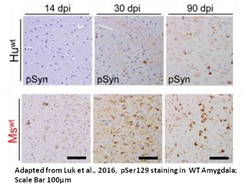
Propagation and Aggregation of Alpha Synuclein in Murine Models

K.R. Walker, W. Arias, M. Berthiaume, P. Calafati, N. Chirichella, K. Cox, M. Hall, S. Nunez, R. Springer, K. Cirillo, D. Hyde, M. Bansal and S. Ramboz

PsychoGenics Inc., Tarrytown, NY, USA

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 that homologous seeding.



Adapted from Luk et al., 2016, pSer129 staining in WT Amygdala; Scale Bar 100µm

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.

➢ ***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 ANALYSES

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®

➢ ***Open Field:*** Locomotor activity was measured over a 30 minute interval in a Plexiglas square chamber (27.3 x 27.3 x 20.3cm with 16 x 16 x 16 infrared photobeam sources (Med Associates Inc., St Albans, VT). Horizontal activity (distance traveled) and Vertical activity (rearing) were measured by consecutive beam breaks.

➢ ***Grip Strength:*** Forelimb and Hindlimb grip strength were measured using Chatillon DFE digital force gauges attached to 8x8cm square mesh grips (hindlimb mesh grip was angled to 45˚). Mice underwent five trials with an inter-trial interval of 7-20secs.

➢ ***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.

➢ ***Tapered Balance Beam:*** The tapered balance beam test consisted of a training session (five trials) followed 24hrs later by a testing session (3 trials with an inter-trial interval of 2-3minutes). The tapered balance beam consisted of a tapered angled beam elevated from the floor with a goal box located at the steepest end. Latency to turn as well as the time taken to traverse the beam were measured (mice were given a maximum time of 120secs to turn). Video recordings of each mouses' three test session traversals were later manually scored for foot-slips. Mice were required to successfully complete a minimum of two beam traversals in their testing session in order to be scored for number of foot-slips. Number of foot-slips for each limb was normalized to total steps (# of foot-slips/total steps).

➢ ***Pole Test:*** Testing apparatus consisted of a 50cm high roughened pole (0.8cm diameter) inserted in a steel base. The pole test consisted of five trials on training day (ITI 1 minute) and 24hrs later five trials on testing day (ITI 1 minute). Each trial consisted of placing the mouse at the top of the pole with their head facing up, the time taken for the mouse to turn around and orientate themselves with their head facing down (turn latency) and the time taken to descend the pole (descent latency) were measured. Mice were given a maximum of 120secs to complete the task, if they failed to descend during this time they were removed from the pole. Each trial was video recorded for later scoring of turn and descent latencies and errors. As an additional output measure the turn and descent latencies were summed to give the $Total_T$. In cases where upon pole placement the mouse fell immediately from the pole or slid down the pole this was recorded as an error and the time taken to reach the bottom of the pole was included in the turn latency. In trials where mice were unable to complete the pole descent without sustained lateral movement down more than 50% of the pole were also recorded as an error.

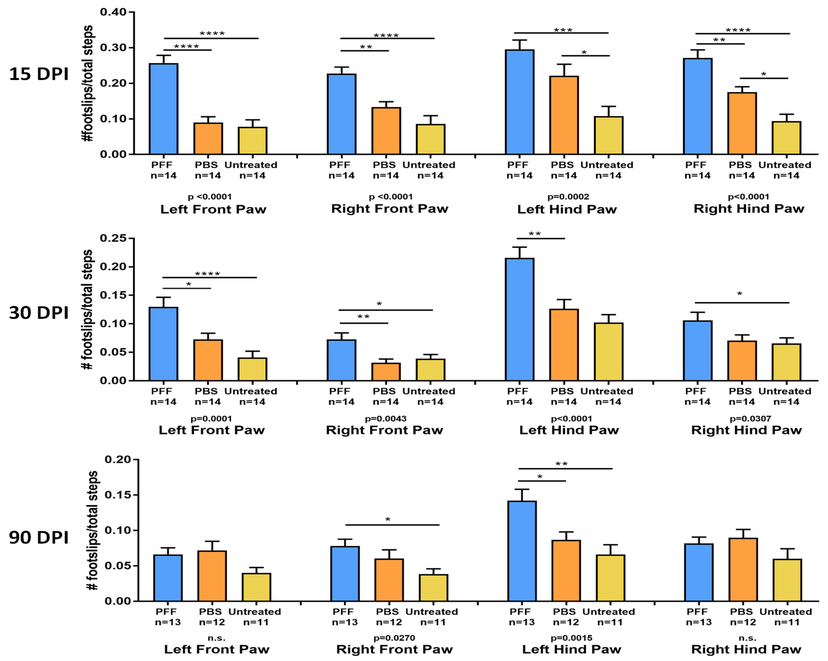
➢ ***Rotarod:*** Mice were given four trials per day (one training trial followed by three testing trials) for a total of three days. Training consisted of a 5 minute trial at a continuous slow speed of 4rpm on the rotarod (Rotamex, Columbus, OH). Testing was carried out at a 4-40rpm acceleration over 5 minutes with an inter-trial interval of 30minutes. The time at which the mouse fell from the rotarod was recorded. Latency to fall (seconds) and speed (rpm) at which the mouse fell was reported.

➢ ***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.

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

RESULTS

Figure 1: Coordination Deficits are Witnessed as Early as 15 days Post-injection of Synthetic Human PFF's Utilizing the Tapered Balance Beam Assay.



➢ **No differences in turn latency or traverse latency where detected at any time-point.**

➢ **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**

Figure 2: Mice Injected with Synthetic Human PFF's Demonstrate Impaired Turn Latency and Commit more Errors During the Pole Test 15days Post-Injection

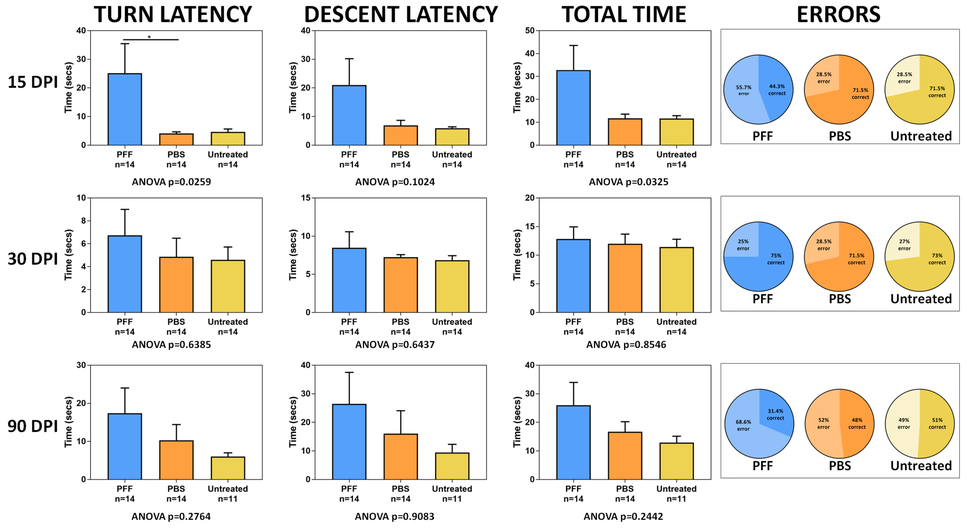


Figure 3: A Reduction in Average Hang-Time is Witnessed in Mice Injected with Synthetic Human PFF's 30 days Post-Injection

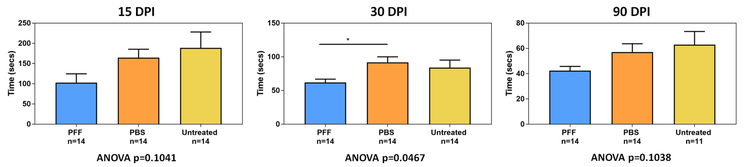
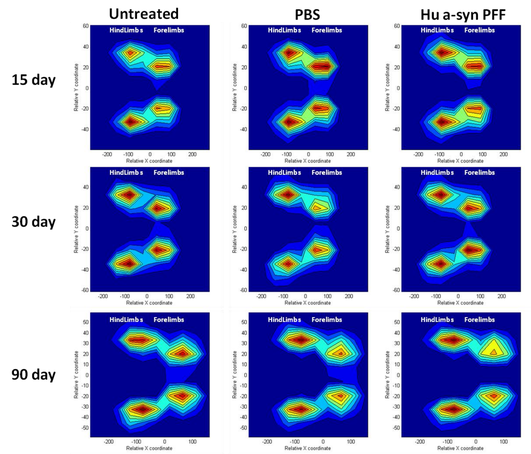


Figure 4: Neurocube® Analysis Reveals Impairments in Correct Forelimb Paw Placement Develop 90days Post-Injection of Synthetic Human PFF's



DISCUSSION

Challenging Motor Tasks Reveal Early Deficits in Co-ordination and Static Muscle Strength Post-Injection of Human Synthetic PFF's in WT Mice.

- ✓ Injections of synthetic human alpha synuclein PFF's do not impair normal ambulatory activity as measured in Open Field or tensile muscle strength in a non-challenging test such as Grip Strength analysis.
- ✓ Injections of synthetic human alpha synuclein PFF's did not induce deficits in gross motor function at early time-points post-injection as measured by Rotarod.
- ✓ While challenging, 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.
- ✓ The tapered balance beam is a challenging motor task with low inter animal variability that can detect co-ordination deficits very early post-injection of human synthetic PFF's.
- ✓ The Neurocube® Platform is able to detect impairments in forelimb paw placement beginning at 90 days post-injection of human synthetic PFF's in WT mice.

WORK WITH THIS MODEL IS ONGOING, VISIT US AT THE ALZHEIMER'S AND PARKINSON'S DISEASE CONGRESS IN VIENNA 2017 FOR FURTHER UPDATES.

ACKNOWLEDGEMENTS

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