



# Dendritic BK Channels activation by N-type voltage-gated Ca<sup>2+</sup> channels in neocortical layer-5 pyramidal neurons





The effects of the diverse

Optical

(black)

and of BK

In the case of N-type

occurred during the first

500 µs following the AP

(i.e. during the

activation). In the case of

L-type VGCCs (A) and of

widening occurred mostly

>500 µs after the AP peak

expected peak of the I<sub>ca</sub>).

(i.e. during or after the

CAKS

1µM iberiotoxi

(BK CAKC)



LABEX ICST





blockades

conditions

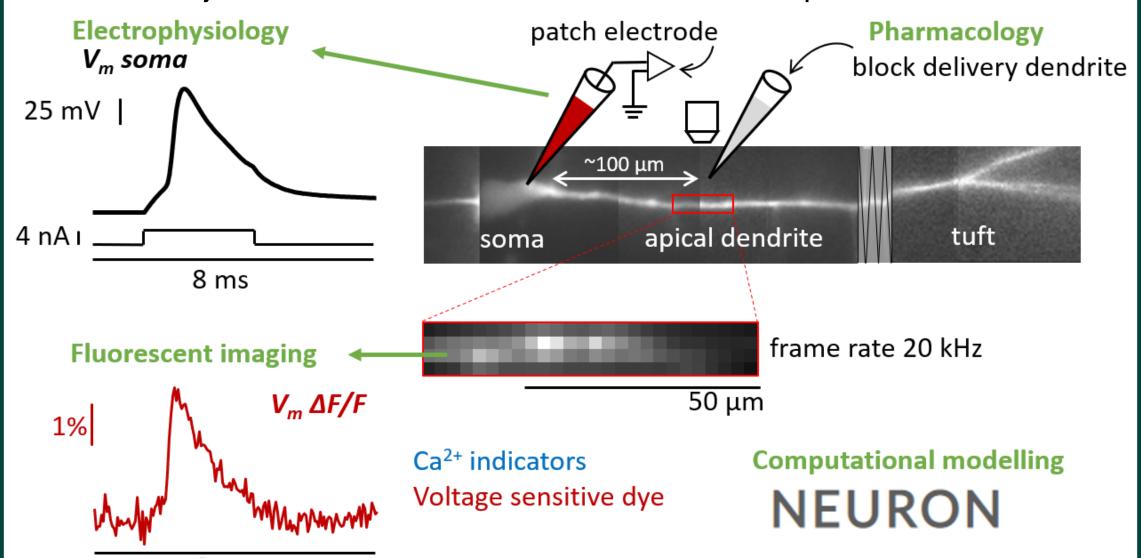
VGCCs (B)

after blocking (red).

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<sub>m</sub>) and Ca<sup>2+</sup> imaging, we show that N-type voltage-gated Ca<sup>2+</sup> channels (VGCCs) in the apical dendrite of layer-5 neocortical pyramidal neurons, selectively target large-conductance Ca<sup>2+</sup>-activated K<sup>+</sup> 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<sup>2+</sup> 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<sup>2+</sup> 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.

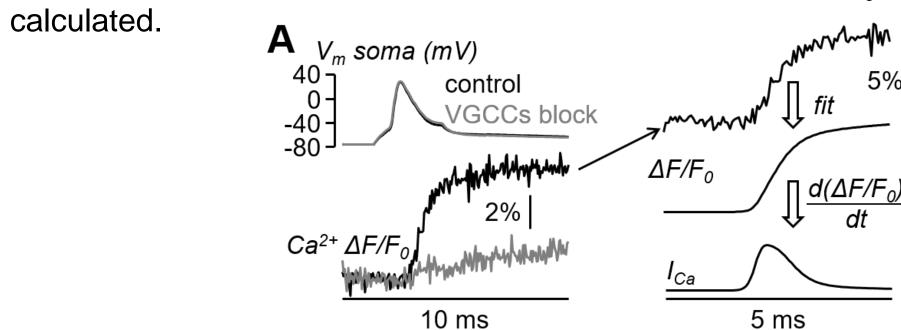
## Electrophysiology

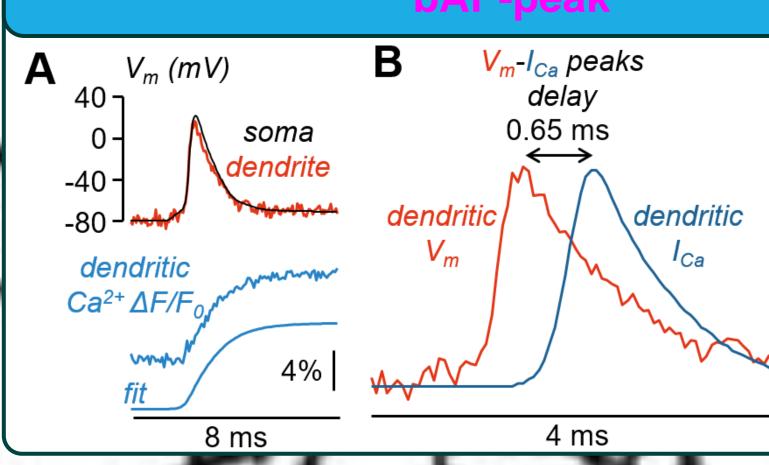
- Sagittal 350 µm cortical brain slices from P21-35 C57Bl/6j mice.
- L5 somatosensory pyramidal neurons in whole-cell patches filled with voltage sensitive dye JPW1114 or a calcium indicator (OG5N or CAL-520FF).
- Current injection at the soma of 3 ms and 1-4 nA amplitude.



## **Imaging**

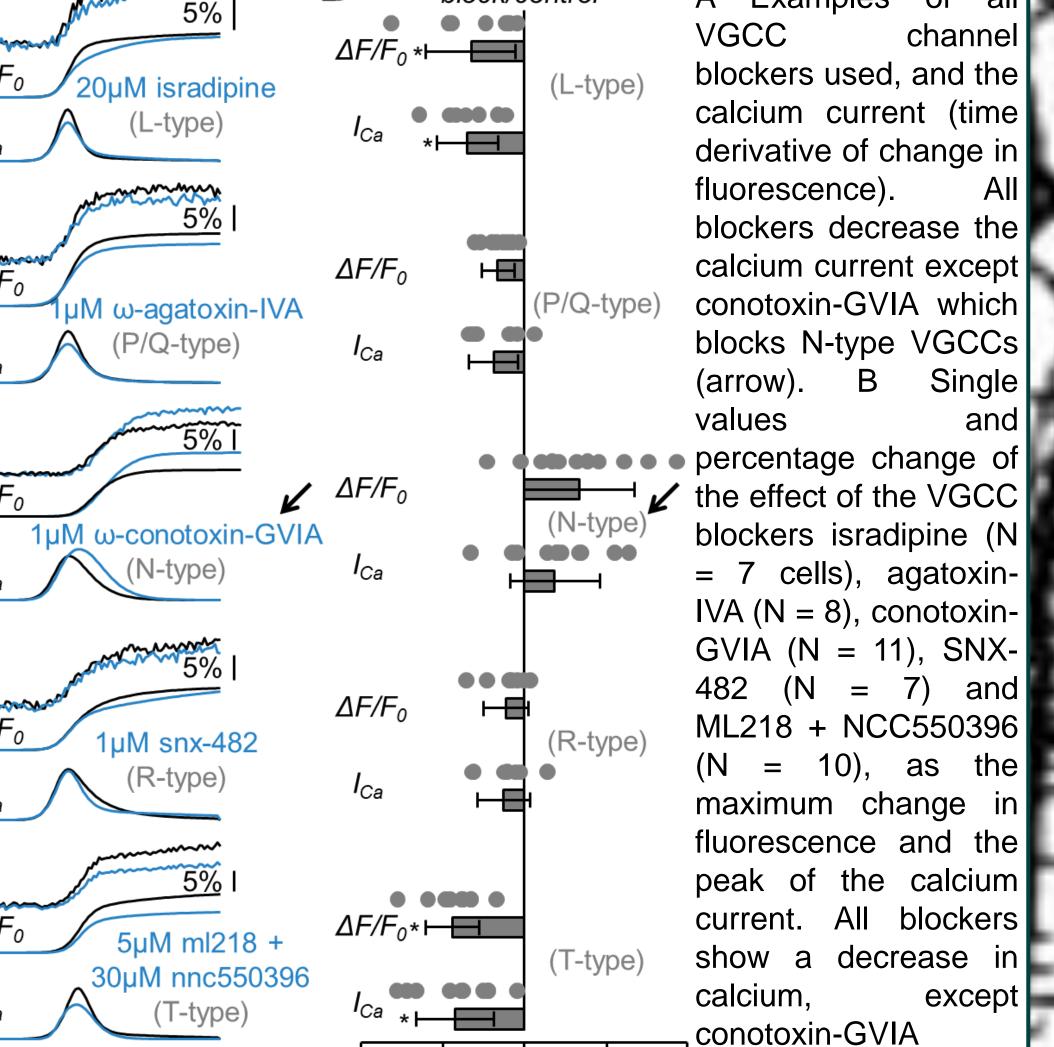
Averaged Ca<sup>2+</sup> and V<sub>m</sub> data were expressed as fractional changes of signals), calculated after subtraction of the autofluorescence background. The Ca<sup>2+</sup>- ΔF/F<sub>0</sub> signal was fitted with a 4sigmoid function. The time derivative of the fitted Ca<sup>2+</sup>-ΔF/F<sub>0</sub> signal was then

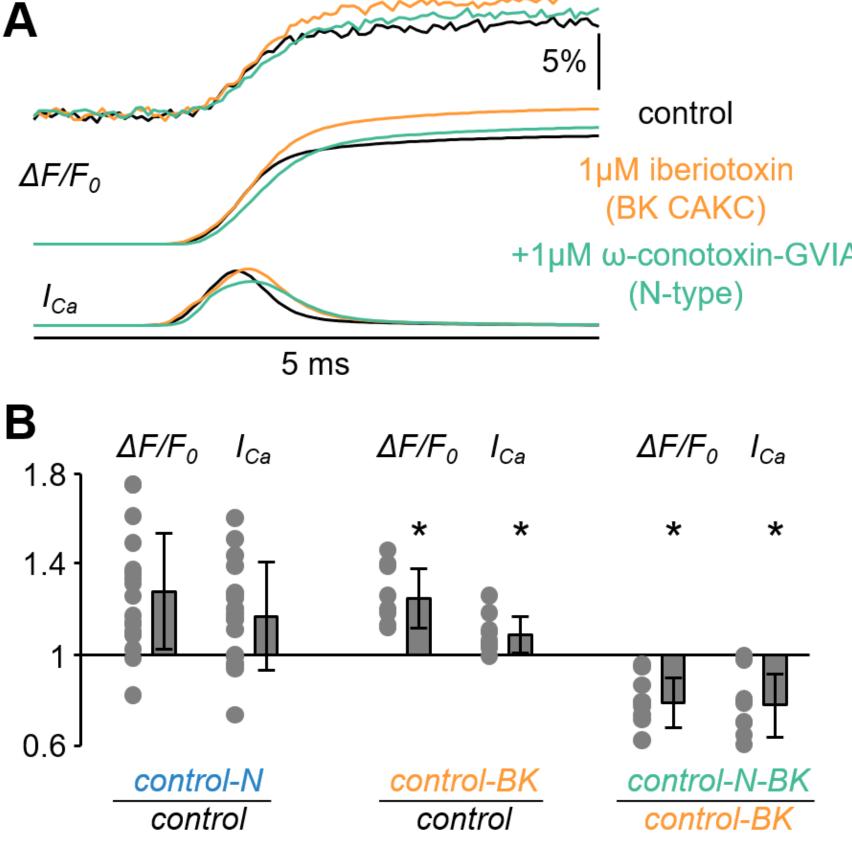




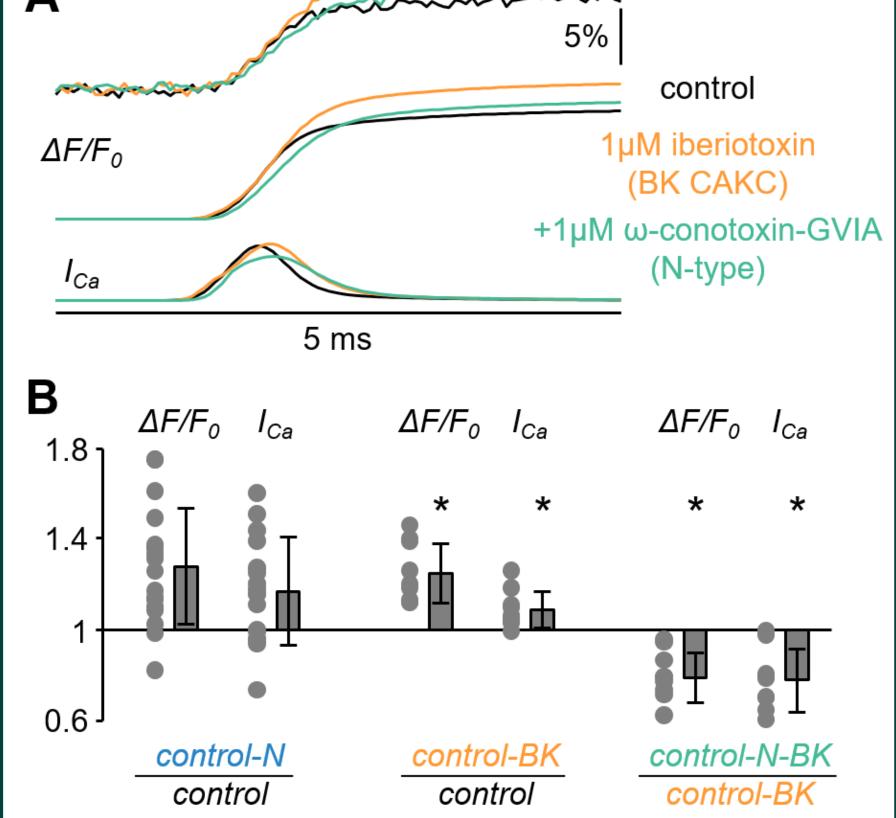
Dual  $V_m$  (red) and Ca<sup>2+</sup> (blue) imaging in the dendrite, showing VGCCs start opening rising the phase of the AP, and remain open during the falling phase of increasing cytosolic Ca<sup>2+</sup>.

## block/control A Examples of all VGCC





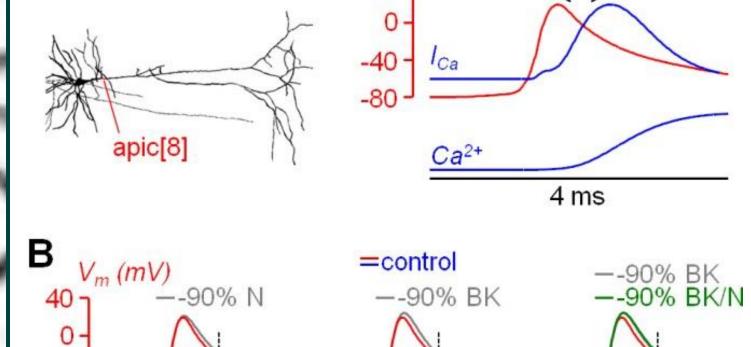
Blocking BK-CAKCs boosts the Ca<sup>2+</sup> transient similarly to the blockade of N-type VGCCs. Blocking N-type VGCCs in the presence of iberiotoxin reduced the Ca<sup>2+</sup> transient. This suggests a functional coupling between N-type VGCCs and BK-CAKCs.



noise

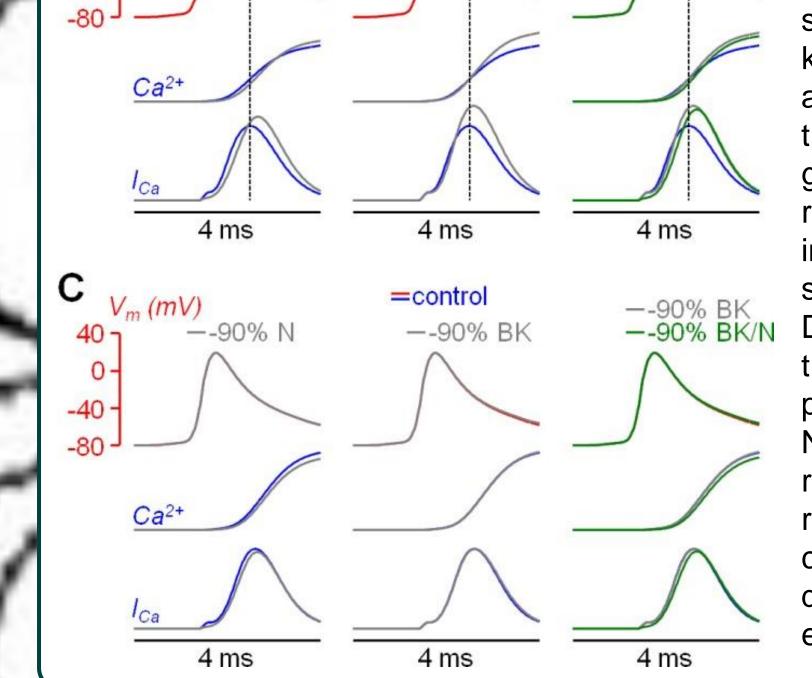
 $V_m$  (mV)

 $V_m$  (mV)



 $\mathbf{A}$  40  $\sqrt{V_m}$  (mV)  $\sqrt{V_m}$  peak

 $IV_m$  peak



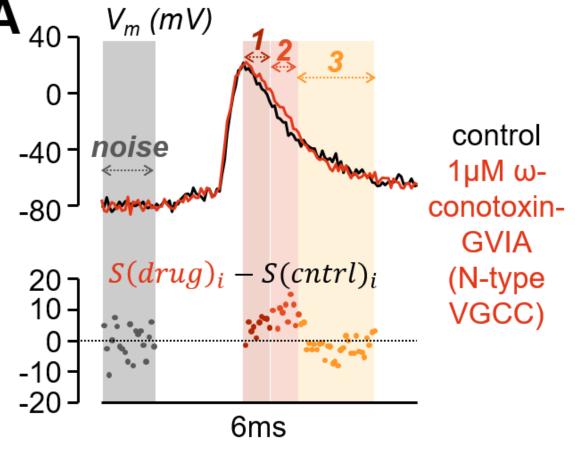
A Left, morphology of L5 pyramidal neuron in the model. Right, dendritic AP evoked by somatic current injection (red) in **NEURON** kinetics of simulation; the associated transient and curren superimposed (blue). B dendritic **NEURON** model simulation kinetics associated and current; removing indicated channel Dotted lines indicate the timing of the I<sub>Ca</sub> peak. In panel B, with strong BK-N coupling, simulations reproduce experimental Without this coupling (C) simulations reproduce experimental results.



1. (arrow).

(n = 8)

 $V_m$  (mV)



A Above voltage imaging (VI) in the **D** proximal dendrite before and after blocking N-type VGCCs. Traces are compared in bins of 500 µs after the V<sub>m</sub> peak (1), 500 µs around the Ca<sup>2+</sup> peak noise (2) and 1.5 ms after the Ca<sup>2+</sup> 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.

## N-type VGCC $V_m (mV)$ $V_m$ (mV) $V_m$ (mV) $V_m (mV)$ $V_m$ (mV) $V_m$ (mV) $V_m$ (mV) (n = 8)(n = 9)**BK CAKC** SK CAKC noise $V_m$ (mV) $V_m$ (mV)

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.

 $V_m$  (mV)

 $V_m$  (mV)

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<sup>2+</sup> concentration associated with the transient activation of VGCCs. Thus, the blockade of N-type VGCCs widens the AP peak prolonging Ca<sup>2+</sup> entry through the other VGCCs and boosting the increase of intracellular Ca<sup>2+</sup> concentration associated with the AP.