Quantitative diffusion tensor imaging of the brain reflects motor impairments in a PD mouse model with intraneuronal [alpha]-synuclein aggregates

G. Vanhoutte[1], A. Buys[1], P. Kahle[2], and A. Van der Linden[1]

[1]Bio-imaging Lab, Department of Biomedical Sciences, University of Antwerp, 2020 Antwerp, Belgium, [2]Laboratory of Functional Neurogenetics, Department of neurodegeneration, Hertie Institute for Clinical Brain Research, University Clinics Tubingen, 72076 Tubingen, Germany

Introduction: Parkinson's Disease (PD) is a progressive neurodegenerative disorder associated with severe movement impairments. The neuropathological hallmark of PD is the loss of dopaminergic neurons in the substantia nigra and the formation of Lewy bodies (LBs). An increasing number of genes associated with PD have been identified to influence the structure and processing of α-synucleine (αSYN), the major component of LBs. Freichel et al. (1) generated the (Thy1)-h[A30P] αSYN transgenic mice in which the α-synucleinopathy manifests itself as intraneuronal αSYN inclusions in the amygdala and the motor cortex. At the age of 4 months, no behavioral deficits were present, cognitive performance was impaired at the age of 12 months and motor impairment became significant beyond 17 months. These behavioral deficits correlated with the distribution of αSYN in the brain (1). In order to gain insight into the molecular mechanisms of α-synucleinopathy at different ages, we performed in vivo diffusion tensor imaging (DTI) on the brains of αSYN mice to discern and quantify disturbed brain diffusion parameters as a correlate for intraneuronal α-synuclein deposition and for impaired connectivity. It was our aim to validate this tool as a potential non-invasive biomarker of PD-associated pathology both in early (synuclein depositions) and progressive stages (loss of connectivity).

Methods: We focused on studying one group of 18 months (n=4) reflecting full blown PD and another group of 5 months (n=6), i.e. the age before any clinical symptoms occurred. All mice (αSYN mice and age matched controls) were anaesthetized using 5% isoflurane (Forene®) for induction and 0.4%-0.8% for maintenance in a mixture of O_2:N_2 (2:4). A refined monitoring system (PC-SAM) controlled the body temperature (37.0±0.2°C) and breaths per minute (150±20). All MR experiments were performed on a 9.4T horizontal bore magnet (Bruker) using multi-slice DTI-FSE, a circular surface coil and diffusion sensitizing gradients along 6 directions. On a pixel-by-pixel basis, the fractional anisotropy and the eigenvalues (FA, λ_1,λ_2 and λ_3) were derived using house-made programs (MathWorks, Natick, MA, USA). After the generation of the parameter maps, anatomy-based ROI analysis was performed for selected regions of the motor circuit: motor cortex (Cm), substantia nigra (SN) and striatum (Str). The Mann-Whitney test - a non-parametric test - was used to detect significant differences.

Results: The ROI analysis is illustrated in the graphs of figure 1 and values are relative compared to the auditory cortex, used as a reference region to overcome inter-subject variability. In the striatum, no differences were observed (not shown). For young mice, no significant changes occurred but there was a trend of decreased FA in the SN which became significant at 18m. The reason for this was an increase in relative FA for the controls (from 1.73 ± 0.36 to 2.49 ± 0.29) (left graph compared to middle graph). Other pathological features in αSYN mice of 18m were present in the SN and the Cm: a decrease for λ_1 and an increase for λ_3 occurred in the SN and in the Cm, a decrease of both λ_1 and λ_2 and of FA was significant. These results were not due to age effects in the controls.

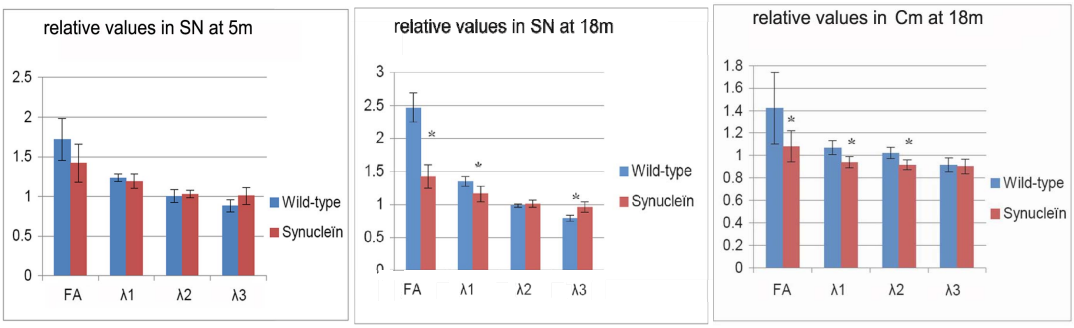

***Figure 1**: ROI analysis of all DTI parameters in substantia nigra (SN) and motor cortex (Cm). All values are relative to the auditory cortex. Significance level * $p<0.05$*

Conclusion and discussion: In our study we observed alteration in microstructural integrity, as measured by DTI, in regions of the motor circuit at 18 months. This is in accordance with the motor impairments which are reflected here as decreases in FA, λ_1 and λ_2 for the Cm and a decrease in λ_1 for SN. The increase in FA in controls at 18m as compared to 5m goes along with no age effect in radial diffusivity and reflects a reduced tortuosity toward more straightened fibers and/or increased axonal fiber organization with age (2). This normal developmental pattern was absent in αSYN mice and explains the decreased λ_1 and increased λ_3 as a loss of microstructural integrity. Importantly, in accordance with the behavioral studies, no abnormal effects were detected at 5m. However, the significant differences in αSYN mice at 18m supports the validity of our findings and suggests that brain tissue changes during the development of PD. In the near future we will explore in a longitudinal study at what time point DTI parameters start changing and correlate this with the known α-synucleinopathy in this model.

References:

(1)Freichel et al. Neurobiology of Aging (2006) Sep;28(9):1421-35

(2)Ashtari et al. Neuroimage (2007) Apr 1;35(2):501-10