PR001 and PR002 Modify Motor Phenotype in a Rat Model of Parkinson's Disease

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1 BACKGROUND

To date, there have been no disease-modifying therapies developed for the treatment of Parkinson's disease (PD). We have previously identified two small molecule compounds, PR001 and PR002, which exhibited putative neuroprotective efficacy in Drosophila melanogaster with PD-relevant mutations (see adjacent poster: 755.23 / EE7).

Here, we evaluated the effects of PR001 and PR002 on general behaviour, locomotor performance as well as tyrosine hydroxylase positive cell counts and α-synuclein staining in substantia nigra pars compacta in rats transfected with adeno-associated virus (AAV) carrying human α-synuclein with A53T mutation. These rats have been previously shown to be a promising Parkinson's disease model sensitive to neuroprotective effects of LRRK2 inhibitors.

2 METHODS

All animal experiments are approved by the National Animal Experiment Board, Finland. Male Wistar rats at 7-8 weeks of age at the time of surgery were used for the experiments. To perform unilateral substantia nigra AAV infusion, rats were anesthetized with 5% isoflurane (in 70% N_2O and 30% O_2; flow 300 mL/min) and placed in a stereotactic frame. During the operation, the concentration of the anesthetic was reduced to 1–5%. The skin was opened by a medial incision and retracted laterally. The right brain hemisphere was exposed through a small craniectomy to the skull. The dura mater was carefully removed with fine forceps and a stereotaxic injection with either empty AAV1/2 or AAV1/2 expressing human A53T alpha-synuclein (1.7×10^{12} vg/mL). A total of 4 µL of AAV-vector was infused at a speed of 0.4 µL/min at the following coordinates: AP -5.2, ML 2.1, DV -7.5 mm from the skull surface. The infusion cannula was left in place for another 5 min before being withdrawn. The skin was closed and disinfected. The rats were allowed to recover from anesthesia and were carefully monitored for possible postsurgical complications. The animals were returned to the home cages with *ad libitum* access to food and water.

The rats were divided into treatment groups as follows:

Group 1: (control): 10 male Wistar rats received AAV-Null/Empty virus.

Group 2: (vehicle): 10 male Wistar rats received AAV1/2-A53T virus. The animals were dosed with vehicle B.I.D. (12/12 h) from Day 1 through Day 28.

Group 3: (PR001): 10 male Wistar rats received AAV1/2-A53T virus. The animals were dosed with PR001 B.I.D. (12/12 h) from Day 1 through Day 27.

Group 3: (PR002): 10 male Wistar rats received AAV1/2-A53T virus. The animals were dosed with PR002 B.I.D. (12/12 h) from Day 1 through Day 28.

On day 28 of the experiment, 30 min following the morning treatment, the rats were subjected to kinematic gait assessment on a MotoRater (TSE Systems, Homburg, Germany). In this test, movements of rats, as they walked along a narrow beam, were captured using a high-speed camera (300 frames per second) from three different dimensions (from below and from the left and right sides). Different gait patterns and movements were analyzed using a custom programmed automated analysis system. The analyzed parameters included: 1) general gait pattern parameters (stride time and speed, step width, stance and swing time during a stride, interlimb coordination), 2) body posture and balance (toe clearance, iliac crest and hip height, hind limb protraction and retraction, tail position and movement), and 3) fine motor skills (swing speed during a stride, angle ranges and deviations of different joints, vertical and horizontal head movement).

Four weeks after the infusion, rat brains were removed and fixed by immersion in 4% paraformaldehyde in 0.1M phosphate buffer (PB) for 24 h. Thereafter, the blocks were cryoprotected in 30% sucrose in 0.1 M PB for 2–3 days, snap-frozen in liquid nitrogen and stored at -80 °C. The tissue block containing substantia nigra pars compacta (SNc) was used for TH/α-syn staining and stereological analysis.

3 RESULTS

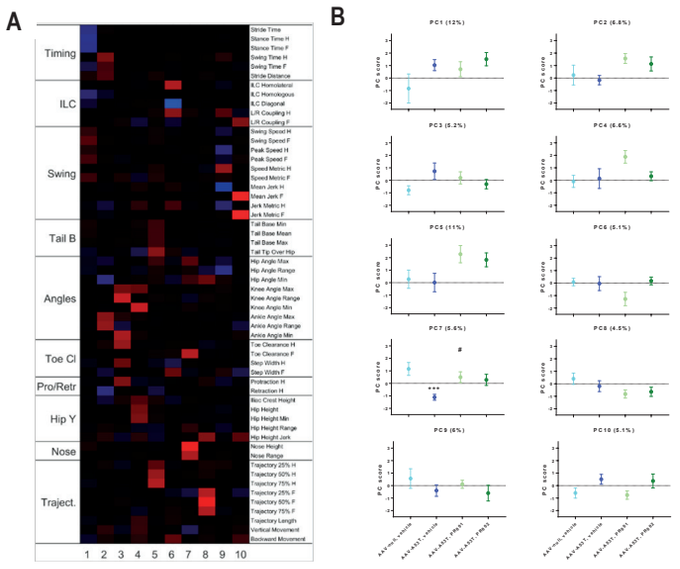

Figure 1. Effects of experimental treatments on fine motor skills and gait properties of male Wistar rats compacted into 10 principal components (PCs). (**A**) Correlation heatmap illustrates the original parameters from the whole data set that differentially influence each of the ten principal components (red = positive correlation, blue = negative correlation, black = no correlation). Numbers on the X-axis represent respective PC numbers. (**B**) Changes in individual PC scores. Percentage values in brackets next to corresponding PC numbers on the top of each panel refer to the percentage of variation in the original data that is explained by the respective PC. Data are presented as the mean ± standard error of mean (N = 9–10 per group). Statistical significance of differences is indicated as follows: *** - significantly different from "AAV-null, vehicle" (P < 0.001); # - significantly different from "AAV-A53T vehicle"..

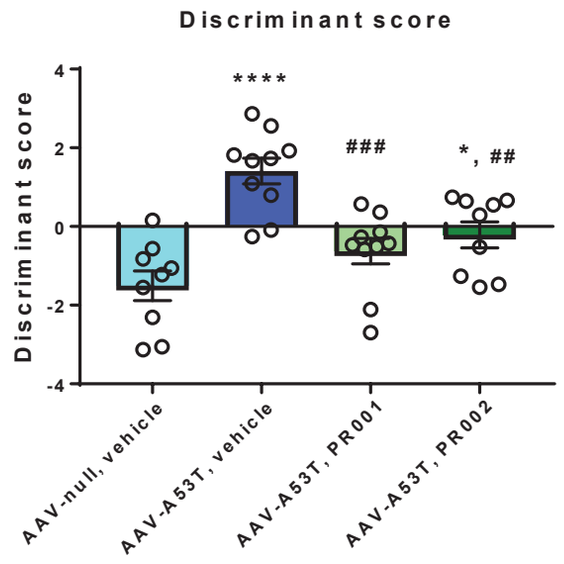

Figure 2. Effects of experimental treatments on overall Fine Motor Phenotype Score (FMPS) Data are presented as the mean ± standard error of mean (N = 9–10 per group). Statistical significance of differences is indicated as follows: *P < 0.05; ****P < 0.0001 - significantly different from "AAV-null, vehicle"; ##P < 0.01; ###P < 0.001; - significantly different from "AAV-A53T, vehicle".

3 RESULTS CONT'D

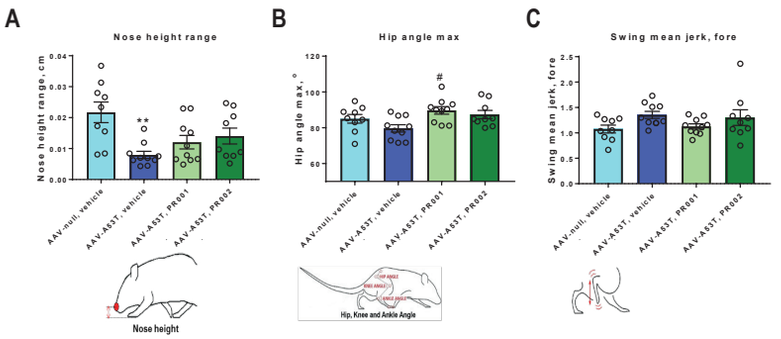

Figure 3. Effects of experimental treatments on selected gait parameters such as nose height range (**A**), maximum hip angle (**B**) and mean amplitude of swing jerk of forepaws (**C**). Data are presented as the mean ± standard error of mean (N = 9–10 per group). Empty circles denote individual values. Statistical significance is indicated as follows: **P < 0.01 - significantly different from "AAV-null; #P < 0.05 - significantly different from "AAV-A53T vehicle".

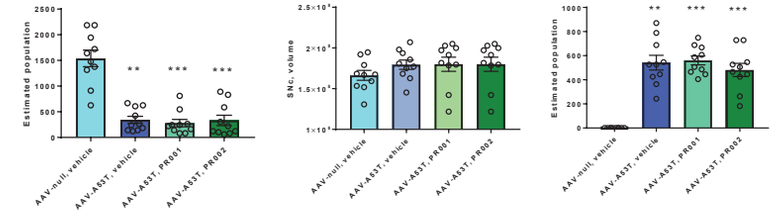

Figure 4. Effects of a unilateral infusion of AAV-Null Empty or AAV-A53T on the (**A**) number of TH-positive cells in substantia nigra pars compacta (SNc; ipsilateral to the side of infusion), (**B**) SNc volume and (**C**) the number of α-synuclein-positive cells in ipsilateral SNc). Data represent the mean ± s.e.m. of measurements in five SNc slices (side ipsilateral to infusion site) from 9–10 animals per group. Empty circles denote individual values. Statistical significance of treatment effects is indicated as follows: **P < 0.01; ***P < 0.001; - significantly different from "AAV-null, vehicle".

4 CONCLUSIONS

- In rats that received vehicle treatment, single unilateral infusion of the AAV1/2 vector carrying DNA sequence of human mutant A53T α-synuclein into SNc led to a pronounced loss of TH-positive cells in ipsilateral SNc compared to TH-positive cell counts in vehicle-treated rats infused with AAV-Null empty virus.
- Administration of PR001 or PR002 did not prevent the decrease in the number of TH-positive cells and did not affect the number of α-synuclein-positive cells in SNc.
- Multiple gait parameters, determined in 4 weeks after A53T-α-synuclein infusion, in vehicle-treated AAV-A53T rats were significantly or nominally different from those in AAV-Null rats, which collectively led to a significantly different Fine Motor Phenotype Score in AAV-A53T (vehicle) animals.
- Treatment with PR001 prevented the change in the Fine Motor Phenotype Score brought by the infusion of AAV-A53T, as the score value was statistically different from the score in AAV-A53T (vehicle) group but not statistically different from the score in AAV-Null group.
- Treatment with PR002 led to moderate attenuation of the motor phenotype caused by the infusion of AAV-A53T, as the value of the Fine Motor Phenotype Score in this group was intermediate between values in AAV-A53T (vehicle) and AAV-Null groups and statistically different from both of them.