The role of GRK6 in animal models of Parkinson's Disease and L-DOPA treatment

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SUPPLEMENTARY INFORMATION includes:

Supplementary Methods

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SUPLEMENTARY METHODS

BRET assay to analyze cAMP regulation and βarrestin 2 recruitment to D_2 dopamine receptor following GRK6 over-expression *in vitro*

All cell culture reagents and buffers were from Invitrogen (Carlsbad, CA) and Sigma (St. Louis, MO). Coelenterazine *h* was purchased from Promega (Madison, WI). All compounds used in this study were obtained from Sigma. GRK6, EPAC biosensor, D_{2L} receptor, D_{2L} receptor fused to Renilla Luciferase (Rluc) and βarrestin 2 fused to YFP have been generated as described in Masri et al (2008)[1] and Gainetdinov et al (2003)[2]. Human embryonic kidney 293 cells (HEK293T) were maintained in Dulbecco's Modified Eagle's medium supplemented with 10% (vol/vol) of FBS, 2mML-glutamine and 0.05 mg/ml of gentamicin at 37°C in a humidified atmosphere at 95% air and 5% CO2. Transient transfections were performed 24 h after cells seeding using lipofectamine 2000, according to manufacturer's protocol (Invitrogen). For the BRET experiments, 24 h after transfection, the cells were plated in poly-D-lysine coated 96-well microplates (well assay plate with clear bottom, Fisher Scientific) at a density of 80,000 cells per well in phenol red free Minimum Essential Medium containing 2% of FBS, 10 mM Hepes, 2 mM L-glutamine. The cells were then cultured for an additional 24 h. For BRET assays, at the day of the experiment the phenol red free medium was removed from HEK293T cells and replaced by Phosphate Buffer Saline (PBS) containing calcium and magnesium and 0.003% (wt/vol) of ascorbic acid. For measurement of cAMP variation using the EPAC biosensor the assay was started by adding 10 µl of the cell-permeant substrate specific for *Renilla* luciferase, coelenterazine *h* to the well to yield a final concentration of 5 µM as described in Masri et al (2008)[1]. The agonistic activity of quinpirole was measured by adding it 5 minutes after the Rluc substrate, and forskolin was then added to the wells 5 minutes following agonists. To follow the βarrestin 2 recruitment to the dopamine D_{2L}R receptor, the same protocol as above was used and the reads started 10 minutes after the addition of Rluc substrate. BRET readings were collected using an Infinite F500 instrument (Tecan, Switzerland) that allows the

sequential integration of the signals detected in the 465 to 505 nm and 515 to 555 nm windows using filters with the appropriate band pass and by using i-control software.

References

1 Masri, B. *et al.* Antagonism of dopamine D2 receptor/beta-arrestin 2 interaction is a common property of clinically effective antipsychotics. *Proc Natl Acad Sci U S A* **105**, 13656-13661 (2008).

2 Gainetdinov, R. R. *et al.* Dopaminergic supersensitivity in G protein-coupled receptor kinase 6-deficient mice. *Neuron* **38**, 291-303 (2003).

SUPLEMENTARY FIGURES

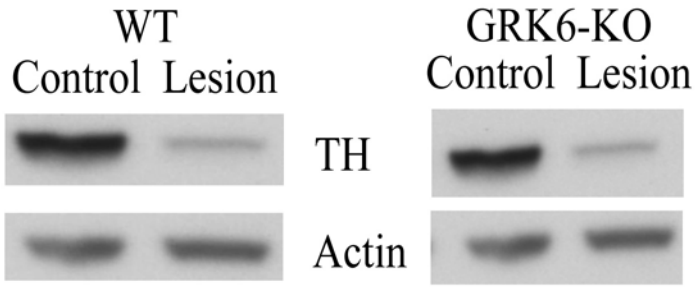

Figure S1. Representative blots of TH immunoreactivity in control and lesioned mice. Western blots showing the Tyrosine Hydroxylase (TH) and Actin levels in the striatum of control and 6-OHDA Lesioned mice for WT and GRK6-KO genotypes.

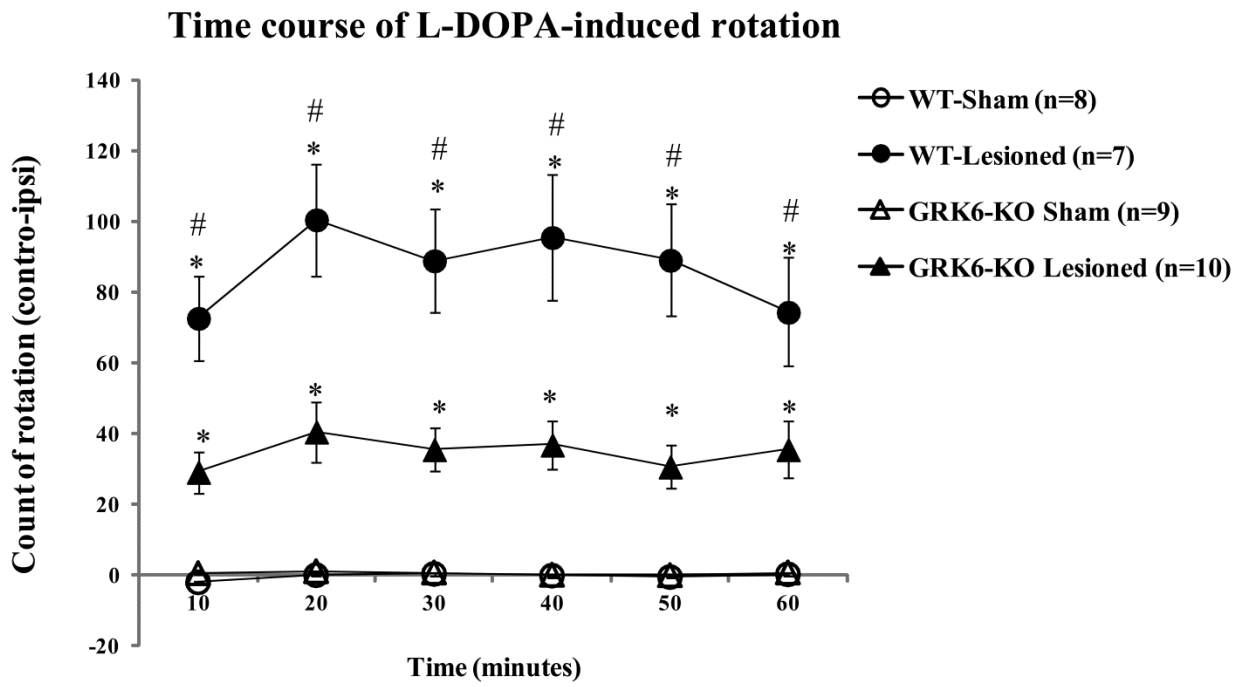

Figure S2. Time course of L-DOPA induced rotation. The graph shows the mean of rotations for each group counted every 10 minutes for 60 minutes at the last day of L-DOPA treatment. Four experimental groups, two groups for WT: Sham and Lesioned and two for GRK6-KO: Sham and Lesioned. All the animals were treated with 20/12 mg/kg of L-DOPA/Carbidopa for 21 days. The rotations (means, ±SEM) are counted as controlateral minus ispilateral rotations. Two-Way Anova for repeated measures, Tukey HSD *post hoc*. *$p<0.05$ vs Sham in the same group of genotype and time point; # $p<0.05$ vs WT-lesioned in the same time point.

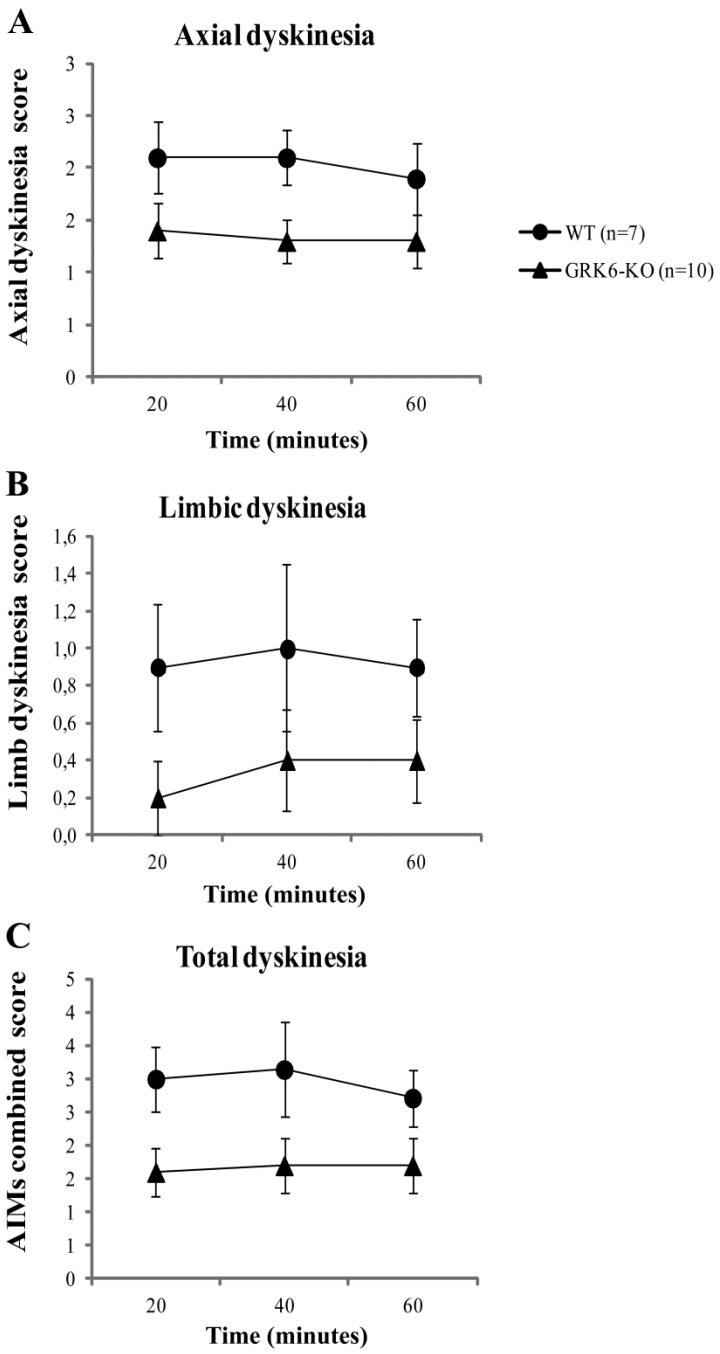

Figure S3. Time course of chronic L-DOPA induced AIMs in GRK6-KO mice.
S4A. Axial AIMs; **S4B.** Limb AIMs; **S4C**. Combined AIMs score**.** The graphs represent the time course of AIMs in the last day of treatment. WT and GRK6-KO 6-OHDA-lesioned animals received L-DOPA treatment for 21 days and AIMs were scored using the rodent abnormal involuntary movements (AIM) rating system described in the text. No significant AIMs were detected in sham-operated controls.

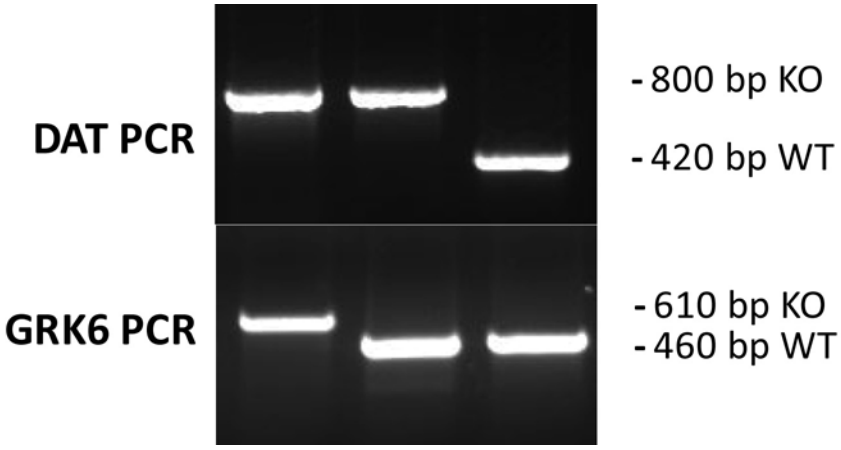

Figure S4. Genotyping of DAT/GRK6-KO mice. DAT-KO, GRK6-KO or DAT/GRK6 double KO mice were distinguished by two separate PCR. The wt GRK6 locus gives a 460 bp band and the GRK6-KO locus gives a 610 bp band; while the wt DAT locus give a 420 bp band and the DAT-KO locus gives a 800 bp band. Genotyping was routinely performed on tail tip DNA.