Structural Analysis of Axonal Degeneration by MRI diffusion in ALS Mice

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Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease characterized by a progressive onset and loss of locomotor symptoms, leading to a rapid functional deterioration and premature death. Among the different multi-compartment MRI diffusion models, neurite orientation dispersion and density imaging (NODDI) has recently added new insights to the complex microstructural changes in white matter (WM) in the spinal cord (SC). The purpose of this study is to determine if NODDI can detect alterations in axonal connectivity in early ALS stages and validate the outputs from this model with histological techniques applied in fluorescent axon-labeled ALS mice.

Experimental

MRI studies: Paraformaldehyde-fixed SC from control mice (n=5) at the presymptomatic (P80) and symptomatic (P120) stages were placed in individual 5 mm NMR tubes (New Era, NJ) and immersed in Fluorinert silicone oil. *MRI Imaging:* MRI scans were performed using Bruker Avance III HD 17.6T 750Hz. magnet and Micro-2.5 gradients (1500 mT/m) with 5- and 25-mm RF coils. All imaging experiments were performed at room temperature. Diffusion-weighted images were acquired using a spin echo sequence with TR/TE 4000/28 msec., interleaved 0.15 mm thick slices, a field of view = 20 x 20 x 3 mm^3 in each block of slices, in-plane acquisition matrix = 133 x 133, for an isotropic image resolution of 150 µm. Diffusion weightings were 2 b-values at 700 in 12 directions and 2500 s/mm^2 in 64 directions with δ of 3.5 msec. and Δ of 17.5 msec. and 12 and 64 directions. *Histological analysis*: SCs were processed for confocal fluorescence microscopy following standard procedures described elsewhere [1,2].

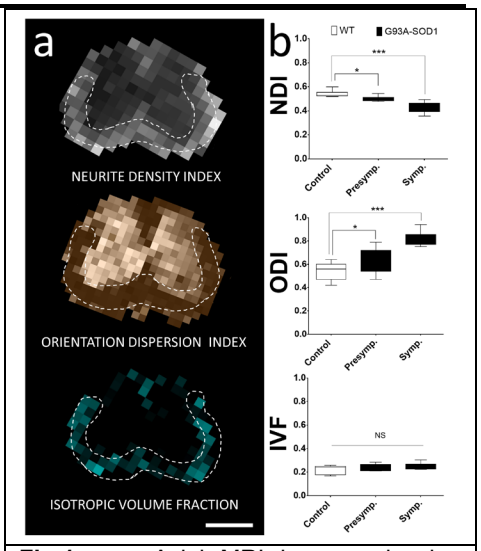

Fig.1: a - Axial MRI images showing different NODDI outputs from individual lumbar mouse spinal cords. **b** - Thee main parameters from NODDI are analyzed: Intracellular volume fraction (ICVF); Orientation dispersion index (ODI) (Extracellular compartment) and isotropic Volume Fraction (IsoVF) representing the free water compartment (n=5).

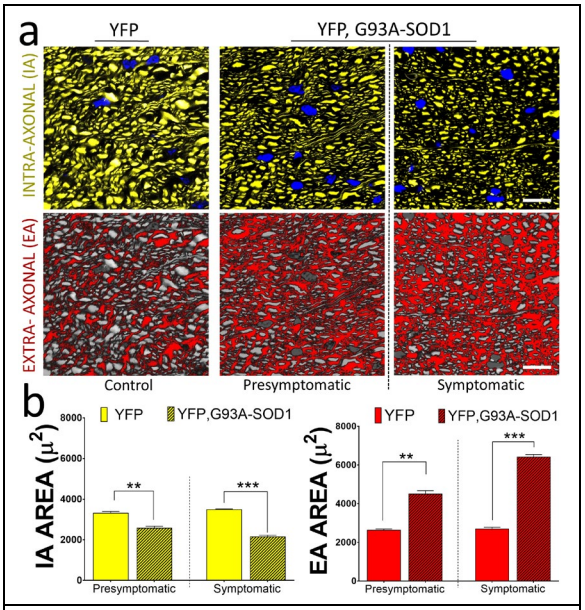

Fig.2: a- Evaluation of ventral white matter compartments in intracellular compartment (Yellow) and extra-axonal compartment (Red) in a fluorescent ALS mouse. **b -** Quantitative analysis centered on the ventral fasciculus of the lumbar spinal cord shows a significant decrease in intra-axonal compartment measured by counting the total area of yellow fluorescence protein (YFP) and increase in extra-axonal compartment (n=5).

Results and Discussion

NODDI showed a decrease in intracellular volume fraction (-24%) and an increase in orientation dispersion index (+35%) and isotropic volume fractions (+33%). In addition, histopathological results demonstrated a reduction in axonal areas (-11%) and myelin content (-29%) **[Fig 1].** A histological decrease in WM intra-axonal space (-71%) and increase in the extra-axonal compartment (+22 %) were also detected. Our studies demonstrate that NODDI may be a suitable technique to detect pre-symptomatic spinal cord WM microstructural degeneration in ALS **[Fig2].**

Conclusions

Our studies demonstrate that NODDI may be a suitable technique to detect presymptomatic spinal cord WM microstructural degeneration in ALS.

Acknowledgements

This work was possible by funds of the Magnet Lab Visiting Scientist program at NSF-AMRIS [VSP #278/ P17430] and the Chicago Biomedical Consortium [KC Award #085740].

References

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[2] *Gatto et. al.,* Translational Neurodegeneration **7**(20), 2-14 (2018)