ABSTRACT – HOPE 2020 (600 words max)

Evaluation of dopaminergic neuronal loss and synaptic changes in an α-synuclein overexpressing model of PD

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Introduction
Parkinson’s disease (PD) is the second most common age-related neurodegenerative disorder, pathologically characterized by a loss of dopaminergic neurons in the substantia nigra (SN). The resulting dopamine deficiency in the striatum underlies some of the observed motor symptoms such as rigidity and tremor. A histopathological hallmark of PD is the presence of Lewy Bodies in the brain; aggregates containing, amongst others, misfolded α-synuclein protein[1]. In the present study, we aim to evaluate neuronal loss and synaptic compensation mechanisms in a rodent PD model that unilaterally over-expresses mutant (A53T) human α-synuclein, a missense mutation found in familiar cases of PD[2].

Materials & Methods
Two cohorts of rats (n_total=16) were unilaterally injected in the SN with AAV2/6 viral vector coding for mutated (A53T) human alpha-synuclein. Animals were evaluated at early (n=8; 6-8 weeks post-injection (wpi)) or late time-point (n=8; 10-12wpi). At both time-points, all animals underwent in vivo behaviour and PET imaging, and post mortem immunohistological and qPCR analysis.

PET imaging was performed using 6-[18F]fluoro-L-m-tyrosine [3][FMT] - a substrate for AADC, and [18F]-LBT999 [4][LBT] - a radioligand for dopamine transporter (DAT). AADC enzymatic rate (Ki) and DAT binding (BPND) were calculated using Patlak and Logan graphical methods respectively, employing the cerebellum as a reference region. For behavioural analysis, rats were subjected during 5 minutes to the cylinder test, in which contralateral and ipsilateral paw use was compared. After the in vivo studies, rats were sacrificed for histological studies using tyrosine hydroxylase immunohistochemistry, and biochemical analyses using qPCR. Paired student t-tests were used to compare the contra- and ipsilateral sides in imaging studies, while an ANOVA was used to compare contralateral paw use to a control group.

Results
The cylinder test revealed a clear motor deficit at both the early (-37%;n=7;p=0.025) and the late time-point (-35%;n=8;p=0.032). FMT-PET imaging did not reveal any significant differences in AADC enzymatic activity in the striatum at either time-point, nor did we observe a difference in AADC mRNA levels in the SN. In contrast, we observed a significant asymmetry in DAT binding in both the early (-
26%;n=6;p=0.027) and the late cohort (-42%;n=4;p=0.003). However we only demonstrated a significant left/right difference in DAT mRNA levels in the SN (-25%;n=5/6;p=0.01) and DAT protein levels in the striatum (-26%;n=8;p=0.005) at the late time-point.

Finally, we demonstrated significant dopaminergic cell loss in the SN with TH stereology in both the early (-14%;n=6;p=0.013) and late cohort (-28%;n=8;p=0.009), and a significant TH protein loss in the striatum at the late time-point (-33%;n=8;p=0.038).

**Discussion/Conclusions**

Based on behavioural data, and compared with previous studies on a complete lesion PD model, the AAV2/6-A53T model shows mild progressive neuronal loss between the early and late time-point. However, we were not able to confirm the dopaminergic neuronal loss using an in vivo PET marker of AADC enzymatic, neither were we able to demonstrate any evidence of AADC compensation mechanisms, as previously suggested in the literature[5]. Although our DAT binding at the early time-point was greater than neuronal loss measured with stereology, we were not able to demonstrate any compensation effect of DAT by post-mortem data. However, this compensation effects were evident at the late time-point, as previously suggested by Lee et al.[6]. Future research will have to unveil whether these compensation effects occur at the ipsilateral and/or contralateral synapse. Mild progressive PD models liked used in this study, unlike full lesion models, will allow to study compensatory mechanisms, and could give us new information about PD pathology.

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**References**


