CHARACTERIZATION OF A MODEL OF PARKINSON'S DISEASE USING SYNTHETIC α-SYNUCLEIN INOCULATION TO EVALUATE MOTOR FUNCTION, SYNUCLEINOPATHY, AND DOPAMINERGIC NEUROTRANSMISSION.

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INTRODUCTION

Alpha-synuclein is a presynaptic neuronal protein that is linked to Parkinson's Disease (PD) and contributes to disease pathogenesis. We sought to build on previous research on PD models by examining the pathogenic effects of synthetic α-synuclein proteins injected into the striatum in male C57Bl6/J WT mice at 8 weeks of age. Previously we have shown behavioral deficits using mouse α-synuclein preformed fibrils. However, follow up studies have shown a lack of reliability and reproducibility in these models. The goal of this work was to identify a model that exhibited motor impairment that was stable, consistent, and robust.
Briefly, α-synuclein were inoculated via stereotaxic surgery into unilateral or bilateral mouse striatum. Animals were evaluated using a battery of behavioral tests to assess motor function as well as for markers of α-synuclein and dopaminergic neurotransmission.
Striatal inoculation with α-synuclein preformed fibrils and synthetic oligomers displayed motor and gait deficits at the highest doses as well as decreases in the concentration of dopamine and its metabolites in the striatum. Concluding remarks are pending additional behavioral data analysis and histological readouts. Our goal is to further characterize this model for testing disease modifying therapies for Parkinson's Disease.

METHODS

Animals: A total of 35 (n=6 to 8 per treatment group), eight-week-old gender mixed C57Bl6/J mice were purchased from The Jackson Laboratories (Bar Harbor, ME). Mice were assigned unique identification numbers and single housed in polycarbonate OptiMice cages. All animals were examined, manipulated, and weighed prior to study initiation to ensure adequate health and suitability for the study.

Alpha-synuclein Pre-Formed Fibrils: Mouse alpha-synuclein preformed fibrils (referred to as mPFFs) (2.5 μg/μl) were prepared as per Luk's preparation protocol (Luk et al, Science, 2012).

Synthetic Oligomers from StressMarq: Kinetically stable alpha-synuclein oligomers (SPR-484, StressMarq) are toxic to dopaminergic neurons and induce phosphorylation of alpha-synuclein Ser129, a pathology associated with Parkinson's disease. Oligomers (2 mg/ml) solutions were diluted in PBS immediately prior to experimentation and used the same day they were thawed.

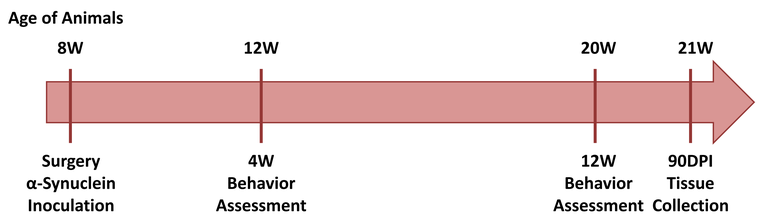
Surgical Method: Mice were anesthetized with isoflurane and stereotactically injected into either the right hemisphere (for unilateral injections - coordinates (anteroposterior: +0.4 mm, mediolateral: ±2.2 mm, dorsoventral: -2.6 mm) or both hemispheres (for bilateral injections) with recombinant mPFF (10.0 μg; 2.5 μg/μl, 4.0 μl total volume per hemisphere) or synthetic oligomers (5 μg or 10 μg, at a dose volume of either 4 μL or 5 μL). Control animals received inoculations with PBS. Injections were performed with a 10 μl syringe (Hamilton, NV) at a rate of 0.1 μl/min with the needle left in place for 5 minutes following administration at each target. All mice received analgesia both pre- and post-operatively. Carprofen (5 mg/kg), subcutaneous (SC) was administered immediately before surgery and Buprenex (0.1 mg/kg, SC) was administered after surgery once the mouse was fully awake. In addition, 1 ml of Ringer's solution was administered (SC) at the conclusion of surgery. Wet feed was provided for post-surgical animals for a period of 3 consecutive days following surgery.

Behavior assessment: At 4 weeks post inoculation, all animals were tested in wire hang, tapered balance beam, and NeuroCube®. The **wire hang test** of motor function was conducted by following a modified protocol described by Santa-Maria et al. Neurobiol. Aging, 2012. Mice were placed on the top of a standard wire cage lid. The lid was lightly shaken to cause the animals to grip the wires and then turned upside down. The latency of mice to fall off the wire grid was measured, and the average values were computed from three trials, with a max trial time of 300 seconds for each trial. Each trial was performed consecutively, with an inter trial interval of 30 seconds. The **tapered balance beam** consisted of a 100 cm in length black acrylic strip tapered from a width of 1.5 cm to 0.5 cm. The beam was angled of 17° from horizontal running from low to high [58 cm from the floor] topped with a goal box. After training, animals were tested 24 hours later. During testing, mice received 3 trials with an intertrial interval (ITI) of 30 seconds. The following measures were captured: the latency to turn [animal turn to face the goal box], latency to traverse [animal traversal of the beam], and the total number of steps and footslips for each paw and expressed as a ratio of the total number of footslips to the total number of steps. **NeuroCube®** is an automated behavioral platform that employs computer vision to detect changes in gait geometry and gait dynamics in rodent models of neurological disorders, pain, and neuropathies and extracts gait and non-gait features (Dave et al. Phenotypic characterization of recessive gene knockout rat models of Parkinson's disease. Neurobiology of Disease, 2014). Mice were placed into the NeuroCube® and were given 5 minutes to move freely inside the apparatus for automated gait measures recording. Digital videos were analyzed through computer assisted segmentation algorithms. Fitted parameters were then used to extract clips of motor behavior that were used to extract information about gait geometry and dynamics. Bioinformatics-driven procedures then guide the discrimination probability between treatment groups to determine behavior phenotypes.

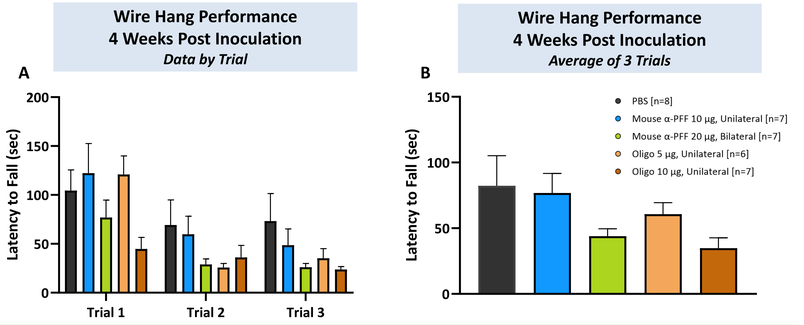
Brain collection: Brain hemispheres were collected according to PGI's standard procedures. Ninety days post inoculation (90DPI), mice were euthanized, and brain tissues were collected. N=3 or 4 animals from each group were collected for immunohistochemistry. Animals were anesthetized with isoflurane and transcardially perfused with PBS until the liver was clear. After perfusion, animals were decapitated and the whole brain will be removed and drop fixed in freshly prepared 4% PFA. N=3 or 4 animals from each group were collected for bioanalysis. Animals were rapidly decapitated and the whole brain will be harvested. The left and right striatum were microdissected, placed in 2.0 mL Eppendorf tubes, and snap frozen in liquid nitrogen.

Bioanalysis: Bioanalysis was carried out on the striatum. The concentrations of dopamine (DA), DOPAC, and HVA were determined using an ultra-high pressure liquid chromatography (UPLC) system equipped with a tandem mass spectrometer (MS/MS) (both from Waters Corp. Milford MA, USA). Striatum samples were processed and then directly injected into a Waters Acquity UPLC system equipped with an YMC™ ODS-AQ™ 2 mm × 100 mm, 3 μm particle column and measured using a Waters Quattro Premier XE triple quadrupole mass spectrometer, operating in the MS/MS mode. Analytes were isolated using a mobile phase consisting of an aqueous component (A: 0.5% formic acid in milliQ water) and an organic component (B: 1% formic acid in acetonitrile) using a 10-minute LC method. Quantification of analytes was achieved by utilization of standard curves and by correction with internal standard of a known concentration.

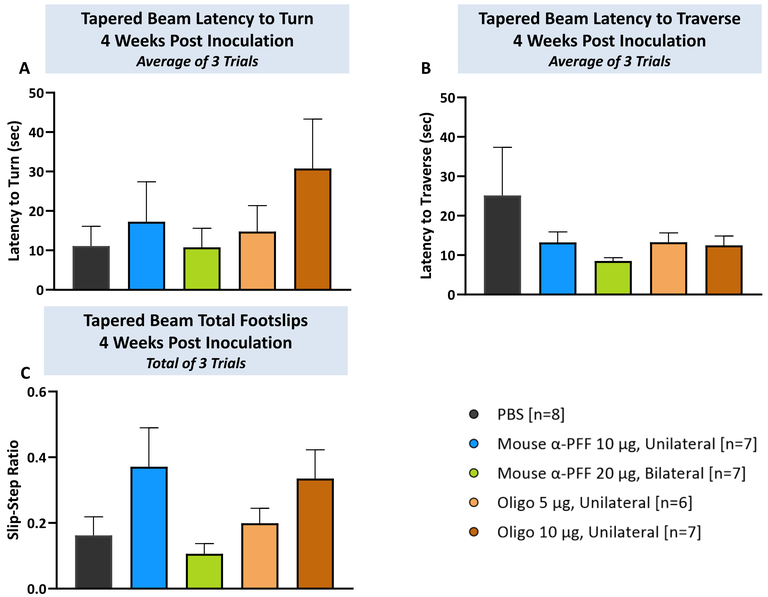
TIMELINE



MOTOR ASSESSMENTS



(A) Wire hang performance by trial 4 weeks post α-synuclein inoculation. The latency of mice to fall off the wire grid was measured across 3 consecutive trials with an inter trial interval of 30 seconds and a max trial time of 300 seconds for each trial. (B) The average wire performance across 3 consecutive trials 4 weeks post α-synuclein inoculation. Trends in the data suggest decreased latency to fall in animals that received either a 20 μg bilateral dose of α-PFF or a 10 μg dose of oligomer, which is suggestive of motor deficits. Data expressed as mean ± sem.

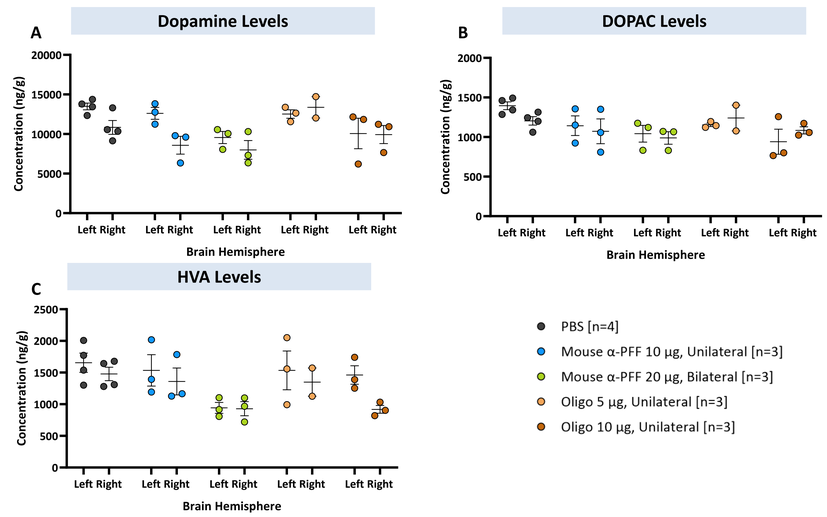


A) Tapered beam average latency to turn on the beam 4 weeks post α-synuclein inoculation. The latency of mice to turn on the beam was measured across 3 consecutive trials with an inter trial interval of 30 seconds and a max turn time of 120 seconds for each trial. (B) Tapered beam average latency to traverse the beam 4 weeks post α-synuclein inoculation. The latency of mice to traverse across the beam was measured across 3 consecutive trials with an inter trial interval of 30 seconds and a max traversal time of 120 seconds for each trial. (C) The total number of footslips measured across all 3 consecutive trials as animals traversed the beam, expressed as a ratio of the total number of steps. Trends in the data suggest increased latency to fall and increased slip-step ratio in animals that received either a 10 μg unilateral dose of α-PFF or a 10 μg dose of oligomer, which is suggestive of motor deficits. Data expressed as mean ± sem.

GAIT ASSESSMENTS

NeuroCube® all features gait analysis assessed at 4 weeks post α-synuclein inoculation. (A) Cloud analysis of PBS treated mice versus mice that received a 10 μg unilateral dose of α-PFF. No significant differences were noted. (B) Cloud analysis of PBS treated mice versus mice that received a 20 μg bilateral dose of α-PFF. No significant differences were noted. (C) Cloud analysis of PBS treated mice versus mice that received a 5 μg unilateral dose of oligomer. No significant differences were noted. (D) Cloud analysis of PBS treated mice versus mice that received a 10 μg unilateral dose of oligomer. Significant differences in gait features were observed.

BIOANALYTICAL DATA



Bioanalytical Data. Tissue samples were collected at 90 days post α-synuclein inoculation. (A) Dopamine levels in the left and right striatum across all treatment groups. Treatment with a 10 μg unilateral dose of α-PFF resulted in a unilateral decrease in dopamine levels while treatment with a 20 μg bilateral dose of α-PFF resulted in a bilateral decrease in dopamine levels. While treatment with a 5 μg unilateral dose of oligomer did not appear to reduce dopamine levels, trends in the data indicated that treatment with a 10 μg unilateral dose of oligomer resulted in decreased dopamine levels in both hemispheres, suggestive of contralateral spreading. (B) DOPAC levels in the left and right striatum across all treatment groups. Trends in the data suggest a bilateral reduction in DOPAC after treatment with either a 20 μg bilateral dose of α-PFF or a 10 μg unilateral dose of oligomer. (C) HVA levels in the left and right striatum across all treatment groups. Trends in the data suggest a bilateral reduction in DOPAC after treatment with either a 20 μg bilateral dose of α-PFF or a 10 μg unilateral dose of oligomer. Data expressed as mean ± sem.

CONCLUSIONS

In summary, we demonstrated that a 10 μg unilateral dose as well as a 20 μg bilateral dose of α-synuclein preformed fibrils resulted in motor deficits as assessed by the tapered balance beam and wire hang assessments at 4 weeks post inoculation. The most robust and reproducible deficits were observed with a 10 μg unilateral dose of synthetic oligomers, which showed motor deficits as assessed by tapered balance beam and wire hang assessments, as well as significant differences in gait as assessed by the NeuroCube®, also at 4 weeks post inoculation. Decreases in the levels of dopamine and its metabolites were observed in the striatum in tissues collected at 90 days post inoculation. Future analysis of the behavioral assessments conducted 12 weeks post inoculation as well as histological readouts of pSer129 and tyrosine hydroxylase from brain tissues collected at 90 days post inoculation will also be conducted. Additionally, future studies with increased power will be used to validate and select the most robust model for testing disease modifying therapies for Parkinson's Disease.