Activity-dependent structural plasticity of cerebellar climbing fibers

Mattia Musto[1,2*], Matilde Bergamini[1,2*], Alessandra La Terra[3], Antonella Marte[3], Fabio Benfenati[1,2,3], Giorgio Grasselli[1,2‡]

[1]Center for Synaptic Neuroscience, Istituto Italiano di Tecnologia, Genoa, Italy

[2]IRCCS Ospedale Policlinico San Martino, Genoa, Italy; [3]Department of Experimental Medicine, University of Genoa, Genoa, Italy [*] equal contribution, [‡] email: giorgio.grasselli@iit.it

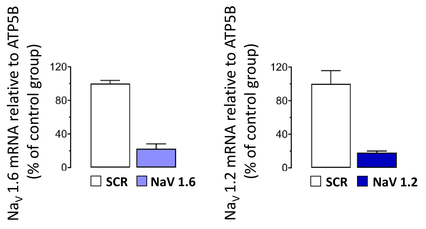
Introduction

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/timing signal to **Purkinje cells (PCs)** that is crucial for learning. These fibers are the neuronal projections of the **inferior olivary (IO) 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 olive[2]. However, it is still unknown whether **activity-dependent structural plasticity** can actually occur in CFs and as a result of their own activity. Clarifying this point can add a new element in the ensemble of forms of activity-dependent plasticity that encode memories in the olivo-cerebellar circuit.

Here we investigate the **pre- and post-synaptic** structural modifications caused by inactivating single CFs by **knocking-down (KD)** the expression of **voltage-dependent sodium channels** (**Na_V 1.1/1.2** or **Na_V 1.6**) in the IO.

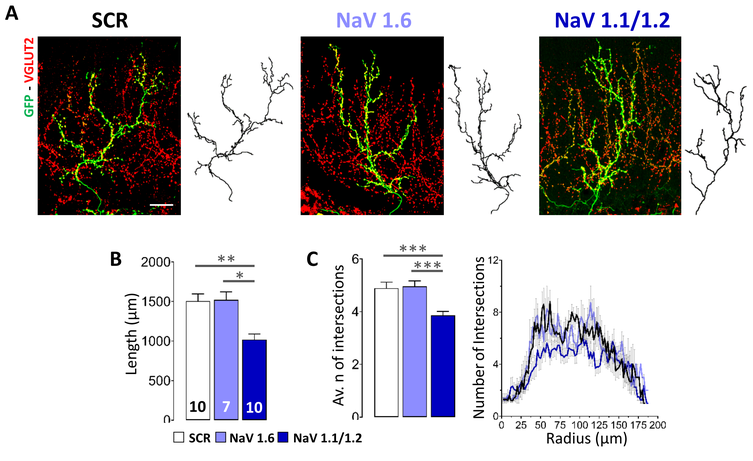
Results

1. *In vitro* Na_V 1.2 and 1.6 knock-down



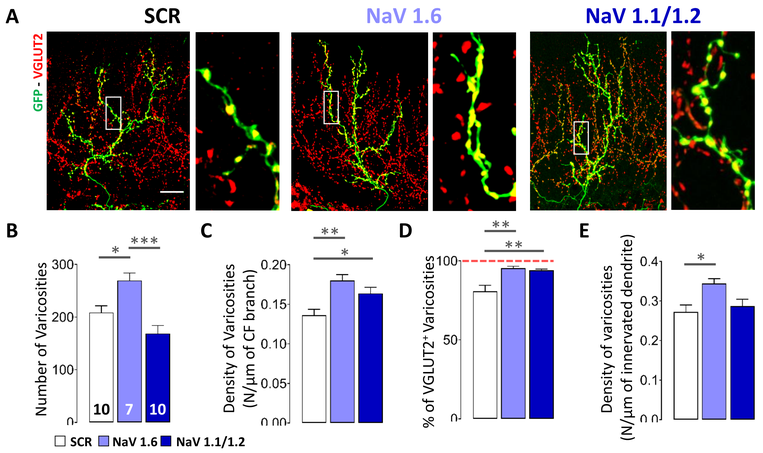
Quantification of Na_v 1.6 (left) and Na_v 1.2 (right) mRNAs level by real-time PCR (TaqMan probes), after lentiviral transduction of NGF-differentiated PC12 cells or primary cerebellar granule cells respectively.

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



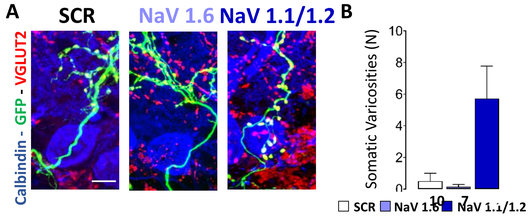
(A) Representative images of GFP$^+$ CFs (green), immunolabeled for VGluT2 (red) and corresponding reconstructed traces (in black). **(B)** Na_v 1.1/1.2-KD causes a reduction in length and branching of CFs **(C).** KD of Na_v 1.6 does not affect either CF length or branching.

3. Compensatory increase of the number of varicosities



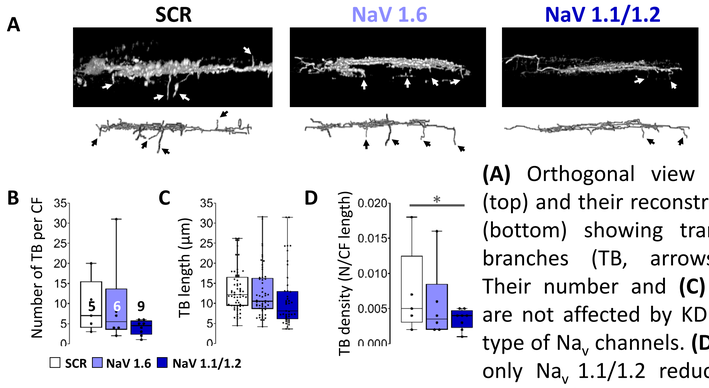
(A) Representative images of GFP$^+$ CFs (green), immunolabeled for VGluT2 (red) to identify synaptic varicosities (scale bar: 25 µm). **(B)** Only KD of Na_v 1.6 (but not of Na_v 1.1/1.2) causes an increase in the total number of synaptic varicosities per CF, due to the atrophy caused by Na_v 1.1/1.2-KD. **(C)** In fact, KD of either Na_v 1.1/1.2 or 1.6 causes an increase in the density of varicosities and **(D)** portion of VGluT2$^+$ varicosities. **(E)** This results in a structural compensation in terms of density of varicosities on unit of PCs dendrite length in Na_v 1.6-KD CFs and an over-compensation (increase) in Na_v 1.1/1.2-KD.CFs.

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



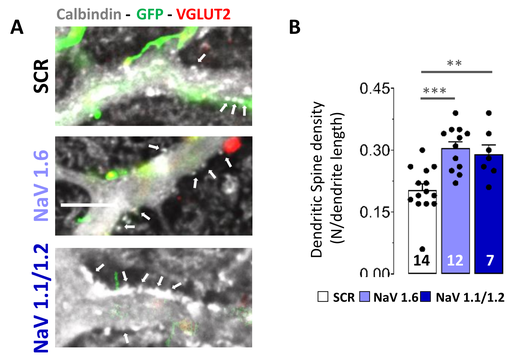
(A) Representative images of GFP$^+$ CFs (green), immunolabeled for VGluT2 (red) (scale bar: 10 µm). **(B)** Na_v 1.1/1.2-KD (but not Na_v 1.6-KD) induces the formation of varicosities on PC cell bodies.

5. Effects of knock-down of Na_V 1.1/1.2 and 1.6 on Transverse Branches



(A) Orthogonal view of CFs (top) and their reconstructions (bottom) showing transverse branches (TB, arrows). **(B)** Their number and **(C)** length are not affected by KD of any type of Na_v channels. **(D)** KD of only Na_v 1.1/1.2 reduces the density of TBs.

6. Knock-down of Na_V 1.1/1.2 and 1.6 causes an increase in dendritic spines on CF-innervated proximal branches



(A) Representative images of dendritic spines on PC proximal thick branches, innervated by GFP$^+$ CF (white arrows; scale bar: 5 µm). **(B)** KD of either Na_v 1.1/1.2 or 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 P28-34 mice.

Immunoistochemistry

Two weeks after viral injection, mice were perfused with 4% PFA fixative. Cerebellar sections (30 µm thick) were immunostained 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.

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 non-parametric one-way ANOVA and post-hoc pairwise comparisons (*p<0.05, **p<0.01, ***p<0.001)

Conclusions

The **knock-down of Na_v 1.1/1.2** in the IO induces:

- an **atrophy of CF** (loss/retraction of branches)
- a **compensatory increase in pre-synaptic terminals** (and of axo-somatic synapses)
- a **compensatory increase in dendritic spines** on the innervated territory

This shows that CF electrical activity is necessary to sustain the length of its branches and that, on the contrary, it suppresses the formation of pre-synaptic terminal to homeostatically maintain constant their total number on PCs.

Aknowledgemnts

We are grateful to Piergiorgio Strata (University of Turin, Turin, Italy), Paolo Cesare (IRCCS Santa Lucia Foundation, Rome, Italy and Natural and Medical Sciences Institute, Reutlingen, Germany) and Georgia Mandolesi (IRCCS San Raffaele Pisana / University of Rome San Raffaele, Rome, Italy) for crucial scientific advice and technical assistance; Fabrizio Loiacono (IRCCS Ospedale Policlinico San Martino, Genoa, Italy) for viral titration.

References

1. Cesa, R. & Strata, P. *Psychoneuroendocrinology* 32: S31-35 (2007).
2. Nishiyama, H., Fukaya, M., Watanabe, M. & Linden, D. J. *Neuron* 56: 472 (2007).
3. Grasselli, G., Mandolesi, G., Strata, P. & Cesare, P. *PLoS One* 6(6): e20791 (2011).
4. Allegra Mascaro, A. L. Cesare, P., Sacconi, L., Grasselli, G., Mandolesi, G., Maco, B., Knott, G. W., Huang, L., Paola, V., Strata, P., Pavone, F., *Proc. Natl. Acad. Sci.* 110: 10824–10829 (2013).

Funding

European Commission Marie Skłodowska-Curie Fellowship (H2020 MSCA-IF-2018 "FunStructure", GA No. 844391); Italian Ministry of Health (Ricerca Corrente).