α-SYNUCLEINOPATHY-INDUCED REGIONAL BRAIN ATROPHY ASSOCIATED WITH OLFAC TORY DEFICITS AND SLEEP DISTURBANCES

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Introduction

Parkinson’s disease (PD) is currently diagnosed based on non-imaging and neuropsychiatric disturbances, although non-motor deficits such as cognitive 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 goal of this project was to develop an inducible mouse model of α-synucleinopathy to characterize early neurodegeneration in non-transgenic mice. We have developed an inducible mouse model of α-synucleinopathy with concomitant neuronal loss and astrogliosis for preclinical studies to accelerate the development of disease-modifying treatments for PD.

Methods

AD1/Tg Mice: α-synucleinopathy

- Induced in 8-10 week-old male M3 hemizygous (+/-) mice
- Performed human α-synuclein fibrils (PFFs) (Luk, 2012) were injected into the left Anterior Olfactory Nucleus (AON)
- Injections of Phosphate-Buffered Saline (PBS) were used as a negative control

Behavioral Testing

Olfactory Dysfunction Testing

- Animals were tested for olfactory deficits at 8, 16 and 24 weeks post-surgery (WPS) and 8-16 WPS
- Buried Pellet Test
- Latency to find the pellet (5 min maximum) was measured on four consecutive days (Fleming, 2008)

Sleep Dysfunction Testing

- Animals were tested for sleep dysfunction at 8, 12, and 16 weeks post-surgery
- The PiezoSleep 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 t0) 3D anatomical MRI scans
- Mice underwent follow-up MRI scans at t8, t12, and t16 weeks post-surgery
- All t0 images were processed using Biospec’s fully-automated, production-level, NIGHTWING™ MRI processing platform

Olfactory Nucleus (AON) Volume Measurements

- Mice brain tissue was fixed and embedded in paraffin
- Tissue sections were collected over ~120 levels covering the entire brain
- Stained for pathological phosphoglucomutase-2 (p-Syn) and NeuN for neuronal cell bodies
- Digitized using an AxioScan.Z1 digital whole slide scanner (Carl Zeiss, Canada)
- Semiautomatic quantitation of the stained sections was performed using Biovia’s PERMITS™ software

Astrogliosis

- Significant astroglia in the regions where there is α-synuclein pathology associated with neuronal cell loss
- Quantitative analysis of changes in cortical thickness over 16 weeks


t0 t12 t16

A. Significant olfactory deficits were observed at 16 weeks post-surgery, measured by the latency to find a buried pellet. (Mean ± SEM t-test *p<0.05; **p<0.01)

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<0.05; **p<0.01; ***p<0.001)

C. Concomitant neuronal density assessment indicates a significant loss of neurons in the ipsilateral Piriform Cortex and a tendency towards significance in the Amygdala and Entorhinal Cortex. This observation suggests that the decrease of α-syn load may be associated with neuronal cell loss. (Mean ± SEM t-test *p<0.05; **p<0.01)

D. Changes in sleep variables over time (2-way ANOVA). Significant decreases in sleep parameters were observed for both ipsilateral and contralateral AON. (Mean ± SEM t-test *p<0.05; **p<0.01; ***p<0.001)

E. Significant decrease in volume of ipsilateral olfactory and connected regions

F. Early significant decreases in cortical thickness

References


Ventricle-wise statistical analysis of changes in cortical thickness over 16 weeks. Parametric maps represent the FDR-thresholded t-statistic (gray is not significant). Average cortical thickness was also assessed in anterior/ROIs. An example is illustrated for the Piriform Cortex (PFC) (C). Significant decreases in cortical thickness over time are not represented in this figure.


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