THY-1 ALPHA-SYNUCLEIN MICE (TNWT61): DISEASE PROGRESSION AFTER WEANING

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BACKGROUND

Thy-1 Alpha-Syn mice are characterized by strong alpha-synuclein pathology combined with severe and stable motor deficits. Although early affliction was described (Chesselet et al. 2012), only little is known about early post-weaning disease progression and nothing is published about brain pathological correlates of the progressive PD motor symptomatic.

MATERIALS AND METHODS

Male Thy-1 TNWT61 mice completed a comprehensive behavioral test battery at 2, 3 and 6 months of age (see table below). Brains of 2 and 6 months old mice were sampled and investigated for soluble and insoluble human alpha-synuclein load, murine alpha-synuclein and tyrosine hydroxylase (TH) levels. Pathology in relevant brain regions was correlated to behavioral deficits. Quantitative image analyses used macro-based automatic and rater-independent algorithms (imaging: Zeiss Axio.Imager Z1 microscope, quantification: ImageProPlus).

	Week 1					week 2			
Treatment/test	Monday	Tuesday	Wednesday	Thursday	Friday	Monday	Tuesday	Wednesday	Thursday
Nesting									
Open Field									
Irwin Test									
Marble burying									
Rota Rod									
Pasta gnawing									
CFC									
Tissue sampling									

Time schedule for behavioral battery

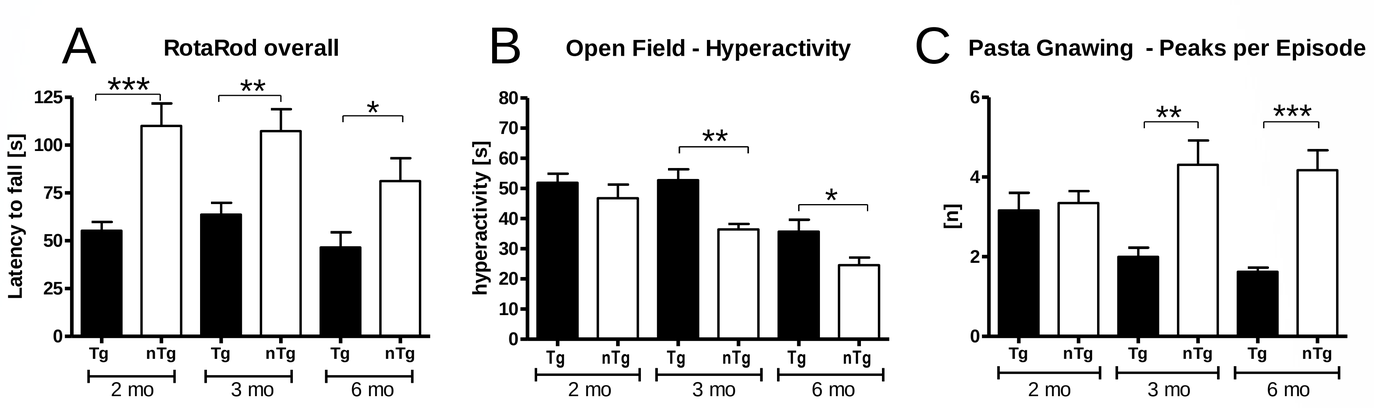


Figure 1. Early behavioral deficits in TNWT61 mice. Male TNWT61 present with early deficits in behavioral tasks, whereas it should be mentioned that several found deficits in other tests of the battery are not shown here. Reduced RotaRod running is found already at 2 months of age, hyperactivity in the Open Field is measurable at the age of three months as well as deficits in Pasta Gnawing. The latter are furthermore progressive thus significant between 2 and 6 months of age ($p<0.05$). Statistical significance between genotypes are indicated by asterisks determined by Two-Way ANOVA (Bonferroni's post test, GraphPadPrism). Data are shown as group mean + SEM (n = 11 to 15).

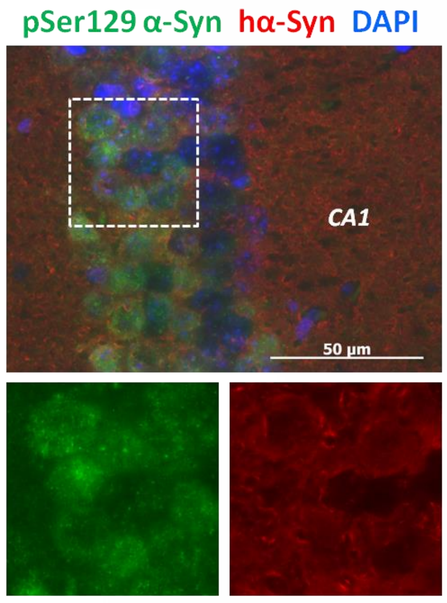


Figure 2. pSer129 alpha-syn in hippocampus. Images show the co-labeling of pSer129 (green) and human (red) alpha-synuclein as detected by clones EP1537Y and 15G7, respectively. DAPI (blue) was used to stain cell nuclei. Phosphorylation is frequent in the medial cerebral cortex and hippocampal pyramidal neurons as shown here (CA1 = cornu ammonis 1). Note that high intense alpha-synuclein is highly probable aggregated, which was separately quantified in overlap with NeuN labeling (not shown).

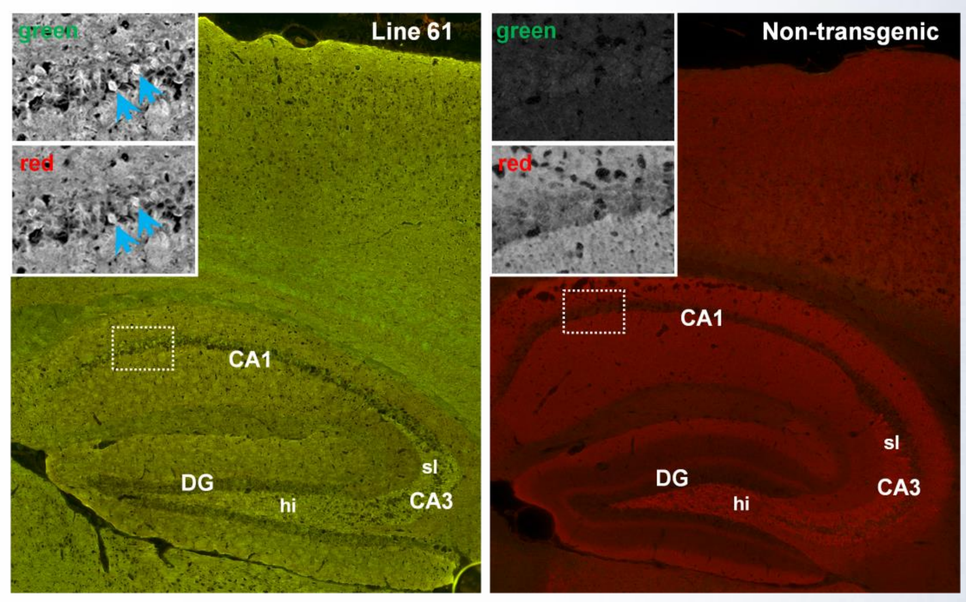


Figure 3. Endo- and exogenous alpha-synuclein. Images show co-labeling of human and pan alpha-synuclein as detected by clones 15G7 and LB09, respectively. The overexpressed human levels add to the endogenous levels, whereas we recently measured an approximate 15% down-regulation of the endogenous alpha-synuclein with six months of age.

Cerebral Cortex

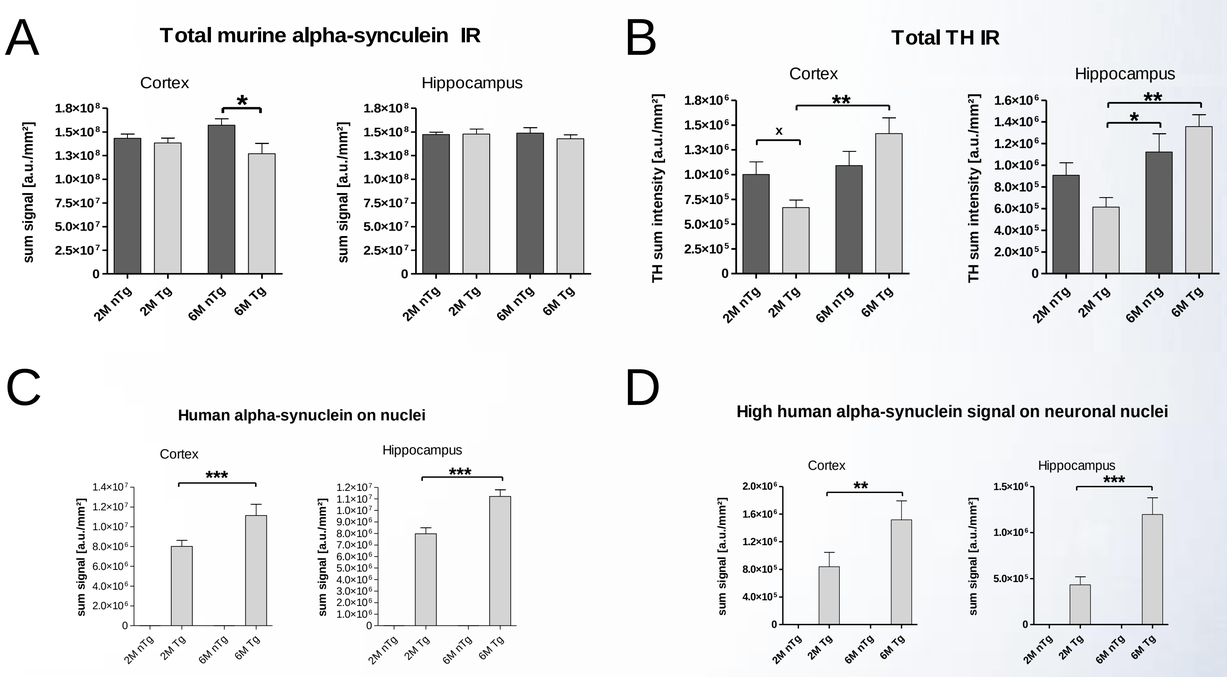
Histology	OF Hyperact	OF Act	OF rearing	OF middle	OF Thigmo	OF Boli	PG interval	PG episodes	RR T1	RR T2	RR T3	RR Mean
Total haSyn	measurement failure											
total haSyn on NN	-0.61 *	-0.6613 **	-0.7526 **	0.2202 ns	-0.2205 ns	0.08189 ns	0.3137 ns	-0.2409 ns	-0.0428 ns	-0.26 ns	-0.1287 ns	-0.1642 ns
High Int hAsyn on NN	-0.4605 ns	-0.5629 *	-0.7447 **	-0.1188 ns	0.1181 ns	0.1287 ns	0.09344 ns	-0.1575 ns	-0.4865 ns	-0.2769 ns	-0.3568 ns	-0.4234 ns
pSer129 aSyn on NN	-0.2205 ns	-0.1807 ns	-0.2956 ns	0.194 ns	-0.1936 ns	-0.1209 ns	0.6422 *	-0.1767 ns	0.289 ns	0.05998 ns	0.3097 ns	0.2378 ns
TH	-0.3873 ns	-0.4708 ns	-0.2237 ns	0.007299 ns	-0.006903 ns	0.5324 *	-0.003411 ns	-0.7299 **	-0.2606 ns	-0.1569 ns	-0.3686 ns	-0.29 ns
maSyn	-0.179 ns	-0.1826 ns	-0.07368 ns	-0.6716 **	0.6721 **	0.4671 ns	-0.2597 ns	0.1348 ns	-0.115 ns	-0.3557 ns	-0.1046 ns	-0.2401 ns

Hippocampus

Histology	OF Hyperact	OF Act	OF rearing	OF middle	OF Thigmo	OF Boli	PG interval	PG episodes	RR T1	RR T2	RR T3	RR Mean
Total haSyn	-0.6954 **	-0.7401 **	-0.3525 ns	-0.2376 ns	0.2364 ns	0.1556 ns	0.2139 ns	-0.5815 *	-0.17 ns	0.00485 ns	-0.2492 ns	-0.144 ns
total haSyn on NN	-0.7675 **	-0.7868 ***	-0.5947 *	0.1404 ns	-0.1414 ns	0.2256 ns	0.1817 ns	-0.3762 ns	-0.03 ns	-0.1202 ns	-0.294 ns	-0.1602 ns
High Int hAsyn on NN	-0.6995 **	-0.7369 **	-0.6326 *	-0.2002 ns	0.2001 ns	0.3503 ns	0.0254 ns	-0.4699 ns	-0.2621 ns	-0.5452 *	-0.612 *	-0.5402 *
pSer129 aSyn on NN	-0.09908 ns	-0.07978 ns	-0.07955 ns	0.5892 *	-0.5886 *	-0.1257 ns	0.7102 **	-0.3214 ns	0.2399 ns	0.2429 ns	0.29 ns	0.2935 ns
TH	-0.2509 ns	-0.2905 ns	-0.3874 ns	0.07789 ns	-0.07808 ns	0.06953 ns	0.1827 ns	-0.0795 ns	-0.0324 ns	-0.08334 ns	-0.1339 ns	-0.09596 ns
maSyn	-0.004774 ns	0.02232 ns	-0.3145 ns	0.05536 ns	-0.05523 ns	0.2525 ns	-0.3247 ns	0.3264 ns	-0.531 *	-0.3989 ns	-0.291 ns	-0.4832 ns

Figure 5. Correlation Table. Linear regression data show Pearson's r and significance of correlation marked by asterisks (two-tailed). Significant coefficients with the direction more pathology leads to more impairment and marked green, otherwise red.

Figure 4. Histological quantifications. Graphs show total murine alpha-synuclein (Cell Signaling # 4179), total Tyrosine hydroxylase (TH) IR, human alpha-synuclein (clone 15G7) overlapping with neuronal nuclei (NeuN) and high signal from aggregated human alpha-synuclein overlapping with NeuN. Statistical significance between genotypes are indicated by asterisks determined by Two-Way ANOVA (Bonferroni's post test, GraphPadPrism). Data are shown as group mean + SEM (n = 8).



RESULTS

Behavior

Motor deficits appear age-dependently and deficits are measurable already at ? months of age in the RotaRod (Fig.1A). Hyperactivity in the Open Field (Fig.1B) and Pasta Gnawing (Fig.1C) follow with three months of age, whereas deficits in the latter are progressive.

Pathology

TNWT61 mice present with decreased endogenous alpha-synuclein in the cortex at six months of age when compared to nTg littermates (Fig.4A), in the hippocampus such an effect cannot be seen. Cortical dopaminergic TH positive projections turned out to be significantly decreased at the young age of two months with the same trend in the hippocampus. However, up to the age of six months this deficit is significantly compensated (Fig.4B). Furthermore these mice strongly accumulate human alpha-synuclein due to high protein overexpression. While total levels do not rise (not shown), the signal that overlaps with neuronal nuclei (Fig.4C) shows a strong progression between 2 and 6 months of age. This effect is even stronger concentrating on high intense signal that widely reflects aggregated nuclear and peri-nuclear alpha-synuclein (Fig.4D) .

Correlations

Strong correlations are given between Open Field hyperactivity and activity and nuclear human alpha-synuclein. Interesting relations exist between aggregated nuclear alpha-synuclein in the hippocampus and RotaRod performance in the repeated trials that include a learning component. Thigmotaxis in the Open Field is related to hippocampal pSer129 alpha-synuclein. The same is also related to an impaired gnawing interval in Pasta Gnawing. More bites per episodes are related to lower cortical TH projections. Positive relations are especially seen for murine alpha-synuclein levels. Higher cortical endogenous levels are coupled with normalized thigmotaxis, also the RotaRod performance in the first trial is positively influenced by higher endogenous alpha-synuclein (Fig.5).

CONCLUSIONS

TNWT61 are a standard model in PD research. The early age deficits seen are likely to give an insight what might happen with the onset of disease. This study for the first time shows real correlation between different behavioral impairments and brain pathology. To note, it is not Proteinase K resistant alpha-synuclein but nuclear accumulating protein that drives deficits. Summarized, the here introduced data argue for early intervention and present valuable read-outs for drug testing.

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