Propagation and Aggregation of Alpha Synuclein in Murine Models

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Introduction

Aggregation of mis-folded proteins is a feature common to many neurodegenerative diseases. Parkinson's Disease is a progressive movement disorder which is characterized neuropathologically by the presence of intraneuronal Lewy Bodies (LB) and Lewy Neurites (LN). Mis-folded and aggregated alpha-synuclein (fibrillar alpha synuclein) is major component of LB's and LN's. Synthetic alpha synuclein fibrils (murine and human) are capable of 'seeding' and propagating alpha synuclein pathology in both alpha synuclein transgenic and non-transgenic (WT) neuronal cultures and mice (Luk, et al., 2012a; Luk, et al., 2012b; Volpicelli-Daley, et al., 2014). Recent studies by Luk et al., 2016 have demonstrated that while both human and mouse synthetic preformed fibrils can 'cross seed' pathology in both and mouse and human substrates respectively (both in-vitro and in-vivo), it is significantly less efficient than homologous seeding. Striatal administration of synthetic murine alpha synuclein PFF's in WT mice has been shown to induce pSyn positive inclusion pathology in the amygdala as early as 14 days post-injection. In contrast, initiation of alpha synculein pathology is significantly delayed when synthetic human alpha-synuclein PFF's are used to 'cross-seed' pathology in WT mice.
However, despite the extensive early alpha-synuclein pathology observed as well as the reduction in striatal dopamine levels witnessed as early as 30 days post injection of synthetic murine PFF injection in WT mice, published accounts of motor deficits do not occur until much later (Luk et al., 2012a, Ramboz et al., 2014). In Luk and colleagues seminal PFF work, despite early pathology, motor deficits were not detected in standard behavioral tests until 90days (wire-hang) or 180days (rotarod) post-injection of murine PFF's. in WT mice.

As a result, we set out to investigate whether the standard battery of behavioral tests we have validated at PsychoGenics for phenotyping murine models of neurodegeneration could be applied to detect motor impairments even in the absence of substantial alpha-synuclein pathology such as that observed when synthetic human alpha synuclein PFF's are used to cross-seed pathology in WT mice.

Methods

Animals:
Male B6C3F1/J mice (8-10 weeks) were purchased from Jackson Laboratories. During the course of the study, 12/12 light/dark cycles will be maintained. The room temperature was maintained between 20 and 23°C with a relative humidity maintained around 50%. Chow and water were provided ad libitum for the duration of the study. Wet chow was placed on the cage floor and was changed daily. The tests were performed during the animal's light cycle phase unless otherwise specified.

Intrastriatal administration of synthetic human alpha-synuclein preformed fibrils (hu a-syn PFF):
Three cohorts of male B6C3F1/J mice were injected in the dorsal neostriatum with either 5µg of synthetic human alpha-synuclein preformed fibrils (hu a-syn PFF; Proteos, Kalamazoo,MI) or sterile PBS as described in Luk et al., 2012a.

Behavioral Assessments:
Three cohorts, with each cohort consisting of a-syn PFF injected, PBS injected and untreated mice (n= 14 per treatment; total n=42 per cohort) underwent a battery of behavioral tests at either, 15 days, 30 days or 90 days post-injection of a-syn PFF's.

TESTING TIMEPOINT	15DPI	30DPI	90DPI
Cohort 1	✓		
Cohort 2		✓	
Cohort 3			✓

OPEN FIELD → GRIP STRENGTH → WIRE HANG → TAPERED BALANCE BEAM → POLE TEST → ROTAROD → NEUROCUBE®

- **Tapered Balance Beam test** consists of a beam angled and elevated from the floor. At the opposite side of the balance beam ('end' portion) there is a goal box which rests on the aforementioned support stand. Following habituation to the testing room, mice are place on the 'starting' end of the balance beam. Mice will receive 2-8 trials per day, with an ITI of at least 60 sec, and will be returned to the home cage between trials. The number of trials and timing will be optimized for each animal model tested on the apparatus, but the maximum number of trials per day will not exceed 8 trials. Latency to traverse the beam (sec), and number of foot slips (left / right; fore / hind) will be recorded. All tests will also be recorded using a video camera for aid in scoring. For longitudinal studies that monitor disease onset and progression, mice could be tested weekly, but will not be tested if they are unable to walk. Testing time points: 6 and 12 weeks of age.
- **Wire Hang:** The four limb hanging test (SOP: DMD_M.2.1.005) from the TREAT-NMD Neuromuscular network was employed. Briefly, mice were placed on top of a steel grid cage lid which was then inverted over a 35cm high circular Plexiglas cylinder. Mice were allowed to grip the steel grid for as long as possible with no maximum testing time cut-off. Mice were given three trials with an ITI of 2-3minutes Testing time point: 10 weeks of age.
- **NeuroCube®** : The Neurocube® system is a platform that employs computer vision to detect changes in gait geometry and gait dynamics. Mice were tested for 5minutes in a rectangular Neurocube® chamber where mice were allowed move freely back and forth through the rectangular walkway. Complex bioinformatics algorithms are employed to subtle phenotypes related to gait.

Statistics Methods: Data was analyzed via multi-factorial analyses of variance (ANOVA) with a Tukey Post-Hoc.

Summary

Challenging motor tasks, like the tapered balance beam and Pole test [data not shown], and static muscle strength tasks like Wire hang, reveal early deficits in co-ordination in synthetic human alpha synuclein PFF's striatal injected WT mice.

Gait assessment revealed a progressive decrease in stride and swing duration and a forelimb paw placement impairment 90 days post-human synthetic PFF's striatal injection.

- ✓ Normal ambulatory activity was measured in Open Field or tensile muscle strength in a non-challenging test such as Grip Strength analysis.
- ✓ Due to the inherent variability in animal performance in the Wire-Hang and Pole Test assays, larger group sizes are required to generate statistical significance.

Reference:

Luk et al., Science.2012a Nov 16;338(6109):949-53; Luk et al., The Journal of Experimental Medicine. 2012b May 7;209(5):975-86; Luk et al., Cell Reports . 2016. Sept 20; 16:3373-87; Ramboz et al., 44th Annual Meeting SFN. 2014 Nov 15-19; Volpicelli-Daley et al., Nature Reports. 2014 Sept; 9(9):2135-46.

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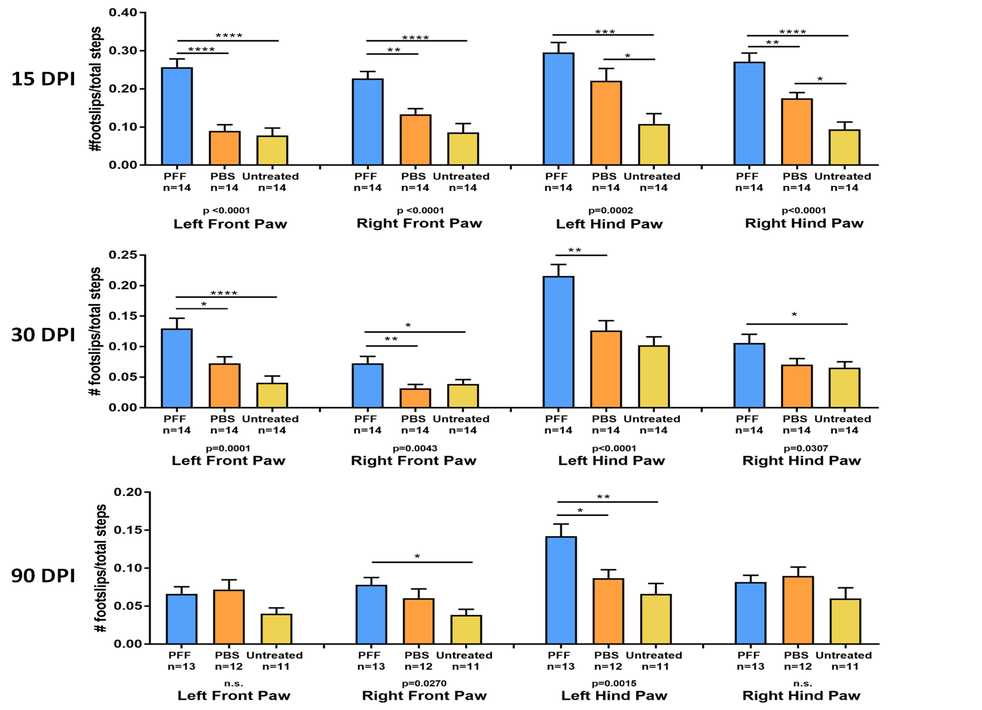
Tapered Balance Beam

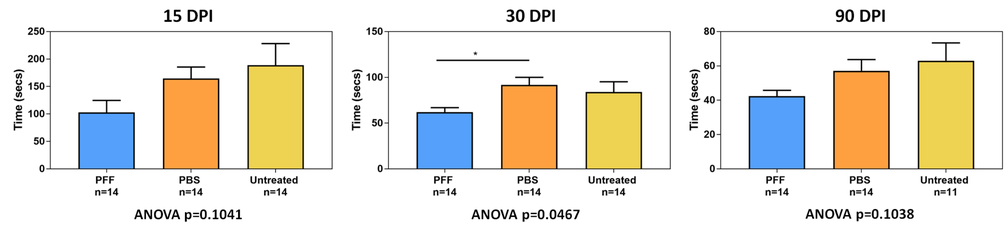
Coordination Deficits are Witnessed as Early as 15 days Post-injection of Synthetic Human PFF's Utilizing the Tapered Balance Beam Assay.



As expected from an injection of synthetic human PFF's in the right dorsal neostriatum the most significant deficits in foot-slips are witnessed in the contralateral hindlimb – the Left Hind Paw

Wire Hang

Reduction in Average Hang-Time is Witnessed in Mice Injected with Synthetic Human PFF's 30 days Post-Injection



NeuroCube®

NeuroCube® Analysis Reveals a Progressive Gait Impairments in Stride and Swing Duration and in Forelimb Paw Placement Develop 90days Post-Injection of Synthetic Human PFF's