NEUROSYS
CNS/PNS IN VITRO
DRUG DEVELOPMENT

Translocation of TDP-43 and FUS in SOD1 Tg and WT spinal cord motor neurons injured with glutamate or AβO$_{1-42}$ peptide: *in vitro* models of ALS

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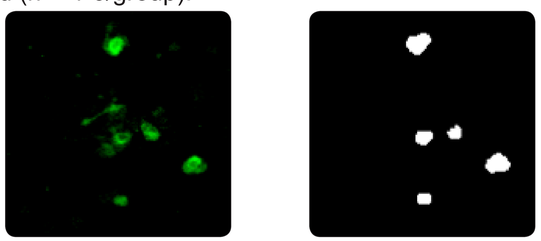
INTRODUCTION

The loss of spinal motor neurons in ALS is caused by complex and multifactorial pathological events. Some familial cases (fALS) are linked to gain-of-function mutations of superoxide dismutase type-1 (SOD1), an antioxidant enzyme whose activity is preserved in most mutant forms. Owing to the similarities in sporadic and fALS forms, mutant SOD1 animal and cellular models are useful tool to study the disease. In addition, mutations on genes involved in RNA metabolism (e.g. tarbp TDP-43, fus) are found in inherited and sporadic forms of ALS. TDP-43 and FUS proteins are abnormally translocated from the nucleus to the cytoplasm where they can form aggregates. Glutamate excitotoxicity is known to participate in neuronal death in ALS and AβO$_{1-42}$ peptide accumulates in the spinal cord of ALS patients which exacerbated neuronal loss.
Here, we investigated the TDP-43 and FUS cytoplasmic translocation and neuronal degeneration, following glutamatergic stress or AβO$_{1-42}$ intoxication in WT and SOD1 (G93A) Tg spinal motor neurons.

METHODS

Primary culture: Wt and Tg SOD1 (G93A, Taconic) Rat spinal cord (E14) were cultured as described by Wang *et al.*, 2013 with modifications. Cells were cultured in 96-well plates pre coated with PLL maintained in a humidified incubator at 37 °C. Aβ$_{1-42}$ (10 % of Aβ oligomers, as determined by WB) and glutamate were applied on day 13.

Immunostaining: After intoxication, neurons were fixed with a solution of acetic acid (5 %) and ethanol (95 %). The cells were incubated with antibodies anti-ChAT, anti-NF-H and anti MAP2 anti-TDP-43 and anti-FUS. Secondary Alexa488 and Alexa568 antibodies were used. Pictures (20x magnification) were acquired an on automated microscope with MetaXpress and automatically analyzed with Custom Module Editor (Molecular Devices). Statistics are described in the figure legend (n = 4-6/group).



Representative picture of a primary culture of spinal neurons (ChAT) and computer-assisted identification motor neurons.

RESULTS

Neuronal maturation is delayed in SOD1 Tg neurons

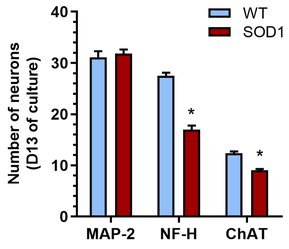


Figure 1: Maturation in a primary WT and SOD1 motor neurons.
Numbers of neurons positive for MAP-2 (pan-neuronal marker), Neurofilament-H (marker of mature neurons) and ChAT (marker of late maturation of motor neuron), after 13 days of culture. *p<0.05, T-test.

TDP-43 and FUS pathology occurs after glutamate and AβO$_{1-42}$ injuries,and is exacerbated in SOD1 Tg neurons

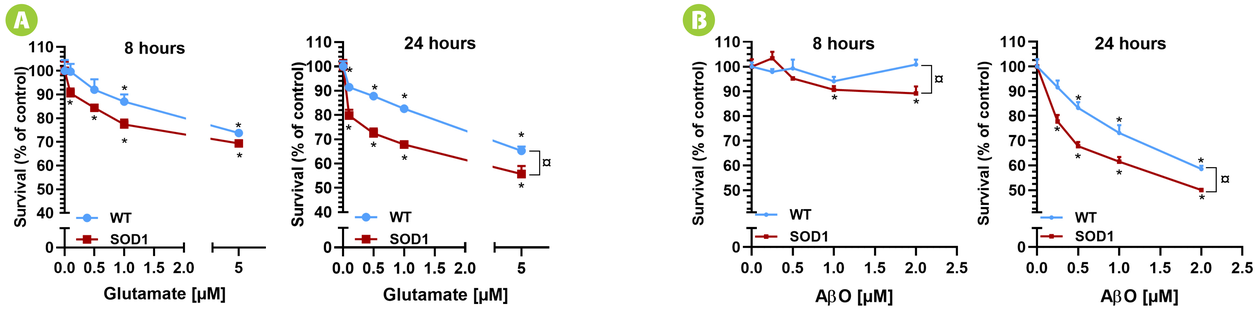


Figure 2: Glutamate and AβO$_{1-42}$ injuries in primary culture of WT and SOD1 Tg motor neurons.
A. Glutamate induced a rapid and dose-dependent neuronal loss. SOD1 Tg motor neurons are more sensitive to glutamate stress when compared to wild type.
B. AβO$_{1-42}$ toxicity was manifest after 24 hours of injury in WT motor neurons. Neuronal loss was observed in the SOD1 Tg culture after only 8 hours of injury and was more marked when compared to the WT culture. *p<0.05 versus vehicle, One-way ANOVA followed by a Dunnett's test; ¤p<0,05, two-way ANOVA.

SOD1 Tg motor neurons are more sensitive to glutamate and AβO$_{1-42}$ injury

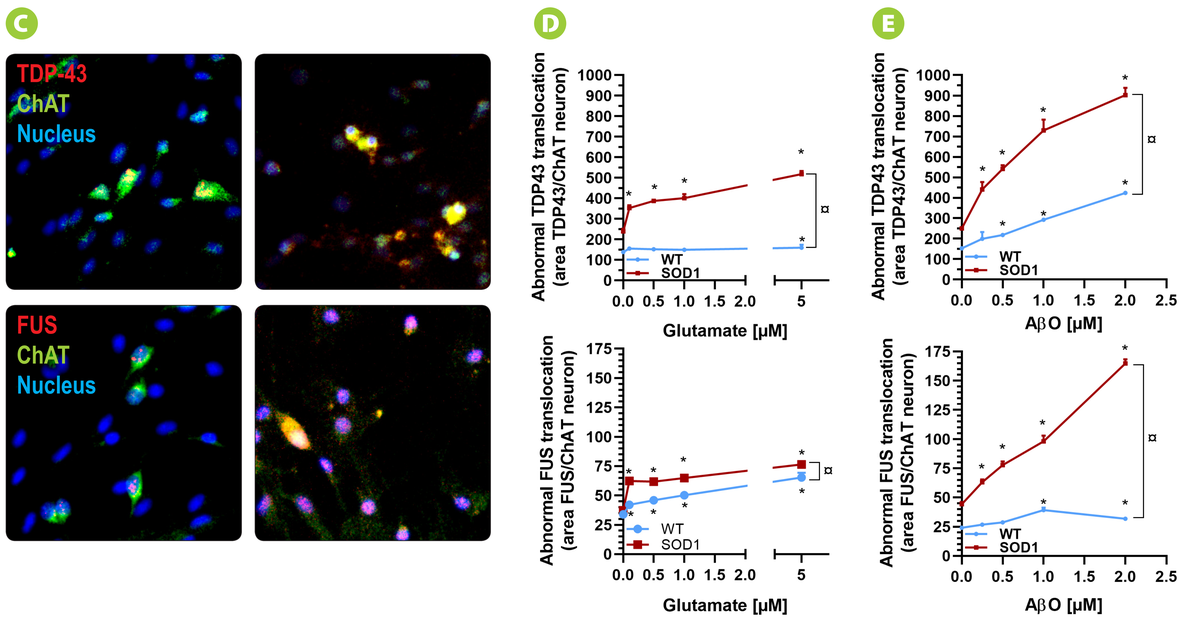


Figure 3: Increased cytoplasmic distribution of TDP-43 and FUS after AβO$_{1-42}$ injury.
C. Representative pictures of TDP-43 (upper panel) and FUS (lower panel) distributions.
D. Glutamatergic stress triggered an abnormal translocation of TDP-43 and FUS at 5 µM in WT motor neurons. The abnormal translocation of TDP-43 and FUS was drastically more marked in SOD1 Tg neurons.
E. AβO$_{1-42}$ injury triggered a dose-dependent translocation of TDP-43 and FUS in WT motor neurons. The abnormal translocation was drastically more marked in SOD1 Tg neurons and occurred at lower doses of AβO$_{1-42}$.*p<0.05 versus vehicle, One-way ANOVA followed by a Dunnett's test; ¤p<0,05, two-way ANOVA.

CONCLUSIONS

- Neuronal maturation, characterized by the expression of neurofilament heavy chain and ChAT proteins, is strongly impaired in primary culture of spinal neurons from SOD1 Tg animal.
- Neurotoxicity following glutamatergic stress or AβO injury is dose- and time-dependent.
- SOD1 Tg MNs are more sensitive to neuronal stress, as toxicity is observed at earlier timepoints and at lowest doses.
- Abnormal translocations of TDP43 and FUS in ChAT motor neurons are observed after glutamatergic stress and after AβO injury, and are exacerbated in SOD1 Tg motor neurons.

Compared to WT, SOD1 Tg motor neurons present with clear defect in neuronal maturation and are markedly more sensitive to glutamatergic stress and AβO$_{1-42}$ injuries.