# Assessment of olfactory dysfunction in an inducible mouse model of α-synucleinopathy via multi-modality imaging

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## Introduction

Parkinson's disease (PD) is currently diagnosed based on motor impairment and neuropsychiatric disturbances, although non-motor deficits, such as olfactory impairment, typically precede the cardinal motor symptoms by several years. This early stage of PD represents an ideal window for therapeutic intervention to prevent development of motor symptoms. PD is associated with progressive loss of neurons, as well as the presence of abnormal aggregates of misfolded α-synuclein. This misfolded α-synuclein is a primary target for novel, disease-modifying therapeutic agents. The overarching objective of this project was to develop an inducible mouse model of α-synucleinopathy to characterize early pathological changes associated with the olfactory system in mice using state-of-the-art, multi-modality imaging techniques in order to provide well-validated tools to accelerate the development of disease-modifying treatments for PD.

## Methods

**1/** The mouse model of α-synucleinopathy was induced in 8 week-old, wild-type (WT) B6/C3H mice (n=40), M83 (human A53T; JAX) hemizygous (+/-) transgenic (Tg) mice (n=10), and M83 homozygous (+/+) Tg mice (n=10). Preformed murine or human α-synuclein fibrils (PFFs) (Luk, 2013) were injected into the anterior olfactory nucleus (AON) (**Figure 1**). Injection of Phosphate-Buffered Saline (PBS) was used as a negative control (n= 40 for WT and n=10 for Tg mice).

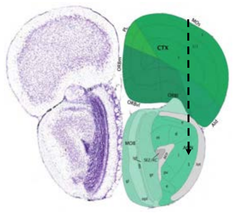
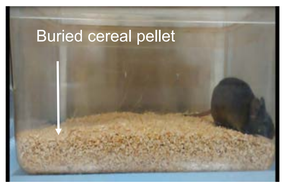



**Figure 1.** AON coordinates = Anterior: 2.8 mm; Left: 1.5 mm; Deep 2mm, from Bregma.

**2/** Animals were tested for olfactory deficits at 15 weeks post-surgery using the buried pellet test (ref). Briefly, after moderate food deprivation, the mice were put into a cage with a cereal pellet buried in the bedding. The amount of time to find the pellet (5 min maximum) was measured on four consecutive days (Fleming, 2008).




**3/** WT mice underwent baseline 3D anatomical MRI and 3D diffusion MRI (dMRI) scans prior to inoculation at 7 weeks-of-age using a 7T animal MRI system (Bruker BioSpec 70/30). Mice were then randomized to PFF injection or PBS control groups, injected, and aged for 18 weeks. At the end of this period, WT mice underwent follow-up MRI/dMRI and manganese-enhanced MRI scans (MEMRI). MEMRI was performed as a 90 minute dynamic scan after intranasal administration of $Mn^{2+}$ (0.1 M; 5 µL; 4 hours prior to scanning). All MR images were processed using Biospective's fully-automated, production-level, NIGHTWING™ MRI processing platform (**Figure 2**).

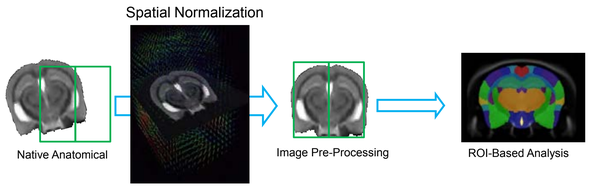



**Figure 2.** NIGHTWING™ MR image processing platform.

As part of this process, an anatomical MRI template and a segmented atlas in reference coordinate space were generated. Representative, orthogonal views of the unbiased, symmetric, customized anatomical MRI template and labeled atlas are shown in **Figure 3**. MEMRI data was analyzed to evaluate the rate of $Mn^{2+}$ accumulation in the olfactory bulb (OB) and the AON regions. A representative parametric map of $Mn^{2+}$ intensity in the OB after 90 minutes of scanning is shown **in Figure 4.** Data was analyzed using repeated-measures, two-way ANOVA.

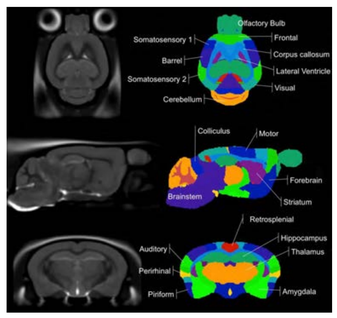



**Figure 3.** Anatomical Template & Volumetric Atlas.

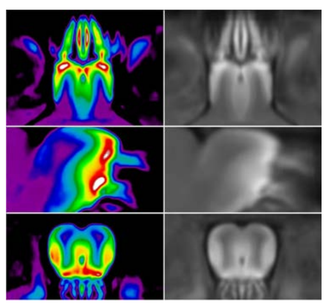


**Figure 4.** Parametric map of $Mn^{2+}$ intensity in OB after 90 minutes.

**4/** Upon completion of MRI scanning, mouse brains were extracted and quantitative immunohistochemistry (qIHC) studies were performed (Zehntner, 2008) to assess α-synucleinopathy, neuronal density, and neuroinflammation using Biospective's PERMITS™ software. PERMITS™ uses multi-step image registration to generate 3D qIHC volumes registered to the MRI coordinate space.

## Results

### 1/ Weight variations of WT and Tg mice injected with mPFFs or PBS

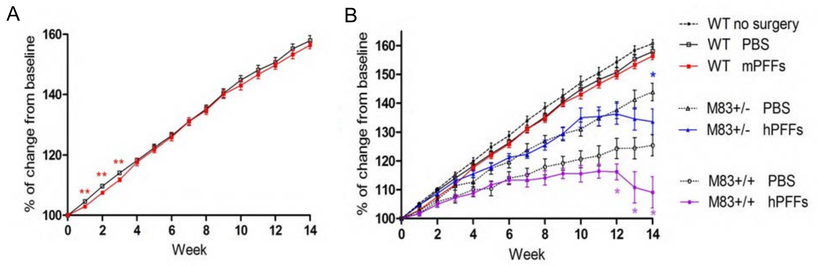



**Figure 5.** A) There was a transient, but significant, weight difference (**p<0.01) between mPFF- or PBS-injected WT mice during the first 3 weeks following stereotaxic surgery. This finding may be due a reduction of appetite for several days following inoculation of α-synuclein fibrils (mean ± SEM; t-test **p<0.01). B) Transgenic mice average weight gain was lower than that of WT B6/C3H mice over the 14 week period, and PFF-injected transgenic mice showed significant weight loss compared to PBS-injected transgenic mice (mean ± SEM; t-test *p<0.05).

### 2/ Injection of PFFs into the AON induced statistically-significant olfactory deficits in WT and Tg mice, measured by latency in the buried pellet test

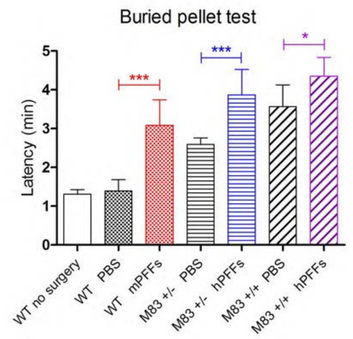
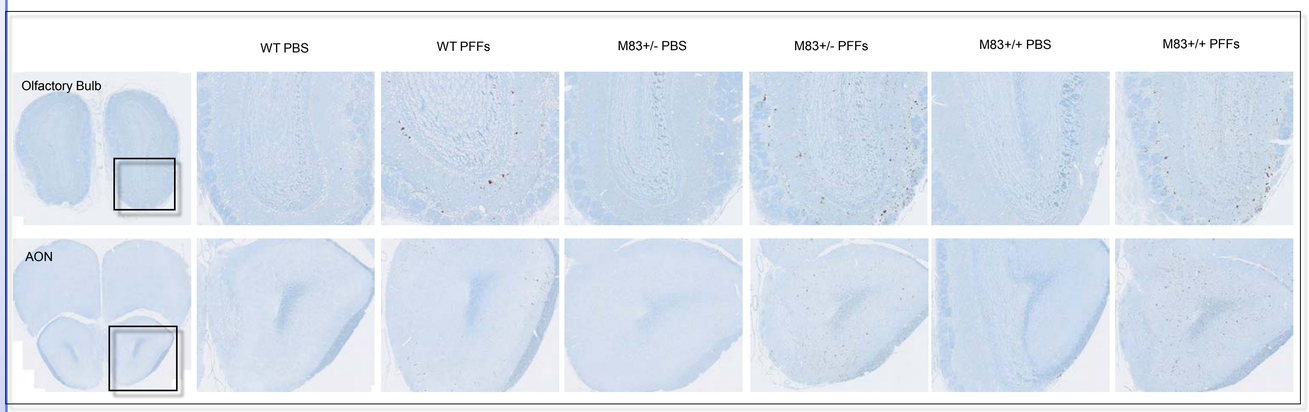



**Figure 6.** Injection of PFFs into the olfactory system induced significant olfactory deficits, measured by the latency to find a buried pellet, in WT B6/C3H, M83 hemizygous, and M83 homozygous mice compared to PBS injection. (t-test with repeated measures; * p<0.05 and *** p<0.0001).

### 3/ Injection of PFFs into the AON led to α-synucleinopathy in anatomically-connected olfactory regions in WT and Tg mice



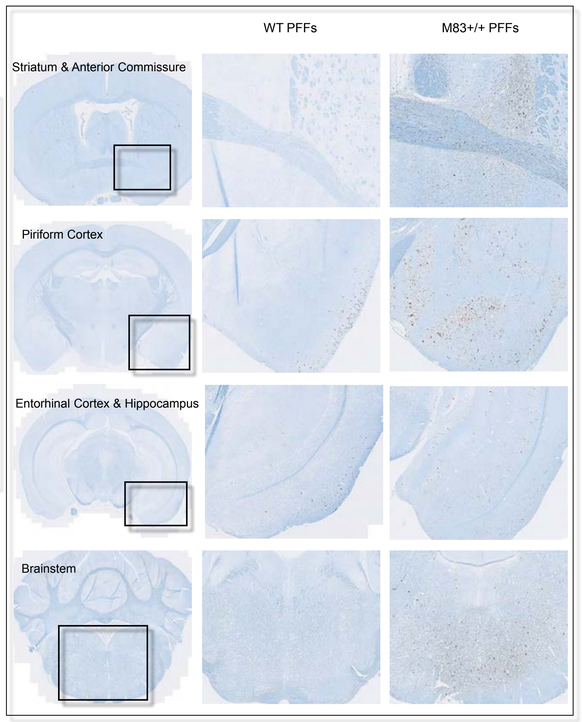




**Figure 7.** IHC staining for the pathological, phosphorylated α-synuclein at 18 weeks post-inoculation of PBS or PFFs.

Generation of 3D quantitative IHC maps of α-synuclein using Biospecitve's PERMITS™ technology to visualize the pattern of spread is currently in progress. We are also assessing neurodegeneration and neuroinflammation.

### 4/ Injection of PFFs into the AON led to a significant decrease in volume of the AON and the OB in M83 hemizygous (+/-) mice

18 weeks post-PBS or PFFs injections, WT and a small group of M83 hemizygous mice underwent 3D anatomical MRI. Based on the anatomical template produced by the Biospective's fully-automated, production-level MRI processing platform, NIGHTWING™, and an atlas defining regions-of-interest (ROIs) (AON, OB), the statistical results for all groups are summarized in **Table 1**. Injection of PFFs into the AON led to a significant decrease in volume of the AON and the OB in M83 hemizygous (+/-) mice. Future studies will require an increased sample size of Tg mice to investigate the potential of structural MRI to assess anatomical changes resulting from α-synucleinopathy.

| | | P value |
|---|---|---|
| AON | WT PBS injected vs. contralateral side | 0.0744 ns |
| | WT PFFs injected vs. contralateral side | 0.2905 ns |
| | WT PBS injected side vs. WT PFFs injected side | 0.0572 ns |
| | Tg PFFs injected vs. contralateral side | **0.0199 *** |
| OB | WT PBS injected vs. contralateral side | 0.2490 ns |
| | WT PFFs injected vs. contralateral side | 0.0815 ns |
| | WT PBS injected side vs. WT PFFs injected side | 0.1709 ns |
| | Tg PFFs injected vs. contralateral side | **0.0037 ** |

**Table 1.** Comparison of the volumes of AON and OB 18 weeks after injection of PBS or PFFs into the AON of WT or M83 hemizygous (+/-) mice (Tg). t-test *p<0.05; **p<0.01; ns=non-significant. WT PBS (n=14), WT PFFs (n=19), Tg PFFs (n=4).

qIHC studies are currently in progress to assess neurodegeneration and neuroinflammation as the potential, underlying cause(s) of these volume differences.

### 5/ Injection of PFFs into the AON of WT mice did not induce significant MRI microstructural or functional changes

18 weeks post-PBS or PFFs injections, WT mice underwent dMRI and MEMRI scans. Data analysis showed no significant difference in the diffusion metrics, fractional anisotropy (FA), axial diffusivity (AD), mean diffusivity (MD) and radial diffusivity (RD), when comparing the PBS- to PFF-injected groups. The t-statistic parametric maps of the main effects are shown in **Figure 8**. MEMRI analysis to assess reduced axonal transport function also showed no significant differences in WT mice receiving PBS compared to PFFs (**Figure 9**).

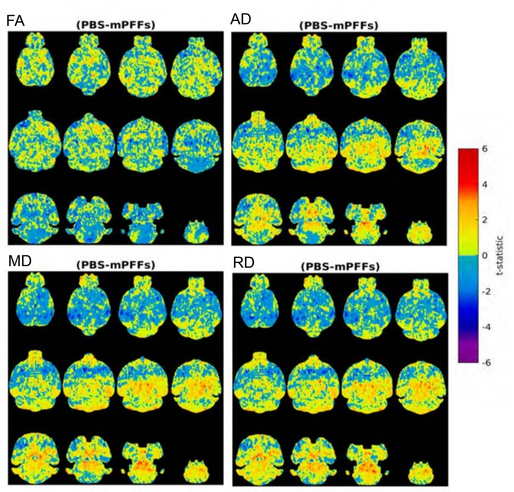



**Figure 8. T-statistic parametric maps (transverse cross-sections).** Main effect of groups comparisons for the four dMRI measures: FA, AD, MD, and RD. There are no significant differences between WT mice injected with PFFs compared to WT mice injected with PBS.

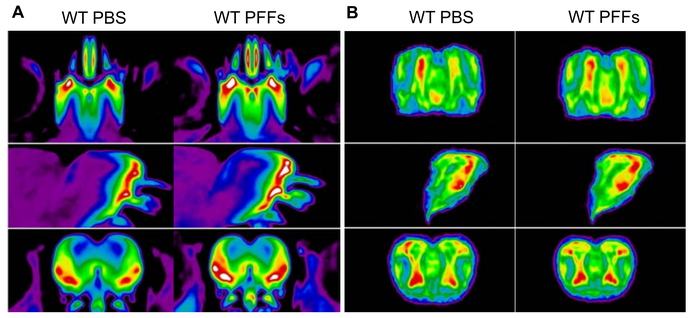



**Figure 9.** Representative parametric maps of $Mn^{2+}$ intensity (A) and the rate of $Mn^{2+}$ accumulation (B) in the OB of WT mice injected with PBS or PFFs after 90 minutes of dynamic scanning. There were no significant functional changes in WT mice injected with PFFs compared to WT mice injected with PBS.

In summary, α-synucleinopathy develops well in WT mice injected with PFFs. These mice demonstrate olfactory deficits (**Figure 6**), but do not show significant structural and functional changes by MRI. The preliminary data from M83 hemizygous and homozygous Tg mice revealed that this model has greater potential for *in vivo* MRI studies.

## References

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## Conclusions

We have developed an inducible mouse model of α-synucleinopathy that demonstrates olfactory dysfunction and a reproducible pattern of spread of pathology through the olfactory network. Future studies will focus on M83 (human A53T) Tg mice as our preliminary studies revealed that this particular model has better potential for MRI studies. Our approach allows for a comprehensive understanding of the alterations underlying *in vivo* MRI-based imaging biomarkers. This rapid, robust, inducible model can be used for preclinical studies to accelerate the development of disease-modifying treatments for PD and other synucleinopathies.

## Acknowledgements

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