Deep cerebellar nuclei circuitry in Amyotrophic Lateral Sclerosis (ALS)

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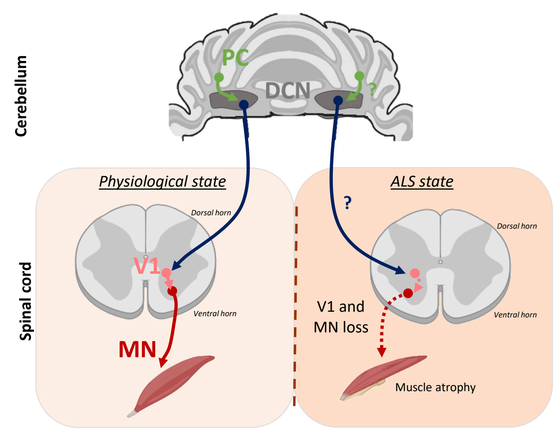
1 Abstract

- **Amyotrophic lateral sclerosis** (ALS) is a fatal **neurodegenerative disease** characterized by progressive degeneration of motor neurons and paralysis.
- There is growing evidence suggesting that dysfunction in circuits beyond the motor cortex and spinal cord contributes to the progression of the disease.
- Recent data[a] identified direct **cerebellum-spinal** tract that target local inhibitory V1 segmental neurons required for skilled movement and locomotion, **positioning the cerebellum as a potential direct modulator of spinal motor circuits**.
- The **cerebellum** is important for motor and non-motor functions, and these functions are dependent on the **deep cerebellar nuclei (DCN**) that represent **the only output** of the cerebellum**.**

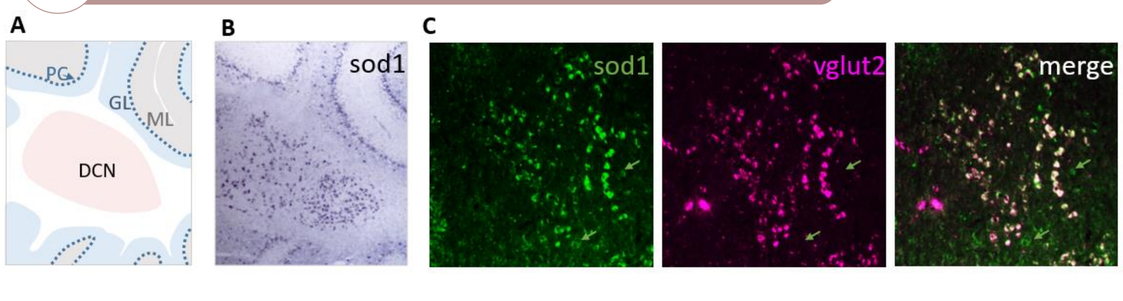
Here we show that in the ALS SOD1^{G93A} mouse model, various dysfunctions were identified prior to the onset of ALS symptoms, both at the molecular and behavioral levels.

Based on these results, we hypothesize that cerebellar circuits, through DCN neurons, may be affected in ALS.



What about cerebellar circuits in ALS ?

2 Characterization of SOD1 expression in the cerebellum

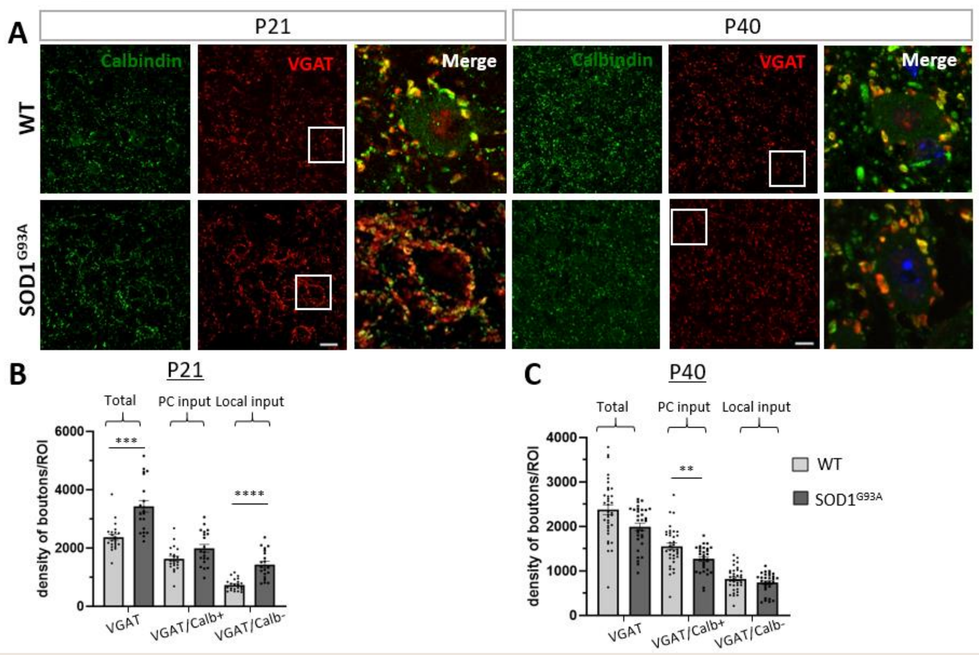


*ISH of WT SOD 1 mice was performed at P16 and P21 in male and female mice independently. Schematic representation allow to identify the layer of the cerebellum, PC= Purkinje cells, GL= Granular layer, ML= Molecular layer. Sod1 is expressed in Purkinje cells, Golgi cells, and some large soma cells in the DNC (B) that are glutamatergic neurons (C). Double ISH shows that **95% of sod1 expressing neurons are Vglut2 neurons** (D).*

Sod1 is mainly express in glutamatergic DCN neurons.

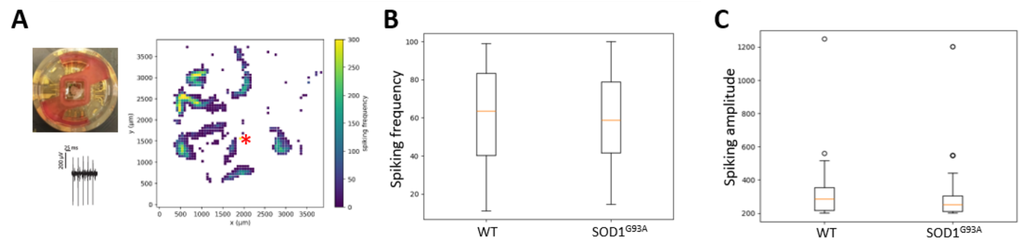
3 Characterization of DCN neurons in ALS mouse model at asymptomatic phase

- Synaptic reorganization occurs during the asymptomatic phase



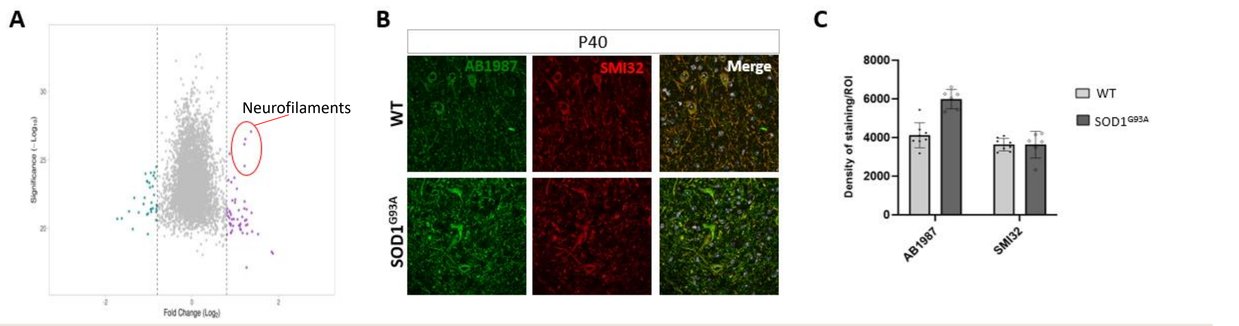
*SOD1G93A is a widely used ALS mouse model in which the overt symptoms appear around P100. Immunostaining of interposed nuclei in cross-sections of cerebellum from WT and asymptomatic ALS mice (SOD1^{G93A}) at P21 and P40 (A). Calbindin labels inhibitory Purkinje cell synapses (uniquely PC inputs) and VGAT labels all presynaptic inhibitory boutons. **An increase in synapses density is observed in ALS model at P21 (B), but this seems to be restored at P40** (C) Scale bar corresponds to 20µm.*

- MEA analysis shows no differences in PC spontaneous activity



*Cerebellar slice on the Multi Electrode Array (MEA) device and its activity heat map. Comparison of the frequency (B) and the amplitude (C) of spikes observed in Purkinje cells in WT mice vs SOD1^{G93A} mice showing **no differences in PC spontaneous activity**. (n=3)*

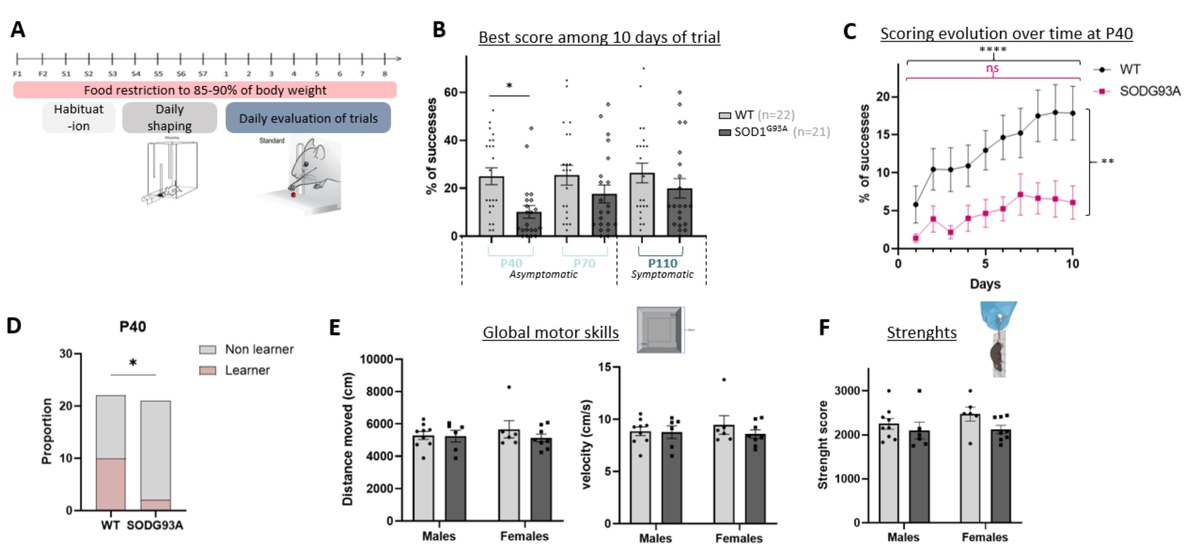
- Proteomic analysis of synaptosome from DCNs identifies altered proteins expression

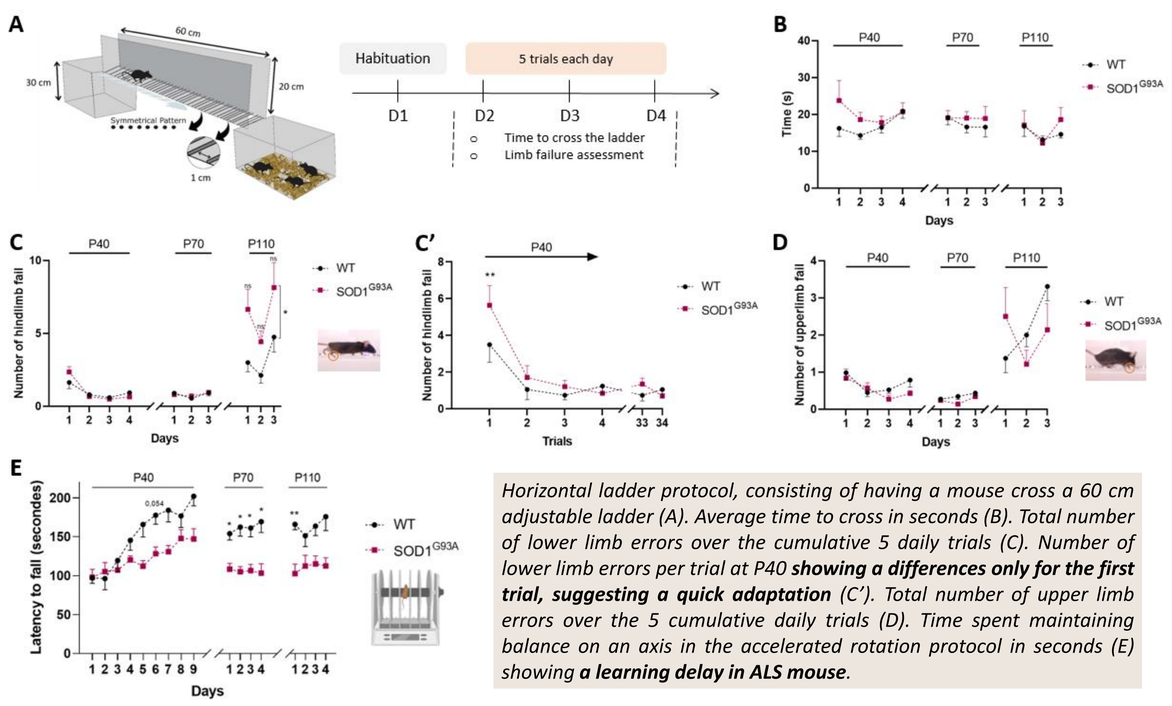


*Volcano plot of mass spectrometry results for synaptosomes obtained from DCNs at P40. The red circle identifies **proteins that are upregulated** in our model and that are associated with neurofilaments (A). Immunostaining of the interposed nucleus at P40 with neurofilament markers: AB1987 labels NF-M and SMI32 labels non-phosphorylated NF-M and NF-H (B). The density of AB1987 labeling appears to be increased in the SOD1^{G93A} model (n= 1) (C), **confirming what we observed in proteomic analysis.***

The synaptic inputs of DCNs are dysregulated in the asymptomatic phase. Although this does not appear to be associated with a change in the spontaneous activity of PCs, modification in the expression of certain proteins could explain this.

4 Fine motor skills impairments associated with learning delays





*Protocol used for the single pellet reaching task test (A). Evaluation of the best percentage of success in catching the pellets over the days **showing a defect at P40 only** (B). Progression of the ability to catch the pellet over the days at P40, showing **clear learning only in the controls** (C). Division of different genotypes into subgroups according to their learning ability: Non-learners (% learning < 50%) and Learners (% learning > 50%) showing a **change in the proportions of each group in the ALS model**. (D). Complementary tests show that the mice do not exhibit any general motor (open field E) or strength deficits (grid test F) at the same stage.*

*Horizontal ladder protocol, consisting of having a mouse cross a 60 cm adjustable ladder (A). Average time to cross in seconds (B). Total number of lower limb errors over the cumulative 5 daily trials (C). Number of lower limb errors per trial at P40 **showing a differences only for the first trial, suggesting a quick adaptation** (C'). Total number of upper limb errors over the 5 cumulative daily trials (D). Time spent maintaining balance on an axis in the accelerated rotation protocol in seconds (E) showing **a learning delay in ALS mouse**.*

A deficit in fine motor skills is observed very early in the asymptomatic phase and appears to be restored later. This deficit could be associated with a motor learning impairment.

5 Conclusion

The aim of this project is to evaluate the contribution of cerebellar circuits in the pathogenesis of ALS and to propose DCNs as new therapeutic targets. After identifying the populations expressing the SOD gene in the cerebellum, we demonstrated **synaptic rearrangements** in DCNs in mice with ALS, **even before the onset of symptoms**. As yet, our results do not demonstrate any alteration in spontaneous cerebellar activity, we quantified the expression of synaptic proteins at these same stages and identified **several families of deregulated proteins**, including neurofilaments. Finally, from a functional point of view, we identified **learning deficits in fine motor tasks, which are known to involve cerebellar circuits**.

Overall, this project will provide a better understanding of the disease and help identify new therapeutic targets.

(a) Sathyamurthy, A. et al. Cerebellospinal Neurons Regulate Motor Performance and Motor Learning. Cell Rep 31, (2020).