A Behavioral Assessment of the Line 61 Mouse Model of Parkinson's Disease Employing the NeuroCube® System of Movement Analysis

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Abstract

Parkinson's Disease (PD) is a neurodegenerative disorder characterized by dopaminergic neuronal loss in the substantia nigra with accumulation of α synuclein containing Lewy bodies. A number of rodent models of PD have been created to recapitulate different aspects of the disease, among these the Line 61 animal, overexpressing the human wild-type alpha-synuclein driven by the murine Thy-1 promoter, in particular has been used extensively to model α synuclein pathology [Rockenstein et al, 2002]. While previous literature has characterized the male Line 61 mice and described a gradual disease progression, female counterparts of this model have been less well studied due to the linkage of the mutation to the X chromosome. In the present study, we performed a number of behavioral assessments in the female Line 61 including wire hang and tapered beam. We also employed a proprietary NeuroCube® assessment to evaluate quantifiable, subtle changes in patterned movement in these animals. This system records and calculates several aspects of ambulation and compares them to detect emerging movement impairments that may otherwise go unnoticed. Results suggest that a mild motor phenotype is present in the female Line 61 compared to both male Line 61 as well as WT littermate counterparts. Implications of these findings, as well as use of the NeuroCube® assessment as a tool in the evaluation of rodent models of disease, are discussed.

Methods

***Subjects*:** Heterozygous (HET) female Thy-1 alpha synuclein (Thy-1 asyn) mice (Line 61) were bred to male C57DBA wild type (WT) mice at PsychoGenics. The offspring were genotyped, and HET and WT animals were enrolled in the present study. Offspring received unique identification numbers, were examined, manipulated and weighed prior to study initiation to ensure adequate health and suitability. Animals were divided into 4 experimental groups comprised of WT and HET animals of both sexes (N=16 WT male, N =12 Het Male, N=5 WT female, N=15 Het female). During the course of the study, 12 hr / 12 hr light/dark cycles and a room temperature of 20 to 23°C were maintained with a relative humidity maintained around 50%. All mice were housed in an enriched environment. Food (#5001) and water were provided *ad libitum* for the duration of the study. Animals were body weighed daily and checked for survival twice per day. All assessments were performed during the animals' light cycle phase.

Tapered Beam Test (TBT): The balance beam test proves to be a sensitive measure of motor impairment and is capable of detecting motor deficits early and even when other measures of motor function, such as the rotarod, failed to show any deficits (Heng et al., 2007; Brooks et al., 2011). The balance beam consists of a strip of smooth black acrylic 100 cm in length, with a square cross section that tapers from a width of 1.5cm to 0.5 cm. The beam also consists of a 0.5cm safety ledge located 2cm below the beam. The ledge maintains a constant width of 0.5cm as the beam tapers. The angle of the beam is 17° from horizontal running from low to high. The highest point of the beam is approximately 58cm 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. The goal box is constructed from black acrylic, measuring 10.5cm3 and containing a 3cm² entrance hole. Mice received 3 trials of testing with an inter trial interval (ITI) of at least 30 seconds. Mice were placed on the bottom of the beam, facing away from the goal box. The time from placement on the beam to turning to face the goal box is recorded as the latency to turn. The maximum amount of time an animal will have to complete the turn is 120 seconds. If the animal is unable or refuses to turn after 120 seconds, it will be positioned on the beam facing the goal box for the next phase of the experiment. Once the animal is facing the goal box, the latency to traverse the beam is recorded. The maximum amount of time an animal will have to complete the traversal is 120 seconds. During the beam traversal, the number of foot slips will be recorded.

Wire Hang: The wire hang test of motor function was conducted by following a modified protocol described by Santa-Maria et al. Neurobiol. Aging, 2012. Mice received 3 trials of testing with an inter trial interval (ITI) of at least 2 minutes. Mice were placed on the top of a wire grid and were gently pulled backwards by the base of the tail until the animal firmly grasped the grid. The grid was then immediately turned upside down. The latency of the mice to fall off the wire grid was recorded. Trials were stopped if the mouse remained on the lid after 10 minutes.

Neuro*Cube*®: 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 are 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. This paradigms is classified as non-invasive for the animal and can be repeated for longitudinal experimental studies.

Summary

- Line 61 mouse model of PD were compared to WT on a battery of behavioral tasks to assess extent of motor deficits in these animals.
- Line 61 show a turning deficit in both genders on the TBT task at 12 weeks of age compared to WT.
- Line 61 show limb weakness as exhibited by decreased hang time on the wire hang compared to WT in both genders.
- Although there was a turning deficits compared to WT, 61SNCA did not show differences in traversal time at 6 or 12 weeks of age on the tapered beam.
- The NeuroCube® assessing gait features shows that deficits in Line 61 HET are more pronounced in male vs. female animals at 9 weeks of age.

Acknowledgments: We would like to thanks our PsychoGenics colleagues who participated in conducting and in the analysis of this study.

11 Week Old Male and Female Line 61 HET show impaired limb strength and endurance on the wire hang task

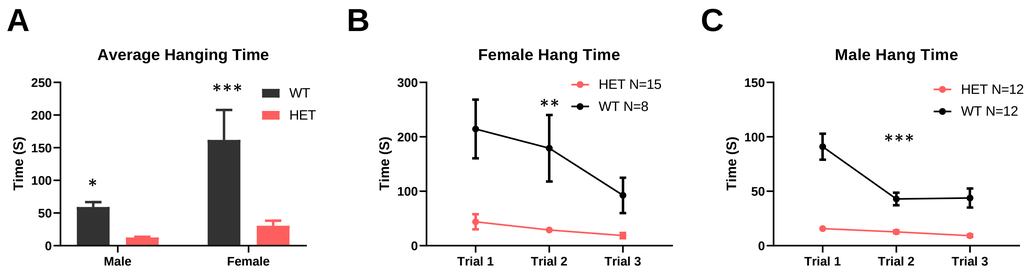

Figure 1. Three Wire Hang trials were conducted in age-matched Line 61 Het and WT littermate mice. In both male and female mice, (A) WT animals spend longer hanging on than their HET counterparts, regardless of gender, on average (F (1, 43) = 27.72, p < 0.0001, male: t(43)=2.02, p<0.05, female: t(43)=5.31, p<0.0001). In addition, through 3 trials, both (B) female (F (1, 21) = 14.57, p < 0.001) and (C) male (F (1, 22) = 38.58, p < 0.0001) WT hang on longer than age-matched Het. Values presented as mean ± one standard error of the mean (SEM). * = p<0.05, ** = p<0.001, *** = p<0.0001

Line 61 HET show turning deficits on the tapered beam task

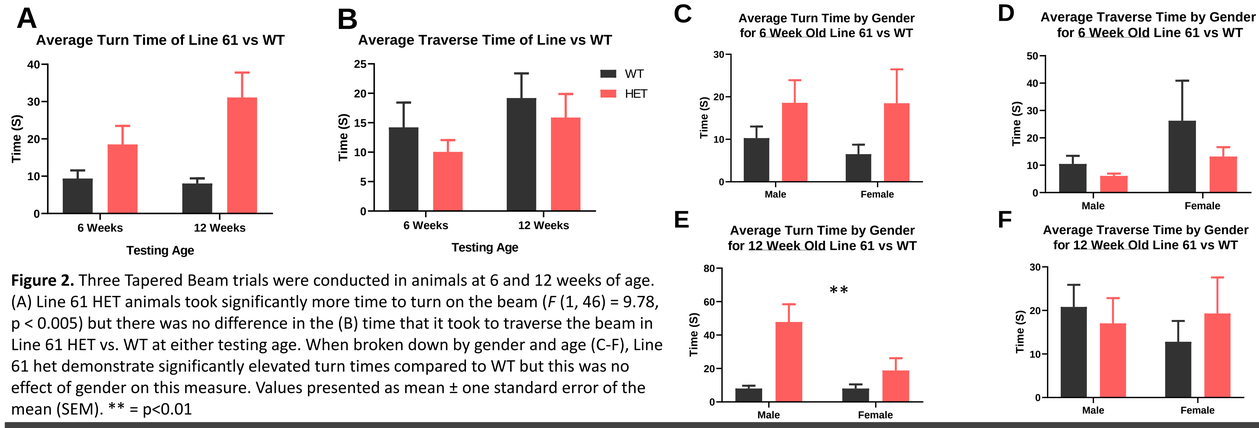

Figure 2. Three Tapered Beam trials were conducted in animals at 6 and 12 weeks of age. (A) Line 61 HET animals took significantly more time to turn on the beam (F (1, 46) = 9.78, p < 0.005) but there was no difference in the (B) time that it took to traverse the beam in Line 61 HET vs. WT at either testing age. When broken down by gender and age (C-F), Line 61 het demonstrate significantly elevated turn times compared to WT but this was no effect of gender on this measure. Values presented as mean ± one standard error of the mean (SEM). ** = p<0.01

Significant discrimination values for gait features in line61 males only.

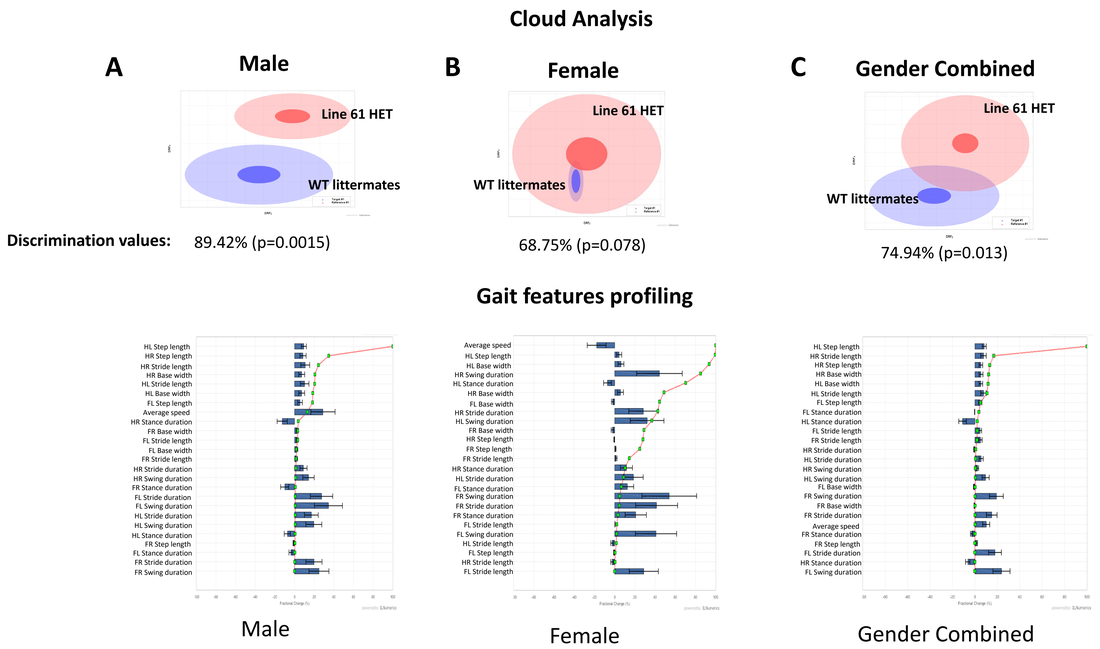

Figure 3. NeuroCube® gait analysis was conducted in Line 61 HET and littermate WT. Cloud analysis comparing Line 61 HET and littermate WT including all features in (A) male only, (B) female only and (C) gender combined. While this analysis is non-significant in the female group, NeuroCube® demonstrates an 89.42% discrimination between male Line 61 HET and WT. This effect in the male animals drives the difference in the gender combined group. Bar graph Y-axis legend: H: Hindlimb; F: Forelimb; R: right; L: left.