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 intraneuronal aggregates of mutated α-synuclein. This misfolded α-synuclein is a primary target for new, 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**

**AON-targeted Model of α-synucleinopathy**
- Induced in 8-10 week-old male MBD hemizygous (+/-) mice
- Performed human α-synuclein fibril (PPF) (Lui, 2012) were injected into the left Anterior Olfactory Nucleus (AON)
- Injects of Phospho-Buffered saline (PBS) were used as a negative control

**Behavioral Testing**

**Olfactory Dysfunction Testing**
- Animals tested for olfactory defects at 6 and 16 weeks post-surgery (t8 & t16)
- Burned Puff Test
- Latency to find the pellet (5 min maximum) was measured on four consecutive days (Fleming, 2008)

**Sleep Dysfunction Testing**
- Animals tested for sleep dysfunction at t8, t12, and t16 weeks post-surgery
- The PicroSleep Mouse Behavior Tracking System was used to record continuous and real-time sleep/wake behavior over a 12 hour period
- Access to food and water was ensured and monitored closely

**Anatomical MRI & Analysis of Volumetric and Cortical Thickness Data**
- Mice received baseline (time point t0) 3D anatomical MRI scans using a 7T Bruker BioSpec70/30 animal MRI scanner
- Animals underwent follow-up MRI scans at t8, t12, and t16 weeks post-surgery
- All MRI images were processed using Biospective’s fully-automated, production-level, NIGHTVISION™ MRI processing platform

2D and 3D Quantitative Immunohistochemistry (qIHC)

- Mouse brain tissue was fixed and embedded in paraffin
- Tissue sections were collected over 120 levels covering the entire brain
- Stained for phosphoSer129 α-synuclein (p-Syn) and NeuN for neuronal cell bodies
- Digitized using an Aperio Scan IT digital whole slide scanner
discovered as a negative control
- 3D reconstruction of the stained sections was performed using Biospective’s PERMITS™ software

- Segmentation and 2D quantitation was also performed with Biospective’s PERMITS™ software
- Manually painted regions were delineated at 12 levels across the brain
- Mean staining density for p-Syn and NeuN were assessed

**References**


**Conclusion**

We have developed an inducible mouse model of α-synucleinopathy that demonstrates behavioral deficits (olfactory dysfunction and sleep disturbances). We observed a reproducible pattern of pathology spreading through the olfactory network with a significant decrease in regional neuroanatomical volumes and cortical thickness over a 16 week period. Our approach allows for a comprehensive understanding of the disease development utilizing in vivo MRI as an imaging biomarker. 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**

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