Olfactory dysfunction, sleep disturbances, neuronal loss, and regional brain atrophy 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 window for therapeutic intervention to prevent development of motor symptoms. PD is associated with progressive loss of neurones, as well as the presence of intraneuronal 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, in order to provide well-validated tools to accelerate the development of disease-modifying treatments for PD.

Methods

Aβ1-40 inhibitor model of α-synucleinopathy
• Induced in 8-10-week-old male MDD hemizygous (+/−) mice
• Performed human α-synuclein fibril (PFFs) (Juk, 2012) were injected into the left anterior olfactory nucleus (AON)
• Injections of Phosphate-Buffered Saline (PBS) were used as a negative control

Behavioural Testing

Olfactory Dysfunction Testing
• Animals were tested for olfactory deficits at 6 and 16 weeks post-surgery (8 & 16)
• Buried Pellet Test
• Latency to find the pellet (5 min maximum) was measured on five consecutive days (PFINING, 2008)

Sleep Dysfunction Testing
• Animals were tested for sleep dysfunction at 6, 11, and 16 weeks post-surgery
• The PInSleep Mouse Behavior Tracking System was used to record continuous and real-time sleep/wake behavior over a 72-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 0) 3D anatomical MRI scans using a T1 Inversion Recovery magnet resonance tubing platform
• Mice underwent follow-up MRI scans at 6, 12, and 16 weeks post-surgery
• All MRI images were processed using Biospective’s fully-automated, production-level, NIGHTING™ software processing platform

Other 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 pathological phosphoSer129 α-synuclein (p-Syn) and NeuN for neuronal cell bodies
• Digitalized using an Aperio ScanStation digital whole slide scanner
• Automated slides
• QIHC of the stained sections was performed using Biospective’s NIGHTING™ software

Quantitative analysis of IHC and qIHC

• 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/NeuN were assessed

Results

1/ Impact on Weight Gain

PPF-injected mice showed a reduction in weight gain starting at week 12 post-surgery, and a significant weight loss compared to PBS-injected mice at week 14 post-surgery.
(Mean ± SEM; t-test: *p<0.05)

2/ Development of Olfactory Deficits

Significant olfactory deficits were observed at 16 weeks post-surgery, measured by the latency to find a buried pellet.
(Mean ± SEM; t-test with repeated measurements: **p<0.01)

3/ Disturbance in the Quality of Sleep

A. Injection of PFFs into the olfactory system induced significant sleep disturbances at 12 and 16 weeks post-surgery in both light and dark phases.
(Mean ± SEM; t-test: ***p<0.001)

B. Significant changes in the type of sleep were observed. There were changes in both the sleep bout length and the wake bout length.
(Mean ± SEM; 2-way ANOVA comparing PFFs t12 and t16 to the PBS group: **p<0.01, ***p<0.001; p<0.001; p<0.001; p<0.001; p<0.001)

4/ Develop Significant α-synucleinopathy with Concomitant Neuronal Loss

(A) Diagram of the projections from the AON to the interconnected neuronal regions and representative p-Syn IHC. Retractive connections exist from the AON to the OB. First order connections in the olfactory regions include (in order): OB, AON, Perforant, Cortex, Entorhinal Areas, the Amygdale, and the posterior Hippocampus. The second order connections occur both at the level of the lateral OB to the contralateral AON, as well as across the corpus callosum (KAY, 2013)

(B) qIHC parametric maps of the p-Syn IHC changes

Representative coronal, sagittal, and transverse views, illustrating p-Syn spreading in olfactory-connected regions (110).

5/ Significant Decrease in Volume of Ipsilateral Olfactory and Connected Regions

Quantitative plots illustrate significant reduction in volume over time in olfactory-connected regions. NIGHTING™ was used to generate volumes from atlas-defined ROIs, 1 tests were performed at each time point compared to the baseline (t0) volume.
(Mean ± SEM in ROI ± test: **p<0.01; ***p<0.001)

6/ Early Significant Decreases in Cortical Thickness

Vertex-wise statistical analysis of changes in cortical thickness over 16 weeks. Parametric maps represent the FDR-thresholded t-scores. Grey to white indicates significant changes. Average cortical thickness was also assessed in anatomical ROIs. An example is illustrated for the Cx (insular) (Thickness [mm] ± SEM ± test: **p<0.01; ***p<0.001), PBS-injected mice showed no significant changes in Cx thickness over time and are not represented in this figure.

References

Acknowledgements

We have developed an inducible mouse model of α-synucleinopathy that demonstrates behavioral deficits (olfactory dysfunction and sleep disturbances). This work was kindly provided by Biospective Inc. and the Quebec Consortium for Drug Discovery (CQ3D).