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CNS histology over age in the Tar6/6 mouse model of ALS and/or FTLD-TDP

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Background

Tar6/6 mice over-express human Tar DNA binding protein (TARDBP) under the control of the Thy1 promoter. Homozygous mice develop an early and severe ALS-like motor phenotype with decreased survival, were described to develop CNS alterations in spinal cord neurons and to accumulate intranuclear pTDP-43 inclusions in the brain (Wils et al. 2010). However, the histopathological description of these mice is still limited. The here presented data entail systematic investigations of several pathological markers, including TARDPB, pTDP-43, GFAP, MAP-2 and NeuN to control for over-expressed protein, toxic pTDP-43 species, neuronal or dendritic network loss, and concomitant astrogliosis.

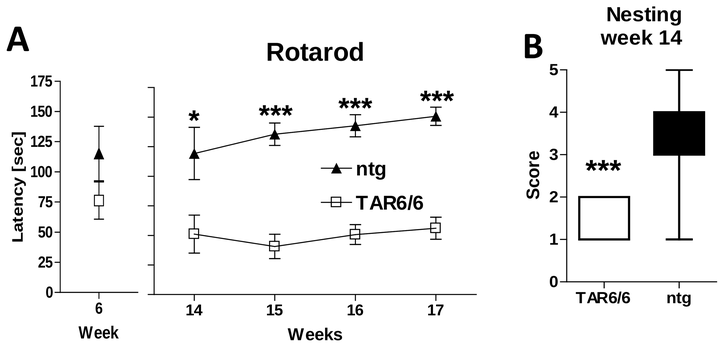

Figure 1: Onset of motor deficits of complex motor tasks and basic motor behavior: RotaRod performance is impaired from 14 weeks of age onwards compared to age-matched non-transgenic littermates (ntg). A: tg n = 5; ntg n = 7; B: tg n = 11-3; ntg n = 16-5. Two-Way ANOVA and Bonferroni post hoc test: P*<0.05; P***<0.001. Also nesting behavior (B) is disturbed at 14 weeks of age in TAR6/6 transgenic mice compared to ntg littermates. tg n = 12; ntg n = 16. Mann Whitney test. Data are shown as box-whisker blot. *** p<0.001. Basic motor behavior as analyzed with the wire hanging test is already disturbed at the age of 6 weeks. Tg n = 6; ntg n = 8. Mann Whitney test.

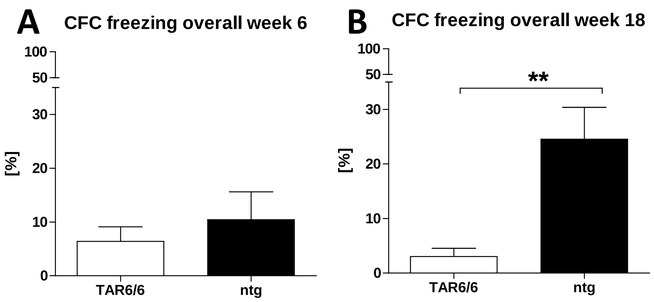

Figure 2: Onset of memory deficits in the CFC: Overall freezing behavior in the CFC of 6 (A) and 18 weeks (B) old TAR6/6 transgenic mice compared to non-transgenic littermates (ntg). A: tg n = 11; ntg n = 12; B: tg n = 11; ntg n = 16. Data are shown as mean ± SEM. Data were analyzed by Mann Whitney test. P**<0.01.

Methods

The mice were behaviorally tested in a motor ability test battery, at 6-8, 12 and 24 weeks of age. This test battery consisted of motor tests, such as nesting building (NB), RotaRod (RR), Elevated Plus Maze (EPM), clasping test and contextual fear conditioning (CFC) , the latter to test memory abilities as well.

Immunofluorescent labeling of TARDPB, pTDP-43, GFAP, MAP-2 and NeuN was investigated in the brains and spinal cords of homo- and heterozygous mice versus non-transgenic littermates.

Results

Behavior: Starting at early age, Tar6/6 mice exhibit strong motor deficits, whereas the onset is at 14 weeks of age for more complex tasks such as RotaRod (Fig. 1A), nesting behavior (Fig. 1B). Basic behavior, such as wire hanging is disturbed even earlier, and at six weeks of age all homozygous transgenics cannot resolve the wire hanging task (Fig. 1C) Memory deficits in the CFC are significant at 18 weeks of age (Fig.2).

Histology: Heterozygous as well as homozygous TAR6/6 mice express high levels of human TARDBP in different brain regions (Fig.3). Homozygous TAR6/6 mice present with increased neuroinflammation as analyzed by GFAP and CD11b labeling (Fig.4). Neuronal loss is measurable in 6 months old hetero-zygous and homozygous TAR6/6 mice, with an increase in the severity relating to the TAR6/6 copy number. The dendritic network of homozygous TAR6/6 mice is severely disturbed compared to non-transgenic littermates (Fig.5).

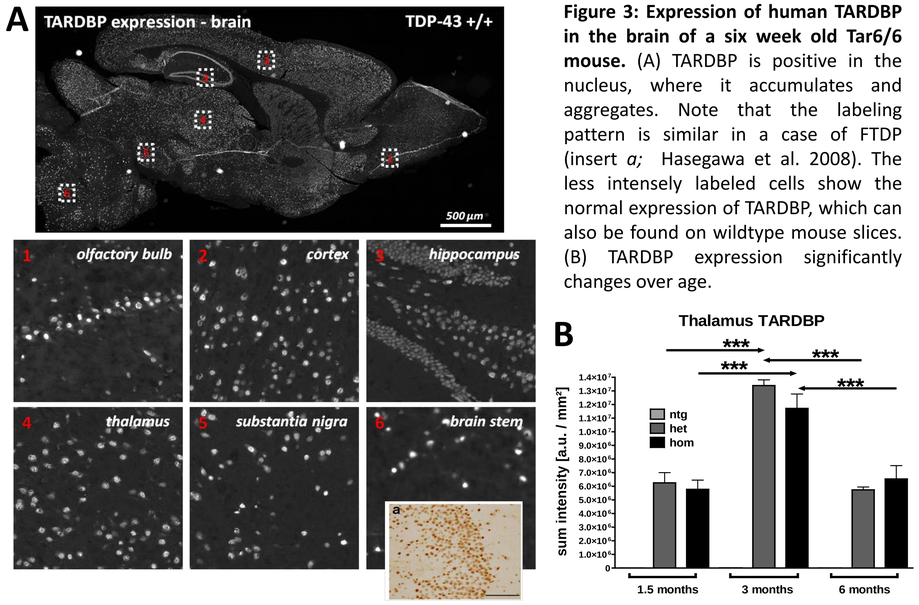

Figure 3: Expression of human TARDBP in the brain of a six week old Tar6/6 mouse. (A) TARDBP is positive in the nucleus, where it accumulates and aggregates. Note that the labeling pattern is similar in a case of FTDP (insert *a;* Hasegawa et al. 2008). The less intensely labeled cells show the normal expression of TARDBP, which can also be found on wildtype mouse slices. (B) TARDBP expression significantly changes over age.

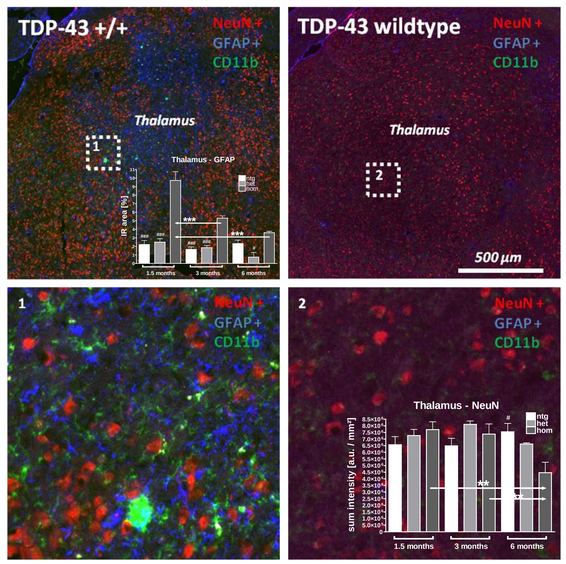

Figure 4: Neuro-inflammation and neuronal loss in the thalamus of a homo-zygous 12 week old Tar6/6 mouse compared to wild type control: Note the loss of NeuN-positive neurons (red) in large areas of the thalamus, and the drastic neuro-inflammation seen as astrogliosis (blue) and microgliosis (green) that is locally increased in brain areas with severe neuronal loss.

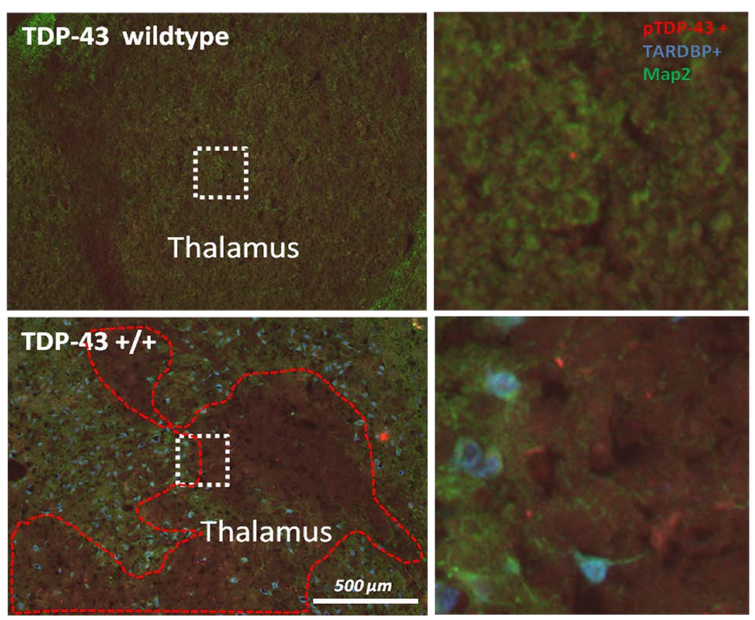

Figure 5: Comparison of dendritic network visualized by MAP2 labeling (green) in a homozygous 6 week old Tar6/6 versus a wildtype control: Note a total loss of dendritic network and TARDBP (blue) positive neurons in large areas of the thalamus. In these areas the concentration of small sized aggregates of the phosphorylated form of TDP-43 (red) is high, while it is hardly traceable in other regions of the brain and spinal cord.

Conclusions

This investigation led to intriguing new insights into the model: Homozygous mice develop an early and severe breakdown of MAP2-positive dendritic network in the thalamus, which is related to the inability of mice to complete the wire suspension task. Furthermore it is accompanied by severe neuronal loss parallel to the appearance of pTDP-43 aggregates in the thalamus and strong astro- and microgliosis. Further alterations such as strong astrogliosis could be found in the medulla oblongata and in the spinal cord. These findings further contribute to the understanding of TDP related alterations in motoneuron diseases.

We would be happy to test your compound in the Tar6/6 transgenic mouse!
Please contact us for our research service to support your drug development program!

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