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, PsycoGenics has established and validated MPTP and 6-GNDA 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, PsycoGenics licensed a well described and validated genetically modified a-synuclein mouse line from Prof. Masliah’s laboratory at UCSD. Line 61 (Rochstein 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 might modulate the formation of protein aggregation like inclusion bodies and fibrils is evident by 9 months of age in this model (Chesselet et al., 2012).

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

Methods
Animals:
Strain pairs were acquired from Masliah’s laboratory from UCSD, California. To generate experimental offspring, female Thy-1 a-synuclein (Thy-1 a-syn) mice (line 61) were bred to male C57DBA wild type mice. Genotyping was performed as per Rockefeller’s protocol at PsycoGenics. Offspring were assigned unique identification numbers per day. All animals were marked, acclimated and housed in polycarbonate cages with filter tops of 6-8 animals. All animals were examined, manipulated and scored prior to initiation of the study to ensure adequate health and suitability of the study as well as minimize non-specific stress associated with manipulations.

During the course of the study, 12/12 light-dark cycles will be maintained. The room temperature was maintained between 20 and 25°C with a relative humidity maintained around 50%. Chow and water were provided ad libitum for the duration of the study. Mice were housed on the cage floor and were changed daily. The tests were performed during the animals’ light phase cycle unless otherwise specified.

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

Behavioral Assessments
- **PhenoCube®** is a proprietary PsycoGenics technology and a high-throughput platform that assesses multiple psychiatric, social and motor behavior exhibited by group-housed mice. Experiments are conducted using open and closed chambers, and pharmacological exposure for time-limited exposure periods. Digital behavioral analysis. IntelliCages have 4 corners with small doors that contain animals to push up the ID from the electronic chips. Inside the corners, two small gates give access to water bottles and allow maintenance of tail-cuff and cognitive performance.

- NeuroCube® is also a proprietary PsycoGenics 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 mice’s, gait, pressure and motor coordination.

Open Field:
The automated open field test was used to measure the locomotor activity (distance travelled) and rearing frequency of mice. Activity chambers (Med Associates, St. Albans, VT: 27 x 27 x 20.3 cm) were equipped with infrared photodetectors. 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 (500-1000 lux).

- Elevated Plus Maze (EPM): Elevated Plus Maze assesses anxiety-like behaviors. The maze (Hamilton Kinder) consists of four open arms (100 cm long and 15 cm wide) and two open arms arms a 25 cm high forming a cross, with a square platform (6 x 6 cm) and elevated of 50 cm 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 100 cm long beam is located at the start point (1.0 cm) and narrows to the end point (0.5 cm) with a 0.5 cm wide beam 2 inches below the top of the beam. The beam is set at an angle of 15°. At the end of the beam, nose will reach a black polystyrene goal box with 2 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 morning day, mice then undergo one test day consisting of 2 trials (3 minutes minimum) separated by a 30 min. A total of five trials were conducted and videotaped for more detailed scoring.

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®.

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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