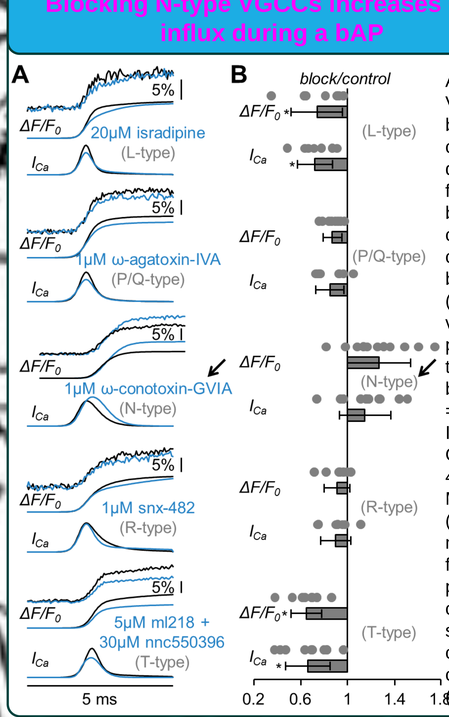


Imaging

Averaged Ca^{2+} and V_m data were 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 was fitted with a 4-sigmoid function. The time derivative of the fitted Ca^{2+}-$\Delta F/F_0$ signal was then calculated.

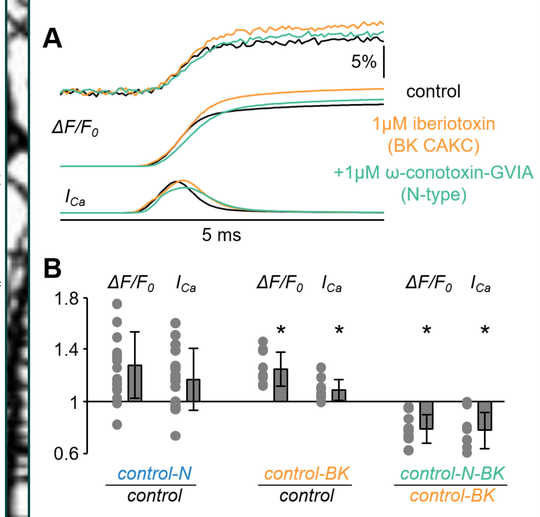


1 Blocking N-type VGCCs increases Ca^{2+} influx during a bAP



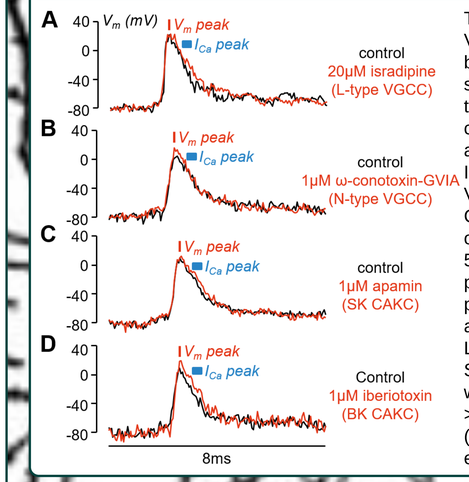
A Examples of all VGCC channel blockers used, and the calcium current (time derivative of change in fluorescence). All blockers decrease the calcium current except conotoxin-GVIA which blocks N-type VGCCs (arrow). B Single values and percentage change of the effect of the VGCC blockers isradipine (N = 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).

2 BK CAKCs are activated by Ca^{2+} influx through N-type VGCCs during a bAP



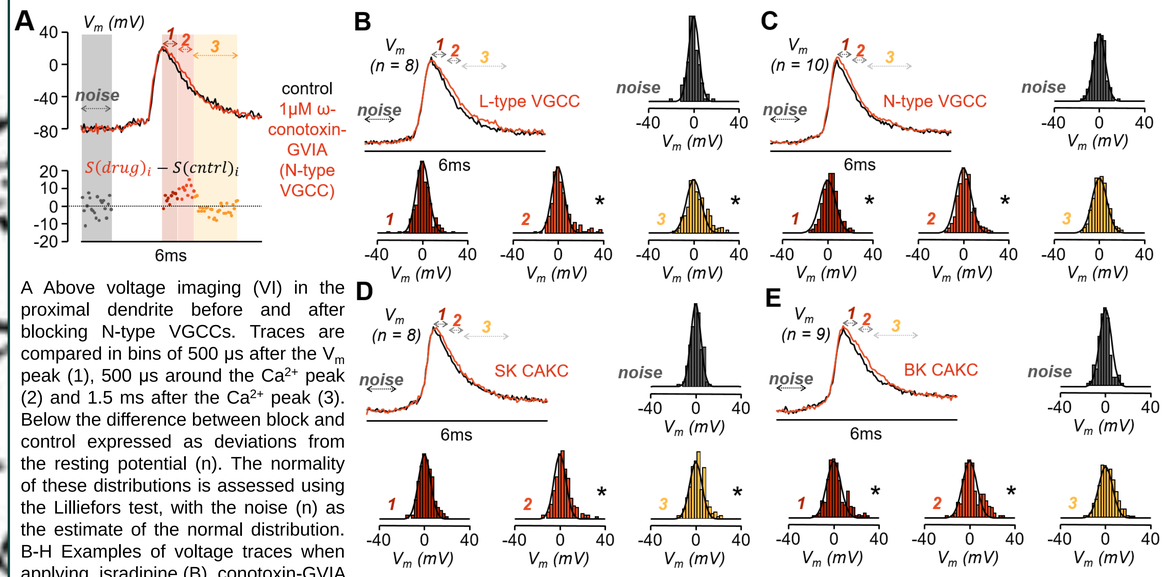
Blocking BK-CAKCs boosts the Ca^{2+} transient similarly to the blockade of N-type VGCCs. Blocking N-type VGCCs in the presence of iberiotoxin reduced the Ca^{2+} transient. This suggests a functional coupling between N-type VGCCs and BK-CAKCs.

4 Blocking N-type VGCCs increases the peak of the bAP



The effects of the diverse VGCC and CAKC blockades on the bAP shape. Optical V_m transient in control conditions (black) and after blocking (red). In the case of N-type VGCCs (B) and of BK CAKS (D) a widening occurred during the first 500 µs following the AP peak (i.e. during the phase of VGCC activation). In the case of L-type VGCCs (A) and of SK CAKS (C), the widening occurred mostly >500 µs after the AP peak (i.e. during or after the expected peak of the I_{Ca}).

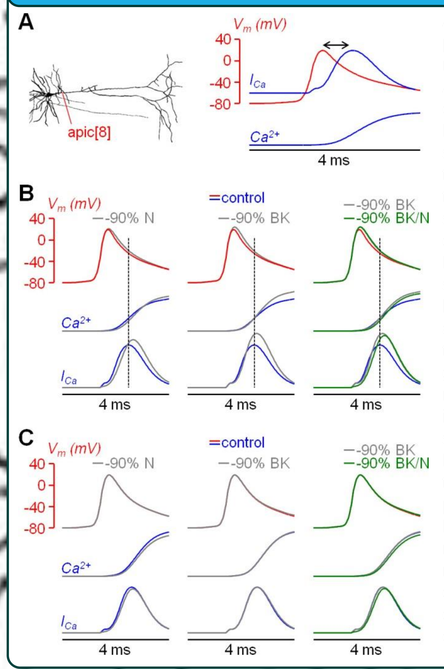
5 The effect of N-type VGCC and BK CAKC coupling occurs in the first 500 µs after the AP peak



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), conotoxin-GVIA (C), apamin (D) or iberiotoxin (E), and histograms of the change in signal for each bin.

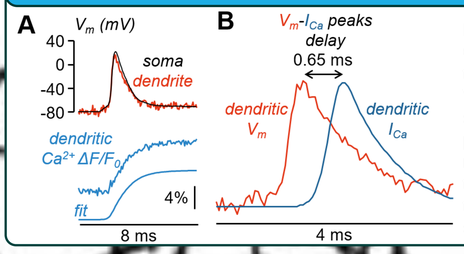
P-values are calculated using the Lilliefors test (based on the Kolomogorov-Smirnov test), which determines if the data follows a normal distribution when the parameters of the normal distribution are unknown.

6 Reproduction of BK and N-type VGCCs interaction in NEURON simulations



A Left, morphology of L5 pyramidal neuron in the model. Right, dendritic AP evoked by somatic current injection (red) in NEURON model simulation; kinetics of the associated Ca^{2+} transient and current superimposed (blue). B dendritic AP in NEURON model simulation and the kinetics of the associated Ca^{2+} transient and current; gray traces are after removing 90% of the indicated channel simulating a block. Dotted lines indicate the timing of the I_{Ca} peak. In panel B, with strong BK-N coupling, simulations reproduce experimental results. Without this coupling (C) simulations do not reproduce experimental results.

3 The inward I_{Ca}-peak is delayed compared to the bAP-peak



Dual V_m (red) and Ca^{2+} (blue) imaging in the dendrite, showing VGCCs start opening during the rising phase of the AP, and remain open during the falling phase of the AP increasing cytosolic Ca^{2+}.

CONCLUSION

N-type VGCCs have an exclusive specific role in neuronal dendrites as activator of BK CAKCs. N-type activation is rapid and is not linear with the increase of intracellular Ca^{2+} concentration associated with the transient activation of VGCCs. Thus, the blockade of N-type VGCCs widens the AP peak prolonging Ca^{2+} entry through the other VGCCs and boosting the increase of intracellular Ca^{2+} concentration associated with the AP.