Behavioral and Gastro-Intestinal Findings in A53T α-synuclein Mouse Model of Parkinson's Disease After LPS Exposure

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BACKGROUND

Multiple commonly used transgenic animal lines exist for Parkinson's Disease (PD), with various approximations of an ideal Parkinsonian phenotype. However, success in creating a genetically altered animal line that shows a PD-like phenotype with robust and stable behavioral motor impairment, as well as pathological tissue changes affecting the dopaminergic system, has proven to be a challenge. Also, many of the models do not express non-motor changes associated with PD, or they have not been investigated thoroughly. However, there is evidence that PD also affects various non-motor related changes in clinical disease, including disturbances in colon motility and fecal output. Targeted over-expression of human α-synuclein in a mouse model shows only a mild PD phenotype, even at 12 months of age. Previous studies have also shown that inflammatory stimulus in A53T transgenic mouse line induces gastrointestinal (GI) changes. Therefore, we attempted to approach the disease model by using additional LPS challenge to enhance disease progression which we assessed by behavioral tests and GI functionality..

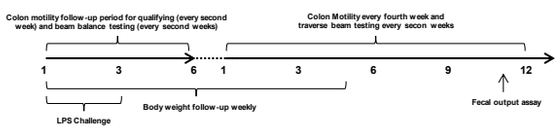
MATERIALS & METHODS

Animals: All animal experiments were carried out according to the National Institute of Health (NIH) guidelines for the care and use of laboratory animals, and approved by the National Animal Experiment Board, Finland. 83 α-synuclein transgenic mice (male and female, homozygous (HOMO A53T) Tg (Prnp-SNCA*A53T) 83 Vle / Tg (Prnp-SNCA*A53T) 83Vle, stock 004479, JAX) and 12 corresponding wild type (WT) mice (male and female) at age of 10 months were used for the experiment. Animals were housed at a standard temperature (22 ± 1°C) and in a light-controlled environment (lights on from 7 am to 8 pm) with *ad libitum* access to food and water.

Experimental groups were:

1. WT mice treated with saline (QW, i.p., 5 ml/kg) on weeks 1-3
2. WT mice treated with LPS 3 mg/kg (QW, i.p., 5 ml/kg) on weeks 1-3
3. HOMO A53T mice treated with saline (QW, i.p., 5 ml/kg) on weeks 1-3
4. HOMO A53T mice treated with LPS mg/kg (QW, i.p., 5 ml/kg) on weeks 1-3

Study outline:



Body weight of the mice was recorded once a week during the study and over all health status was monitored. Mice were divided into treatments groups as they recorded 10% increase in extrusion time (colon motility test results). After qualifying from colon motility, mice were followed up for 12 weeks of additional testing. Colon motility, traverse beam, and beam balance tests were conducted every second week and fecal output assay was performed once at 11th week of follow-up period.

Colon motility: Colonic motility was assessed by measuring time to extrusion of a glass bead inserted into the colon. A single bead (2 mm) was inserted 2 cm into the distal colon of WT and HOMO A53T mice fasted 12 h prior to experimentation. Extrusion time was determined for each mouse.

Fecal output: Each mouse was placed in a separate beaker and observed throughout the 60 min collection period. The stool water content was calculated from the difference between the wet and dry stool weights.

Beam balance: Balance was determined by measuring the time (max 120 seconds) that the mouse was able to stay on a round wood beam. Three beams of decreasing diameter were used (20 mm, 10 mm, 8 mm).

Traverse beam: Motor coordination and balance in mice were tested using a traverse beam test. The beam is wooden and tapers from 1.2cm to 6mm over 1.2 meters. The beam is beveled at the top and that forces the mouse to hang tight to the sides. The dark box placed at the end of the beam acts as a stimulus for the mice to escape the lighted room. Before the first test trial, the mouse was placed in the black box and was allowed to stay in the black box for 1 min. Five consecutive trials were recorded on each testing day. Traverse time was recorded (max 120 seconds).

RESULTS

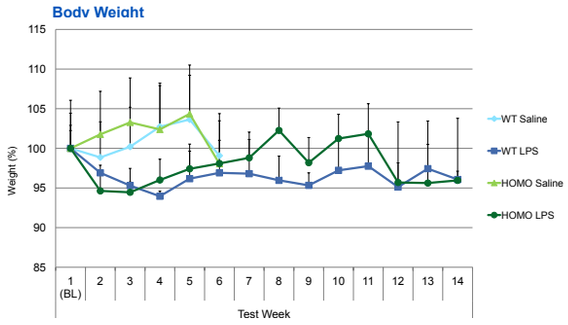


Figure 1. Body weight over time relative to baseline. Data represented as mean ± SEM

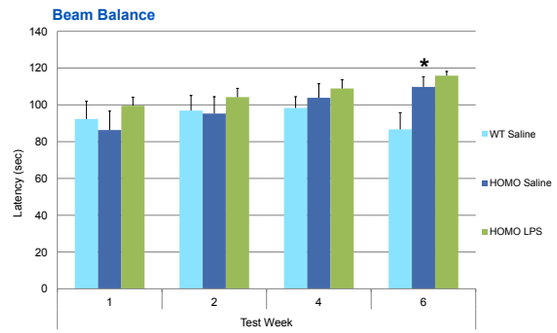


Figure 2. Beam balance test. No significant changes between saline and LPS treated mice. Data represented as mean ± SEM (*<0.05, t-test).

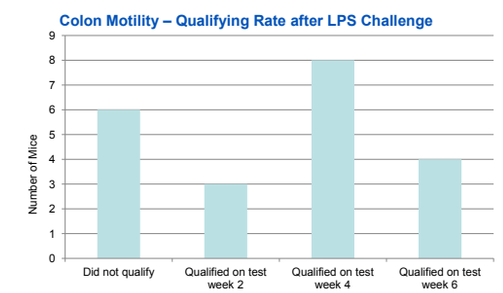


Figure 3. Qualifying rate of LPS treated HOMO mice based on colon motility performance on test weeks 1-6. Qualifying rate of LPS treated HOMO mice was 71% on test weeks 2-6.

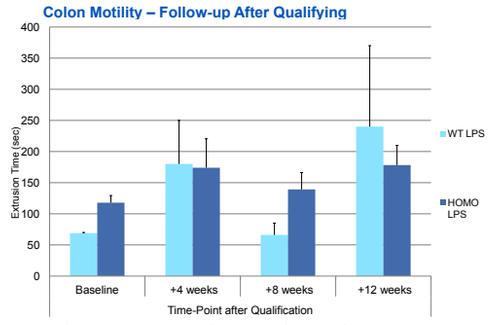


Figure 4. Colon motility testing follow-up after qualifying. Data represented as mean ± SEM.

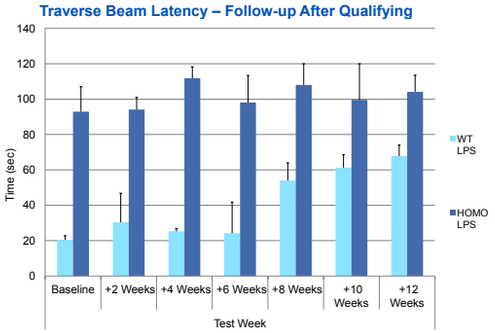


Figure 5. Traverse beam testing follow-up after qualifying. Data represented as mean ± SEM.

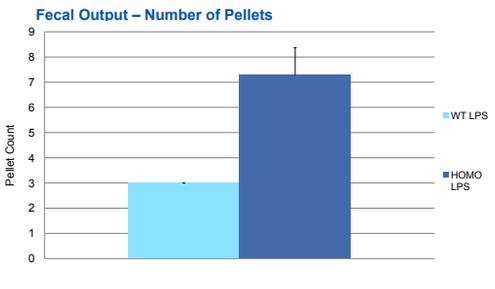


Figure 6. Fecal output – number of pellets. Data represented as mean ± SEM.

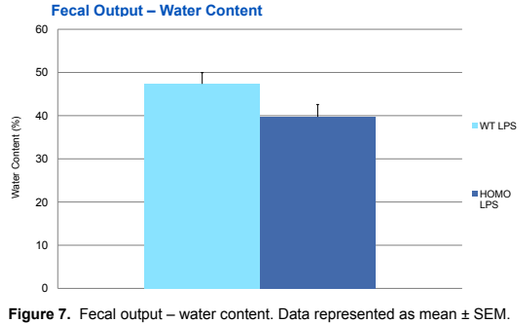


Figure 7. Fecal output – water content. Data represented as mean ± SEM.

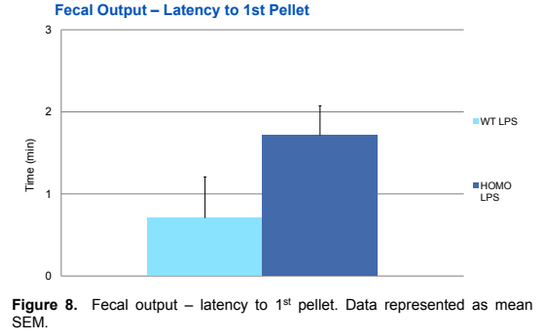


Figure 8. Fecal output – latency to 1st pellet. Data represented as mean ± SEM.

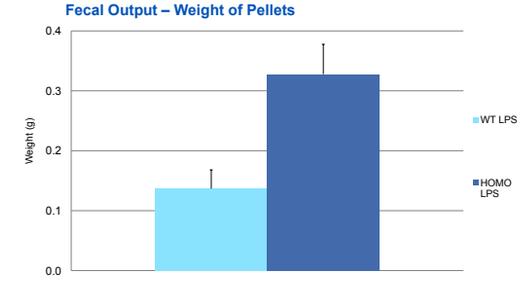


Figure 9. Fecal output – total weight of pellets. Data represented as mean ± SEM.

CONCLUSIONS

This study examined the effect of repeated LPS challenge on motor and gastrointestinal functionality in aged HOMO A53T transgenic mice. Challenge with LPS did not result in significant changes in beam balance test during the first 6 weeks after challenge. Mice fullfilling the qualification criteria of 10% increase in extrusion time (colon motility) were enrolled to the continuous monitoring of up to 12 weeks after LPS challenge. As a result of LPS challenge, HOMO A53T mice showed a trend towards increased colon motility from the baseline but was not signficantly different when compared to WT group. However, in beam traverse test, the latency to traverse across beam was greatly higher in LPS-treated HOMO A53T mice compared to WT mice. In fecal output tests, no significant differences between vehicle-treated WT and HOMO A53T mice (data not shown) were seen although there was a trend towards differences in mutiple fecal output parameters between LPS-treated HOMO A53T and WT mice.