Characterisation of two genetic rat models of Parkinson Disease through two presynaptic PET tracers; [18F]-LBT999 and 6-[18F]fluoro-L-m-tyrosine

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INTRODUCTION

Parkinson's disease (PD) is the second most prevalent age-related neurodegenerative disorder, characterized by several motor-symptoms, pathologically caused by a loss of the dopaminergic neurons in the substantia nigra (SN), resulting in a dopamine (DA) deficiency in the striatum [1]. Previously we developed a pathologically relevant rodent PD model; overexpressing the mutant (A53T) human alpha-synuclein protein in the SN [2]. This resulted in 20% less use of the contralateral forepaw in the cylinder test and 40% decrease of dopaminergic neurons in the SN at 15 weeks post injection [2].

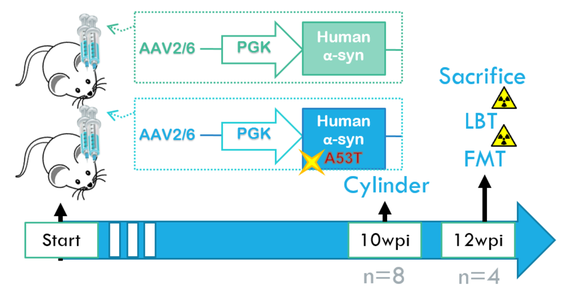
Our aim is to evaluate and compare neuronal loss in two genetic models of PD with *in vivo* imaging techniques, in order to evaluate therapeutic strategies in these models in the future. To asses neuronal loss and DA deficiency two different pre-synaptic PET tracers were used, in combination with behaviour tests and histological stereology.

METHODS

Two cohorts of rats were double injected in the SN unilaterally with a viral vector (AAV2/6-PGK; 1.00E+11 vgc) overexpressing wildtype (WT; n=4, 573±40g) or mutated (A53T; n=4, 589±39g) human alpha-synuclein (α-syn), and were studied at 10 to 12 weeks post injection (wpi) using PET imaging, cylinder test and stereological counting. Behavioural evaluation was performed using the cylinder test; forepaw use was calculated in percentage of total touches for the first 5 minutes of observation time. The *in vivo* studies were followed by sacrifice for stereological counting using tyrosine hydroxylase (TH) immunohistochemistry. All animal experiments are in accordance with French legislation.

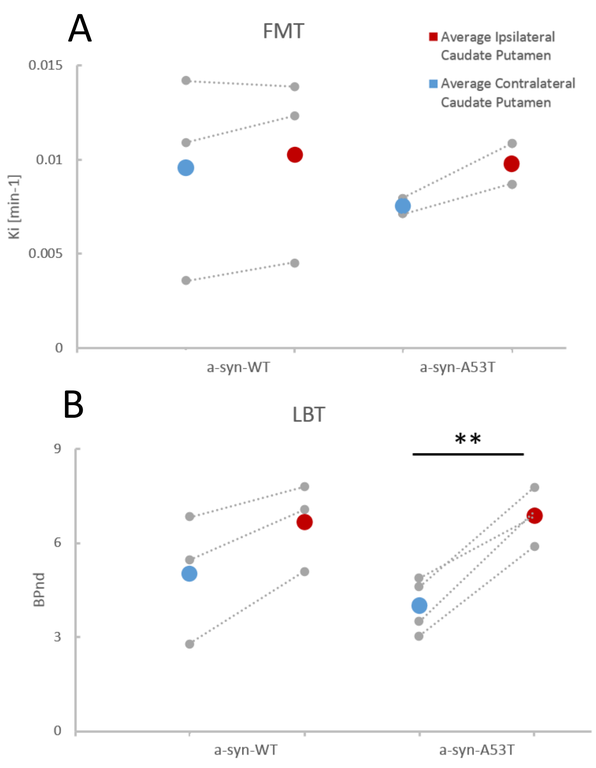
PET imaging was performed using a ligand substrate for AADC, 6-[18F]fluoro-L-m-tyrosine ("FMT", 60min acquisition, 36.4-46.5MBq; pre-treatment by IP injection of 10mg/kg benserazide 30' before imaging [3]), or a ligand for DA transporter (DAT), [18F]-LBT999 [4] ("LBT", 90min acquisition, 54.4-63.0MBq). Quantitative uptake images (BPnd and Ki) were calculated using Logan and Patlak graphical methods, respectively, with the cerebellum as a reference, Ki images were subsequently smoothed.

Probability values (*p<0.05, **p<0.01) were calculated using paired Student t-tests with the contralateral striatum as internal control. For stereology and behaviour studies one-way ANOVA with Scheffe-F post-hoc test was used. Results are expressed as the mean ±SEM.



RESULTS & DISCUSSION

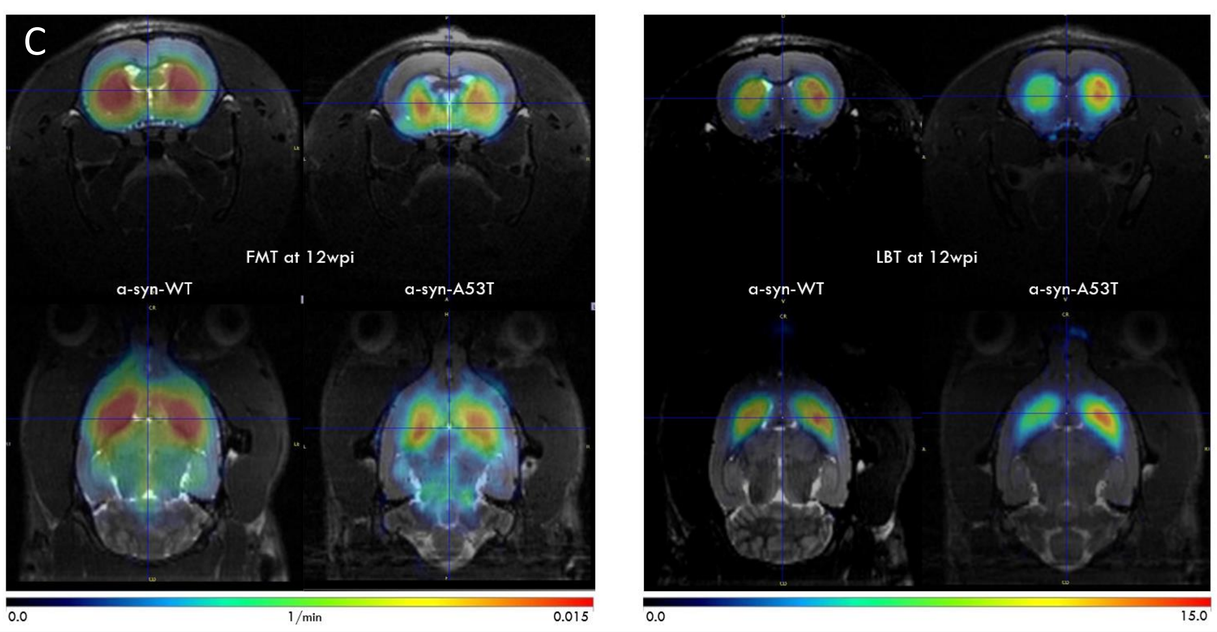
PET RESULTS



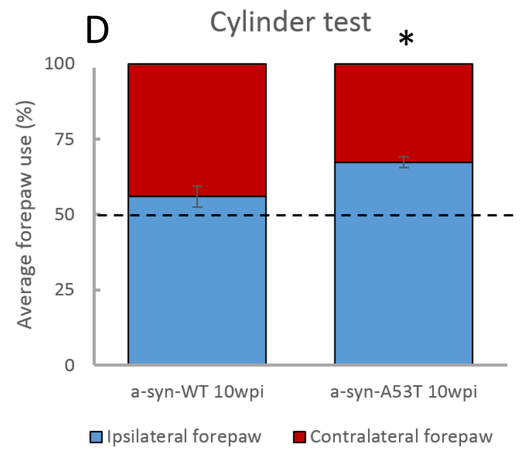
A) 18F-FMTyr of α-syn-WT and A53T
Blocking of COMT with benserazide was not effective in 42% of the animals, thus Ki values could not reasonably be estimated for FMT. Neither for α-syn-WT (n=3, p=0.318) nor α-syn-A53T rats (n=2, p=0.177) a difference was observed in AADC metabolism between ipsilateral and contralateral striata.

B) 18F-LBT999 of α-syn-WT and A53T
The DAT tracer (LBT999) shows a significant difference for α-syn-A53T (n=4, p=0.003) but not for α-syn-WT rats (n=3, p=0.051).

C) Representative PET images of 18F-FMTyr (left) **and 18F-LBT999** (right) **of both α-syn-WT and A53T.** Ki images show lower contrast to background than LBT BPnd images.

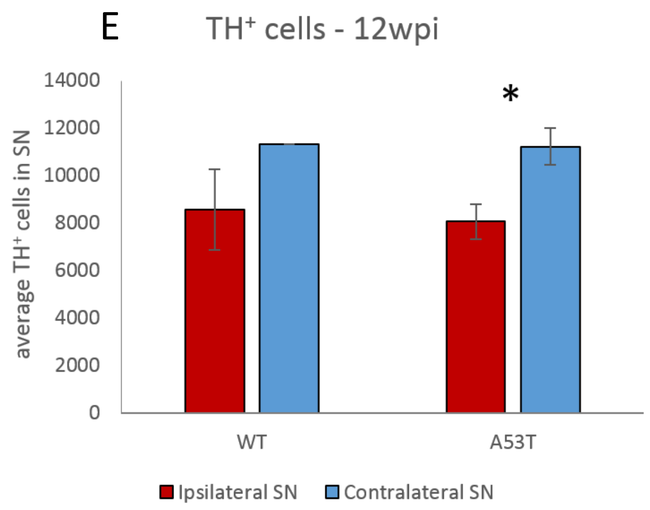


BEHAVIOUR RESULTS



D) Cylinder test of α-syn-WT and A53T at 10wpi
Cylinder test at 10wpi detected motor deficits only in α-syn-A53T overexpressing rats (n=8, p=0.045), but not for α-syn-WT (n=7, p=0.949). PET results are in concordance with the behavioural observations, showing roughly 35% less use of the contralateral forepaw for a-syn-A53T at 10wpi. No significant correlations between PET data and behaviour were observed.

HISTOLOGICAL RESULTS



E) TH⁺ cells in SN of α-syn-WT and A53T at 12wpi
Preliminary results of stereological counting of TH-positive cells in the SN of animals sacrificed at 12wpi. Preliminary results for α-syn-WT group show no significant difference (n=2), while α-syn-A53T does show a significant lower TH-positive cells in the ipsilateral SN compared to the contralateral SN (n=8, p=0.009.

DISCUSSION

Our parametric data suggest that the DAT tracer is more sensitive to detect a mild PD phenotype as compared to the AADC substrate. This phenomenon has previously been described, and is possibly due to a combination of reduced nerve terminal DAT binding sites and downregulation of DAT in surviving neurons, in an attempt to increase DA availability [5]. More FMT scans will have to be done to increase numbers and compensate for ineffective benserazide blocking.

CONCLUSIONS

We have shown here that the α-syn-A53T model, but not the α-syn-WT model, is able to generate neuronal loss and dopamine deficiency, which can be visualized by DAT PET, cylinder test and TH-stereology. The α-syn-A53T model has a shorter time window to develop than our previous model, which makes it more interesting for testing therapeutic strategies *in vivo.* Additionally we have defined DAT transporter imaging, with LBT, as more sensitive and robust as compared to AADC substrate imaging with FMT.

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Acknowledgements

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