Olfactory dysfunction, regional brain atrophy, and pathologic spreading in an inducible mouse model of α-synucleinopathy

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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 disease (PD) is currently diagnosed based on motor deficits, such as olfactory impairment, typically precede the cardinal motor symptoms by several years. This early stage of PD represents an ideal disease intervention opportunity.

Methods

1. The mouse model of α-synucleinopathy was induced in 8-week-old, male (WT) C57/BL6J mice (Charles River, n=40), M83 hemizygous (+/-) mice (Tg), and M83 homozygous (+/+) mice (n=10). Preferred mouse or human α-synuclein (Tg) (Sak, 2013) transgenic mice were used as a negative control (n=40 for WT mice).

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

3. WT mice underwent baseline 3D anatomical MRI scans prior to infection at 7 weeks of age using a 3T animal MRI system (Bruker BioSpec 70/30). Mice were then randomized to PFF injection or PBS control groups, injected, and aged for 17 weeks. At the end of the study period, WT and M83 hemizygous (+/-) mice underwent follow-up anatomical MRI. All MRI images were processed using a fully-automated, production-level, NICHTWING™ MRI processing platform.

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

We generated 3D quantitative IHC maps of the phosphoSer129 α-synuclein using Bioptive’s PERMITS™ technology to visualize the pattern of spread. Representative coronal and sagittal sections of the average phosphoSer129 α-synuclein qIHC parametric maps for the WT (n=4 [PBS] and n=9 [mPFFs]), M83 Tg +/-(n=7 [PBS] and n=6 [PFFs]), and M83 Tg ++ (n=6 [PBS] and n=5 [PFFs]) animals at four months post-injection are shown in Figure 5. The qIHC parametric maps illustrate the 3D reconstructed qIHC data in template space, allowing for quantitative analysis of phosphoSer129. Summary qIHC measures, expressed as staining density, are provided in Table 4 for WT and M83 Tg mice injected with PFFs.

5/ Injection of PFFs into the AON resulted in significant decreases in regional neuroanatomical volumes in WT and M83 Tg mice

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

Acknowledgements

This work was funded by Bioptive Inc. and the Quebec Consortium for Drug Discovery (CQDDM).

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Perfusion-preparation and imaging

3D reconstruction of the olfactory epiphrase was performed using Bioptive’s PERMITS™ software. Image registration employed between-section anatomical MRI. All MR images were processed using a fully-automated, production-level, NICHTWING™ MRI processing platform.

PERMITS™ uses multi-step, image-registration to generate 3D qIHC volumes registered to the MRI coordinate space (Figure 6).

Mouse brains were fixed and embedded in paraffin, then sectioned into 5 μm sections with 120 levels covering the entire brain. Tissue sections underwent IHC staining for phosphoSer129 α-synuclein (approximately 90% of α-synuclein detected in Liley bodies is phosphorylated at serine 129 in a pathological brain tissue) and counterstained with Anti-NeuN (Zimmer, 2000). IHC sections were digitized using an Aperio® digital whole slide scanner (Carl Zeiss, Canada).

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