Complementary Phenotypic Characterization of a Genetically Modified Animal Model of Parkinson's Disease: Line 61.

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Introduction

Numerous genetic and pharmacological animal models have been generated to mimic different aspects of Parkinson's Disease, a neurodegenerative disorder estimated to affect more than 1% of the over-65 population. Parkinson's Disease is associated with the loss of nigral dopaminergic cells leading to a decline in dopamine levels. Due to this observation, depletion of nigral dopaminergic cells using lesion models have been developed and used to investigate the basis of symptomatic treatment.

In the last couple of years, PsychoGenics has established and validated MPTP and 6OHDA lesions models which are broadly used to assess neuroprotective drug treatments. Like any model, the lesions have a down side, provoking a very rapid loss of dopaminergic neurons, which would have taken decades in human patients. The slow cell loss and dopamine depletion witnessed in human sufferers would allow some compensatory mechanisms to occur, which may be absent in lesion models..

Recently, PsychoGenics licensed a well described and validated genetically modified a-synuclein mouse line from Prof. Masliah's laboratory at UCSD: **Line 61** (Rockenstein et al., 2002). This line is an a-synuclein transgenic mouse model expressing the human a-synuclein cDNA under the murine Thy-1 promoter . Line 61 presents most of the characteristics of parkinsonism symptoms, including lack of coordination at 4 months, cognition deficit at 4.5 months, increased total activity in open field by 7 months, hypolocomotion by 14 months and presence of a-synuclein positive aggregates histopathologically. Accumulation of phosphorylated Serine 129 residues in the striatum and substantia nigra which might modulate the formation of protein aggregation likes inclusion bodies and fibrils is evident by 9 months of age in this model (Chesselet et la., 2012).

We are assessing the line 61 animal model using PsychoGenics proprietary technologies (SmartCube®, PhenoCube® and NeuroCube®) to detect early onset phenotype and establish high throughput preclinical readouts (Alexandrov et al., 2015).

Methods

Animals:
Breeding pairs were acquired from Masliah's laboratory from UCSD, California. To generate experimental offspring, female Thy-1 alpha synuclein (Thy-1 asyn) mice (line 61) were bred to male C57DBA wild type mice. Genotyping was performed as per Rockenstein's protocol at PsychoGenics. Offspring were assigned unique identification numbers (ear notch), implanted with unique RFID chips (DataMars) and housed in polycarbonate cages with filter tops in groups of 7-8 animals. All animals were examined, manipulated and weighed prior to initiation of the study to assure adequate health and suitability and to minimize non-specific stress associated with manipulation.
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.

Stat Methods:
A simple student's *t*-test (unpaired) was used for two group comparisons, while a 2-way RM ANOVA was used for two group comparison with repeated measures.

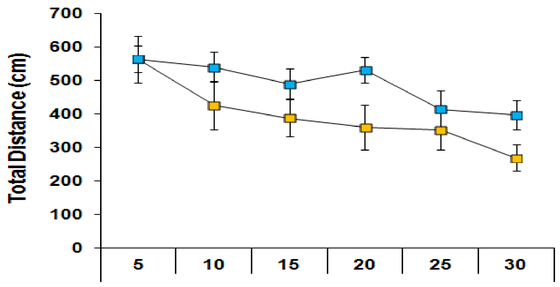
Behavioral Assessments

- PhenoCube ® (PC): PhenoCube ® is a proprietary PsychoGenics technology and a high-throughput platform that assesses circadian, cognitive, social and motor behavior exhibited by group-housed mice. Experiments are conducted using modified Intellicage® units (New Behavior AG), each with a camera mounted on top of the cage for computer vision analysis. IntelliCages have 4 corners with small doors that contain antennas to pick up the ID from the electronic chips. Inside the corners, two small gates give access to water bottles and allow measurement of nosepoking and cognitive performance.
- NeuroCube ® (NC): NeuroCube ® is also a proprietary PsychoGenics technology which is used to gather information on the gait of mice or rats. Animals were tested for 4 minute sessions. NC uses computer vision to capture and score the gait, paw pressure, and motor coordination.
- Open Field (OF): The automated open field test was used to measure the locomotor activity (distance traveled) and rearing frequency of mice. Activity chambers (Med Associates, St. Albans, VT; 27 x 27 x 20.3 cm) were equipped with infrared beams. Mice were placed in the center of the chambers which were then covered by a transparent acrylic lid. Mouse activity was recorded for 30 min under normal conditions of lighting (300-500 lux).
- Elevated Plus Maze (EPM): Elevated Plus Maze assess anxiety-like behaviors. The maze (Hamilton Kinder) consists of two closed arms (14.5cm h x 5cm w x 35cm l) and two open arms (6cm w x 35cm l) forming a cross, with a square center platform (6 x 6 cm) and elevated of 56cm above the floor. All visible surfaces are made of black acrylic. Animals were allowed to acclimate to the experimental room at least 1 hr before the test. Mice were placed in the center of the elevated plus maze facing the open arm for a 5-min run.
- Tapered beam test: The 100cm long beam is broad at the start point (1.5cm) and narrow at the end point (0.5cm) with a 0.5cm ledge positioned 2 inches below the top of the beam. The beam is set at an angle of 17°. At the end of the beam, mice will reach a black plexiglass goal box with a lid used as a refuge after mice traverse the full length of the beam. After 5 minute acclimation, the mouse was placed on the beam close to the goal box and trained to return to the goal box. Following the training day, mice then underwent one test day consisting of 3 trials (2 minutes maximum) separated by a 30s ITI. All trials were live scored and also videotaped for more detailed scoring.

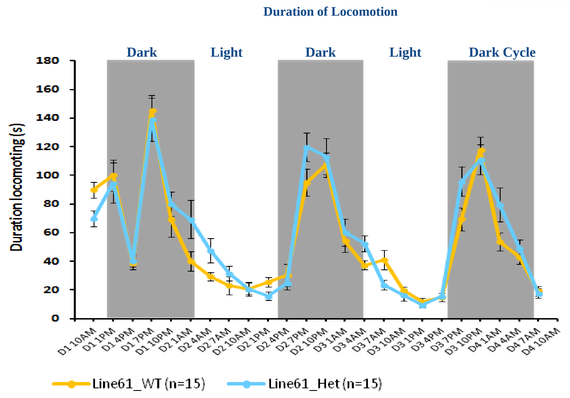
References
Rockenstein, E. et al. Differential neuropathological alterations in transgenic mice expressing alpha-synuclein from the platelet-derived growth factor and Thy-1 promoters. *Journal of neuroscience research* **68**, 568-578 (2002).
Alexandrov, V., Brunner, D., Hanania, T. & Leahy, E. Reprint of: Highthroughtput analysis of behavior for drug discovery. *European journal of pharmacology* **753**, 127-134 (2015).
Chesselet, M.F. et al. A progressive mouse model of Parkinson's disease: the Thy1-aSyn ("Line 61") mice. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics* **9**, 297-314 (2012).

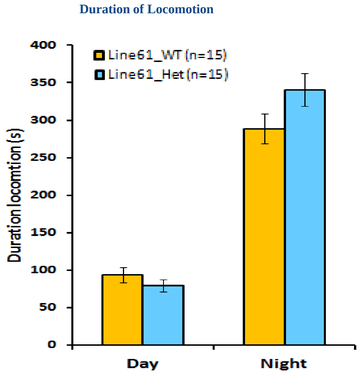
No Difference in Horizontal Activity by 11 Weeks

Open Field



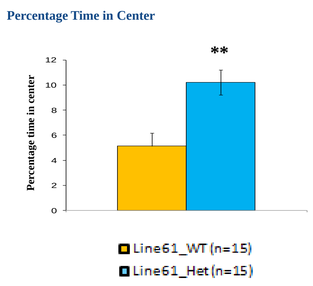
PhenoCube ®



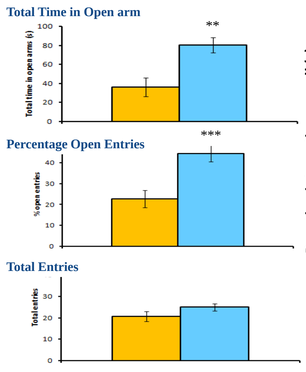


Increase in Risk Taking Behavior by 11 Weeks

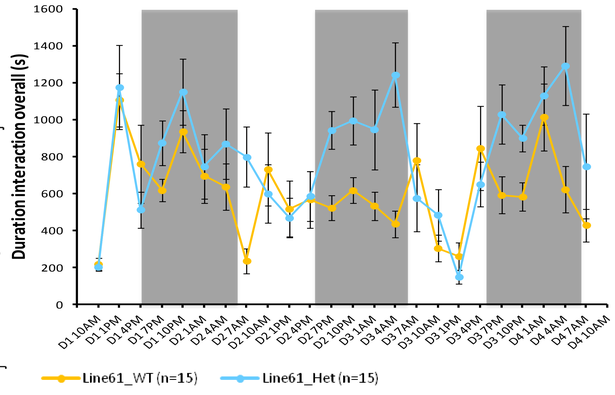
Open Field

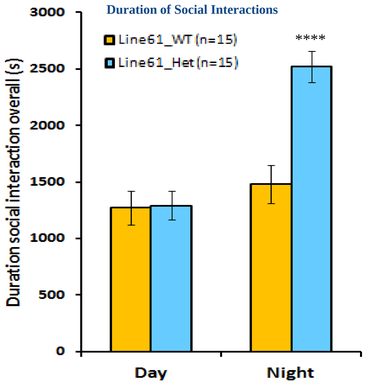


EPM



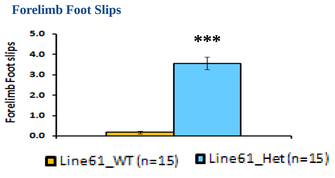
PhenoCube ®

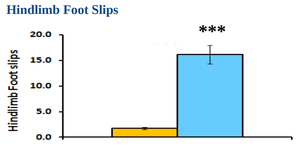




Increase in Motor Impairment by 11 Weeks

Tapered Beam





NeuroCube®

	7 weeks	11 weeks
Overall	87% (p<0.001)	99% (p<0.0001)
Gait	62% (p>0.24)	99% (p<0.0001)
Paw Positioning	89% (p<0.0001)	95% (p<0.0001)

Summary - Discussion

As an early stage of our longitudinal phenotypic characterization, we were able to reproduce much of the published data like lack of motor coordination; however in contrast to others we were able to detect a phenotype at a much earlier time point (~2 months of age) via increased foot slips in tapered beam and high discrimination in overall gait measures and paw positioning in NeuroCube®.

Interestingly line 61 animals display more social interactions during the dark cycle in PhenoCube® which could be interpretated as an increase in risk taking behavior. This finding correlates with the increased time Line 61 HET mice spent in the center of the Open Field and in the Open Arm of the EPM compared to WT animals.

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