Activity-dependent synaptic and structural plasticity of cerebellar climbing fibers

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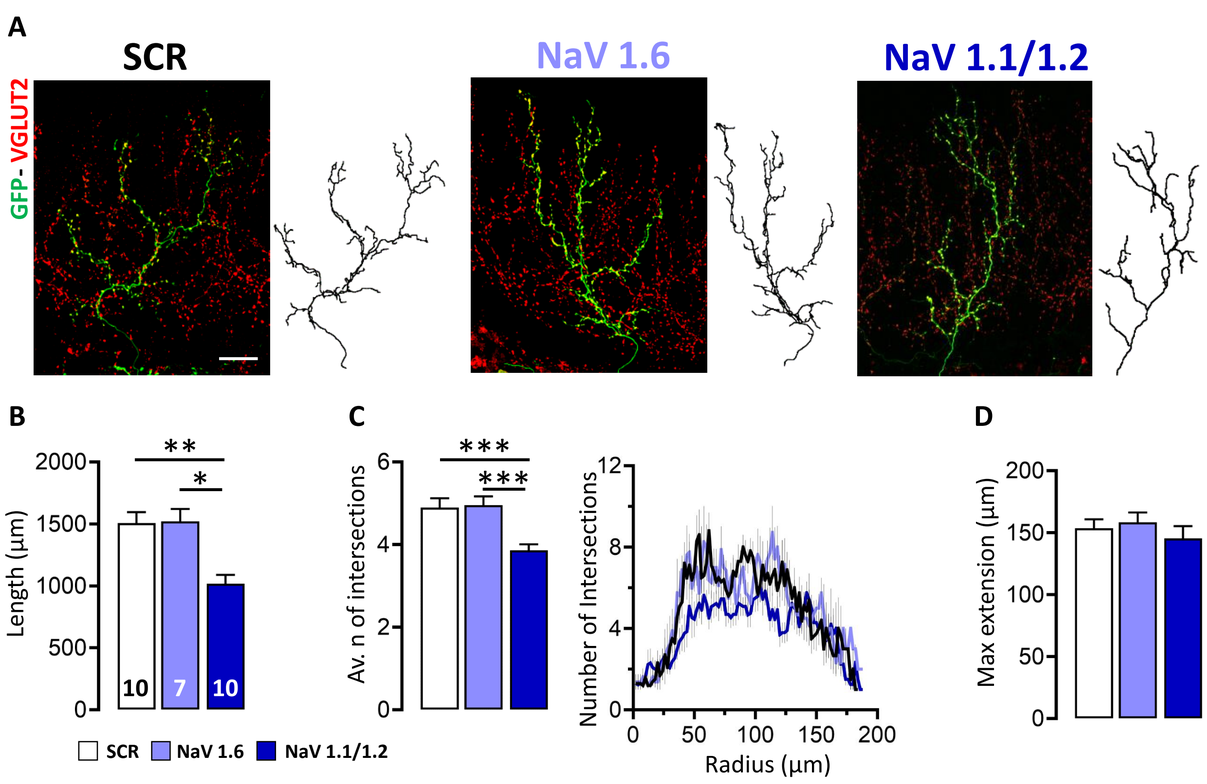
Introduction

The structure and function of neuronal circuits can be modified by experience during the encoding of memories, or under pathological conditions. Cerebellar climbing fibers (CFs) convey a teaching signal to Purkinje cells (PCs) that is crucial for learning. These fibers are the neuronal projections of the inferior olivary nucleus, localized in the brainstem. It was suggested that CFs may undergo structural changes after a general block of neuronal activity in the cerebellar cortex[1] or, to a lesser extent, by increased activity in the inferior olive2. However, it is still unknown whether this form of plasticity is dependent directly on CFs intrinsic activity[3,4].

Here we investigate how modifications of the CF intrinsic excitability affect their structure, by knocking-down (KD) the expression of voltage-dependent sodium channels (Nav 1.1/1.2 and Nav 1.6) of inferior olivary neurons.

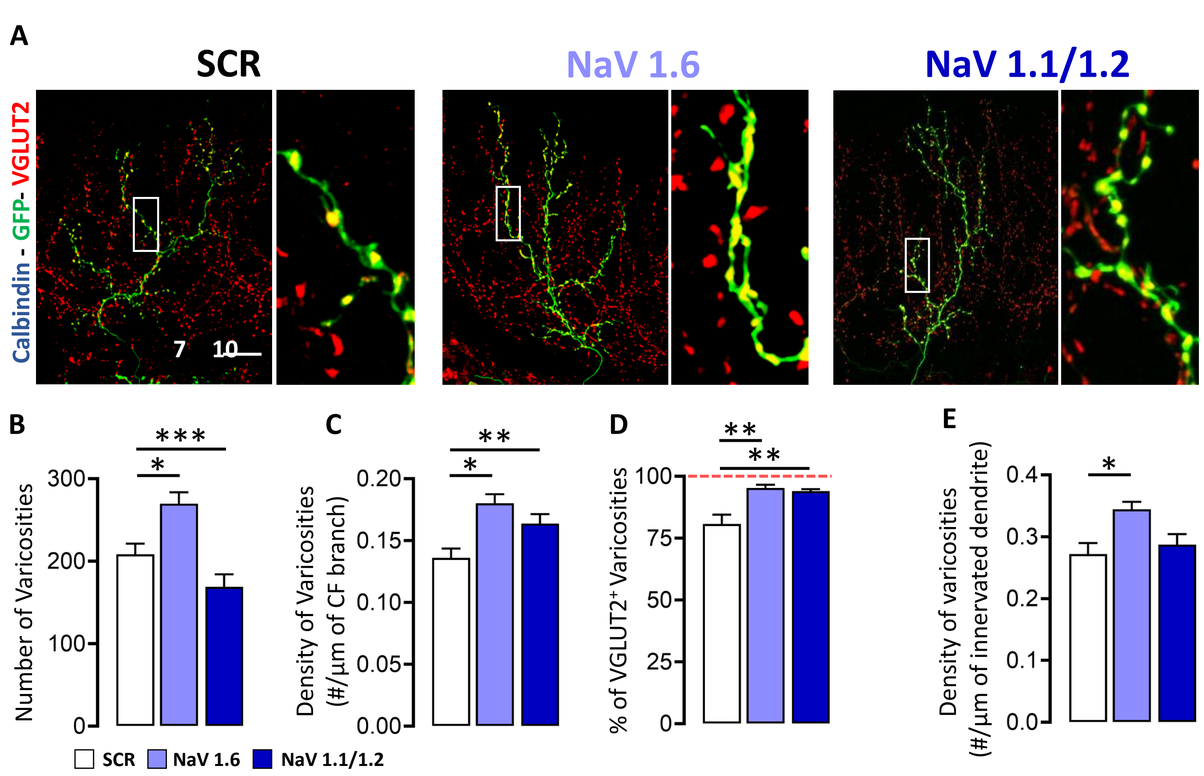
Results

1. Knock-down of Na_V 1.1/1.2 induces CF atrophy



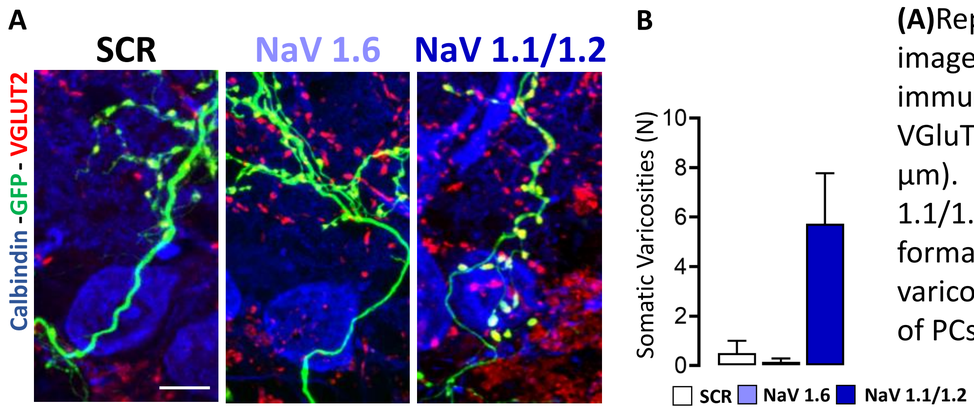
(A) Representative images of GFP⁺ CFs (green), immunolabeled for VGluT2 (red). Traces reconstruction of each CF are represented in black. **(B)** Na_v 1.1/1.2-KD causes a reduction in length and branching of CFs **(C)**, but does not affect its maximum extension (maximum distance from PCs soma) **(D).** KD of Na_v 1.6 does not affect either CF length, branching or maximum extension.

2. Compensatory increase of the number of varicosities



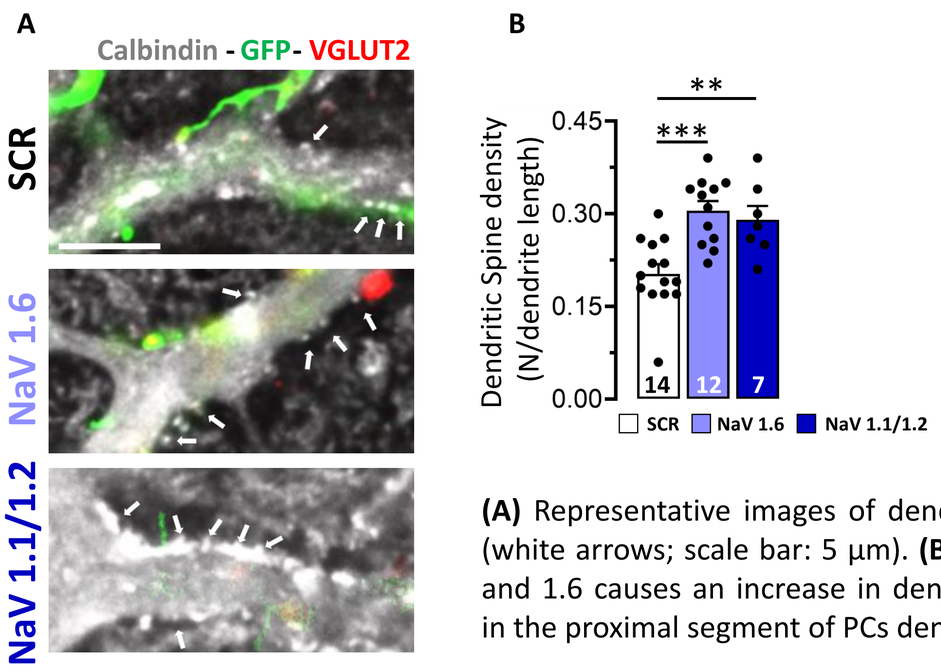
(A) Representative images of GFP⁺ CFs (green), immunolabeled for VGluT2 (red) to identify synaptic varicosities (scale bar: 25 µm). **(B)** KD of Na_v 1.6 causes an increase of synaptic varicosities while KD of Na_v 1.1/1.2 has no effect. **(C)** KD of Na_v 1.1/1.2 and 1.6 causes an increase in density of varicosities and **(D)** portion of VGluT2⁺ varicosities. **(E)** Density of varicosities on unit of PCs dendrite length is increased in KD of Na_v 1.6 but not in Na_v 1.1/1.2.

3. Knock-down of Na_V 1.1/1.2 induces CF somatic innervation on PCs



(A) Representative images of GFP⁺ CFs immunolabeled for VGluT2 (scale bar: 10 µm). **(B)**KD of Na_v 1.1/1.2 induces the formation of synaptic varicosities on somas of PCs.

5. Knock-down of Na_V 1.1/1.2 and 1.6 causes an increase in proximal dendritic spines



(A) Representative images of dendritic spines of PCs (white arrows; scale bar: 5 µm). **(B)** KD of Na_v 1.1/1.2 and 1.6 causes an increase in dendritic spine density in the proximal segment of PCs dendrites.

Methods

Viral injections

Lentiviral preparations, encoding both GFP and a specific short-hairpin RNA (shRNA) targeting Nav 1.1/1.2 or NaV 1.6 mRNA sequence, were stereotaxically injected into the IO of P30 mice.

Immunoistochemistry

Two weeks after viral injection, mice were perfused with 4% PFA fixative. Cerebellar sections (30 µm thick) were incubated overnight with: anti-calbindin (1:1000, mouse, Swant) to stain PCs and anti-VGLuT2 (1:500, rabbit, SYSY) to stain the excitatory pre-synaptic terminals of CFs. To stain dendritic spines, a 3-days permeabilization was performed to increase the tissue-penetration of primary antibodies and anti-calbindin dilution was increase to 1:200.

Quantitative confocal analysis

Tracings from selected CFs were 3D reconstructed with simple neurite tracer (SNT) plugin of ImageJ and analyzed by Sholl's method. The number of varicosities, identified either morphologically or by VGLUT2 staining, was manually counted using ImageJ. PCs dendritic spine density was calculated by counting only the spines emerging from the lateral side of proximal dendrites.

Statistical analysis

Differences between groups were analyzed by Kruskal-Wallis analysis (*p<0.05, **p<0.01, ***p<0.001)

Conclusions

The knock-down of voltage-gated sodium channels (Na_V 1.1/1.2/1.6) can induce a cell-autonomous atrophy in climbing fibers, consisting in:

- **Retraction of the fibers**
- **Compensatory increase in the number of synaptic terminals (and of axo-somatic synapses)**
- **Increase in dendritic spines**

References

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