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LRRK2 G2019S Transgenic Mice Display Increased Susceptibility to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-Mediated Neurotoxicity

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Author contribution statement

V.L.D., and T.M.D supervised the project. S.S.K., V.L.D., and T.M.D. formulated the hypothesis. S.S.K., I.M., S.A., V.L.D., and T.M.D designed experiments. Y.X., provided the mice. S.S.K. performed the MPTP injections, HPLC analysis, and behavior tests. S.S.K., and Y.L., performed blinded stereological counting of tyrosine hydroxylase neurons and M.C.L., D.K., Nissl counts. S.B., M.K., performed TH-fiber density and E.N., P.G., A.J., analyzed the results. S.S.K., V.L.D., and T.M.D., initiated and organized the study and wrote the manuscript. All authors contributed to writing the final manuscript.

Conflict of interest

The authors report no conflict of interest.

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Abstract

Mutations in leucine-rich repeat kinase 2 (LRRK2) are the most common causes of late onset autosomal dominant form of Parkinson disease (PD). Gain of kinase activity due to the substitution of Gly 2019 to Ser (G2019S) is the most common mutation in the kinase domain of LRRK2. Genetic predisposition and environmental toxins contribute to the susceptibility of neurodegeneration in PD. To identify whether the genetic mutations in LRRK2 increase the susceptibility to environmental toxins in PD models, we exposed transgenic mice expressing human G2019S mutant or wild type (WT) LRRK2 to the environmental toxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). MPTP treatment resulted in a greater loss of tyrosine hydroxylase-positive neurons in the substantia nigra pars compacta (SNpc) in LRRK2 G2019S transgenic mice compared to the LRRK2 WT overexpressing mice. Similarly loss of dopamine levels were greater in the striatum of LRRK2 G2019S mice when compared to the LRRK2 WT mice when both were treated with MPTP. This study suggests a likely interaction between genetic and environmental risk factors in the PD pathogenesis and that the G2019S mutation in LRRK2 increases the susceptibility of dopamine neurons to PD-causing toxins.

Keywords

Parkinson's Disease; LRRK2; Dopamine; Stereology; MPTP; Genetic factor; Environmental factor

1. Introduction

Parkinson's Disease (PD) is a common age-related neurodegenerative disorder with unknown etiology. It is characterized by the loss of dopaminergic neurons in substantia nigra pars compacta (SNpc), thereby leading to the depletion of dopamine (DA) in the striatum. Loss of striatal DA is responsible for the majority of PD's cardinal motor symptoms (Dawson *et al.*, 2010; Lees *et al.*, 2009; Savitt *et al.*, 2006). Though most cases of PD are sporadic, it is well established that many cases of PD have a strong genetic component (Di Monte *et al.*, 2002; Satake *et al.*, 2009).

Mutations in the leucine-rich repeat kinase 2 (LRRK2), which leads to late-onset PD with an autosomal dominant pattern of inheritance, are prime examples of genetic variants that cause PD susceptibility (Dawson *et al.*, 2010; Martin *et al.*, 2011; Martin *et al.*, 2014b; Rajput *et al.*, 2006; Yue *et al.*, 2015; Zimprich *et al.*, 2004). LRRK2 is a large multi-domain protein that includes central catalytic domains with GTPase and kinase activities and surrounding protein-protein interaction domains (Sanders *et al.*, 2014; Xiong *et al.*, 2010). Of particular interest to PD is the G2019S mutation in the kinase domain of LRRK2, leading to a gain of kinase activity (Smith *et al.*, 2006; West *et al.*, 2005; West *et al.*, 2007).

Since the majority of PD cases cannot solely be explained by genetic risk factors, the general consensus is that PD develops as a result of the interactions between genetic and environmental risk factors (Dauer *et al.*, 2002; Veldman *et al.*, 1998). 1-methyl 4-phenyl 1,2,3,6-tetrahydropyridine (MPTP), an environmental cause of PD, is a potent neurotoxin

which is specifically taken up by DA transporter and inhibit mitochondrial complex I in dopaminergic neurons. MPTP has been used to generate an acute PD model that closely resembles the PD-like phenotype. MPTP intoxication rodent models mainly provide a valuable tool to study molecular cascades leading to degeneration of DA neurons, and for the drug screening in PD (Blesa and Przedborski, 2014; Karuppagounder *et al.*, 2014; Langston *et al.*, 1984).

Stereological counting is employed mainly in biology, engineering and geography scientific fields. In biology, stereological methods advance the understanding of disease, such as PD and Alzheimer's disease by providing a quantitative assessment of neuronal loss. Recent developments in Design-based Stereological methods allowed improvement in accuracy of estimating the number of neurons in the whole brain region and determining amount of loss of specific cell populations (Schmitz and Hof, 2000, 2005). In characterization of PD models (either genetic or toxin) stereology methods are the most valuable tools to assess the total neuronal loss.

To study the interaction between genetic and environmental factors leading to PD pathogenesis, we asked whether genetic mutations within LRRK2 increase the susceptibility of dopaminergic neurons to MPTP. For these studies we used a mouse model in which the CMVE-PDGF β promoter driven expression of WT or LRRK2 G2019S leads to a two-fold increase in LRRK2 protein levels in the ventral mid brain region as compared to the endogenous LRRK2 levels. Although, LRRK2 G2019S expression in this model was relatively modest, it was sufficient to induce age dependent DA neurodegeneration (Ramonet *et al.*, 2011). We took advantage of this model, to investigate the combination of genetic and environmental hazards, and the subsequent effects on DA neurodegeneration. These mice were injected with MPTP, and stereological techniques were used to evaluate the development of PD pathology and neurodegeneration.

2. Material and Methods

2.1. Animals

All procedures involving animals were approved by and conformed to the guidelines of the Institutional Animal Care Committee of Johns Hopkins University. Male LRRK2 WT and LRRK2 G2019S transgenic mice (Tg) (3-month old, 25–30 g) were produced from in-house breeding. In this study, hemizygous Tg mice were used and backcrossed with C57BL/6J mice for more than 10 generation. Animals were housed in a 12 h dark and light cycle with free access to water and food. All mice were transferred and acclimatized for 3 days in procedure room before starting any experiments.

2.2. Treatment groups and MPTP administration

Mice were segregated into 3 group based on genotyping as following, non-transgenic (non-Tg), LRRK2 wild type (WT) and LRRK2 G2019S mice. The mice either received saline or MPTP (see Fig. 1E for more detail). MPTP was solubilized in normal saline. On day zero, the saline and MPTP group received four intra-peritoneal (i.p.) injections of either saline or MPTP-HCl (18 mg/kg free base) at 2 h intervals. The Pole test were performed on the 5th

day. All the mice were sacrificed on the 7th day and brain samples were processed for neurochemical, and immunohistochemistry studies as previously described (Jackson-Lewis and Przedborski, 2007; Karuppagounder *et al.*, 2014).

2.3. Monoamine Analysis

Biogenic amine concentrations were measured by high-performance liquid chromatography with electrochemical detection (HPLC-ECD). Briefly, mice were sacrificed by decapitation and the striatum was quickly removed. Striatal tissue was weighed and sonicated in 0.2 ml ice cold 0.01 mM perchloric acid containing 0.01 % EDTA and 60 ng 3, 4-dihydroxybenzylamine (DHBA) as an internal standard. After centrifugation ($15,000 \times g$, 30 min, 4 °C), the supernatant was passed through a 0.2 µm filter. Twenty microliters of the supernatant were analyzed in the HPLC column (4.6 mm $\times$ 150 mm C-18 reverse phase column, MC Medical, Tokyo, Japan) by a dual channel coulometer III electrochemical detector (Model 5300, ESA, Inc Chelmsford, MA, USA). The protein concentrations of tissue homogenates were measured using the BCA protein assay kit (Pierce, Rockford, IL, USA). Data were normalized to protein concentrations and expressed in ng/mg protein as previously described (Karuppagounder *et al.*, 2014).

2.4. Pole Test

Animals were acclimatized in the behavioral procedure room. The pole is made up 2.5 ft metal rod of 9 mm diameter and wrapped with bandage gauze. Briefly, the mice were placed on the top of the pole (3 inch from the top of the pole) facing head-up. Total time taken to reach the base of the pole was recorded. Before the actual test the mice were trained for two consecutive days and each training session consists of three test trials. On the day of the test, mice were evaluated in three sessions and total times were recorded. The maximum cutoff of time to stop the test and recording was 120 sec. Results were expressed in total time (in sec) (Karl *et al.*, 2003; Karuppagounder *et al.*, 2014).

2.5. Immunohistochemistry and quantitative analysis

Mice were perfused with ice-cold phosphate buffered saline (PBS), followed by 4% paraformaldehyde/PBS (pH 7.4). Brains were removed and post fixed for 4 h in the same fixative. After cryoprotection in 30 % sucrose/PBS (pH 7.4), brains were frozen and serial coronal sections (60 µm sections) were cut with a microtome. Free-floating sections were blocked with 4 % goat serum/PBS plus 0.2% Triton X-100 and incubated with an antibody against TH (rabbit polyclonal; Novus Biologicals) and striatal sections against GFAP (rabbit polyclonal, Wako) followed by incubation with biotin-conjugated anti-rabbit antibody (anti-rabbit polyclonal; Vector Labs), ABC reagents (Vector Labs), and SigmaFast DAB Peroxidase Substrate (Sigma-Aldrich). Sections were counterstained with Nissl (0.09 % thionin). TH-positive and Nissl positive DA neurons from the SNpc region were counted through an optical fractionator, the unbiased method for cell counting. GFAP positive cells in striatum were counted as mentioned above. This method was carried out by using a computer-assisted image analysis system consisting of an Axiophot photomicroscope (Carl Zeiss Vision) equipped with a computer controlled motorized stage (Ludl Electronics), a Hitachi HV C20 camera, and Stereo Investigator software (MicroBright-Field). The total number of TH- stained neurons and Nissl counts was calculated as described

(Karuppagounder *et al.*, 2014). Serial striatal sections were processed for TH staining following the same procedure as above. Fiber density in the striatum was quantified by optical density (OD). ImageJ software (NIH) was used to analyze the OD as previously described (Karuppagounder *et al.*, 2014; Lane *et al.*, 2006).

2.6. Statistical analysis

All quantitative data are expressed as the mean $\pm$ SEM. Statistical significance was determined by one-way ANOVA followed by Turkey's post-hoc analysis for comparison among multiple treatment groups. p values lower than 0.05 were considered to be significant. Respective p values are indicated in the figure legends. Asterisk (^{ns}p>0.05, *p 0.05, **p 0.01, ***p 0.001, ****p 0.0001) sign indicate statistical significance between non-Tg saline versus LRRK2 WT and/or LRRK2 G2019S MPTP groups and the pound (^{ns}p>0.05, #p 0.05, ##p 0.01, ###p 0.001) sign indicate statistical significance between LRRK2 WT MPTP versus LRRK2 G2019S MPTP groups.

3. Results

3.1. Validation of WT and LRRK2 G2019S transgenic mice and experimental strategy

Transgenic mice constitutively overexpressing LRRK2 were bred as previously described (Ramonet *et al.*, 2011). In order to avoid variation due to the strain background in the susceptibility to MPTP-induced neurodegeneration, the LRRK2 transgenic mice were back-crossed for over 10 generations with C57BL/6J mice. Full length human LRRK2 WT and LRRK2 G2019S are driven by the CMV-enhanced human platelet-derived growth factor β -chain (CMVE-PDGF β) promoter (Fig. 1 A). The transgenic mice express either LRRK2 WT or LRRK2 G2019S transgene as determined by quantitative PCR (qPCR) for the CMVE promoter using tail genomic DNA as a template (Fig. 1 B). LRRK2 protein levels were determined in ventral midbrain lysates by western blot using a LRRK2 antibody. As previously described (Ramonet *et al.*, 2011), CMVE-PDGF β promoter driven LRRK2 transgene expression leads to an almost a two fold increase in the levels of LRRK2 WT or G2019S mice when compared to the endogenous LRRK2 levels in non-TG mice (Fig. 1 C&D).

In this study, we designed an experiment plan to evaluate the contribution of environmental risk factors to PD pathogenesis leading to the neurodegeneration (Fig. 1E). Experiments were performed in 3 months old male mice. We injected the mice with MPTP (18 mg/kg, i.p, 2h interval, 4 times) to compare the neurotoxicity among the different genotypes (Fig. 1E).

3.2. LRRK2 G2019S enhances the susceptibility to MPTP-Induced Dopaminergic Neurodegeneration

To evaluate DA neuronal loss, we stained for tyrosine hydroxylase (TH) and counter stained for Nissl. Unbiased stereological counting was performed to estimate the number of TH-positive and Nissl-positive neurons in the SNpc. Representative immunostaining images for TH (Fig. 2A) and quantification for stereology counting of TH- and Nissl-positive stained DA neurons (Fig. 4B and C) show a significant loss of dopaminergic neurons in non-TG, LRRK2 WT and G2019S mice treated with MPTP compared to saline-treated controls.

Overexpression of LRRK2 G2019S significantly enhances the loss of DA neurons in response to MPTP when compared to non-Tg or LRRK2 WT-Tg (Fig. 2A, B and C). Consistent with prior reports we find that the expression of LRRK2 WT or G2019S has no effect on the number of TH positive DA neurons (Fig. 2A, B and C) at the 3 month time point (Ramonet *et al.*, 2011).

Striatal TH-immunoreactive fiber density was examined by staining striatal sections for TH. Acquired images were analyzed by optical densitometry using ImageJ software (NIH). MPTP injections significantly reduce striatal dopaminergic fiber density in non-TG, LRRK2 WT and G2019S mice compared to saline-treated controls (Fig. 2D and E). Though, there is a trend toward enhanced fiber density loss in LRRK2 G2019S-Tg mice but we did not observe a significant difference with MPTP injected LRRK2 WT (Fig. 2D and E). LRRK2 G2019S alone did not reduce TH-fiber density (Fig. 2D and E). Immunostaining for glial fibrillary acidic protein (GFAP) indicates that non-TG, LRRK2 WT and G2019S mice exhibit significant increased GFAP immunoreactivity following MPTP intoxication with the LRRK2 G2019S mice showing more GFAP activation compared to non-Tg and LRRK2 WT mice injected with MPTP (Fig. 2F and G).

3.3. LRRK2 G2019S mutation enhances DA depletion, but not behavioral deficits following MPTP intoxication

To evaluate the effects of MPTP and LRRK2 G2019S on DA metabolism the levels of DA and its metabolites in the striatum were measured by employing reverse-phase HPLC-electrochemical detection (ECD). MPTP induces a significant reduction in the DA levels in all genotypes (Fig. 3A). Notably, LRRK2 G2019S Tg mice injected with MPTP show a significantly greater reduction in DA levels (Fig. 3A) as compared to MPTP treated non-Tg and LRRK2 WT transgene mice (Fig. 3A). MPTP-injected mice also show a significant reduction in 3,4-dihydroxyphenylacetic acid (DOPAC) in all genotypes when compared with saline, but we do not observe a significant difference between LRRK2 WT compared to LRRK2 G2019S transgene mice (Fig. 3B). Similar effects were observed in the levels of homovanillic acid (HVA) (Fig. 3C) and 3-methoxytyramine (3MT) (Fig. 3D). We assessed the DA turnover ratio to analyze whether the catabolism of DA is modulated by MPTP treatment in the LRRK2 transgenic mice. The DA turnover ratio is increased significantly in all genotypes, whereas these effects were not significantly aggravated by LRRK2 G2019S (Fig. 3E and F).

To examine the behavioral deficits induced by the MPTP administration, motor deficits were assessed by the pole test. MPTP intoxication results in a significant increase in the time to reach the base of the pole in non-TG, LRRK2 WT and G2019S mice compared to the saline-treated controls (Fig. 3G). In the MPTP injected LRRK2 G2019S mice, we do not observe a significant increase in duration to reach the base of the pole but we observe a trend in this group (Fig. 3G) compared to the LRRK2 WT MPTP group.

4. Discussion

PD is a complex-multifactorial disorder that cannot be fully explained by genetic risk factors (Chung *et al.*, 2003). Current models postulate that both genetic and environmental factors

may play important roles in initiating the DA neurodegeneration (Mattson *et al.*, 2002; Veldman *et al.*, 1998). The main purpose of this study was to determine whether a specific combination of genetic and an environmental factor, MPTP, enhances the degeneration of DA neurons. The G2019S mutation in LRRK2 is a disease causing mutation that is a common causes of familial and sporadic PD. We employed MPTP as an exogenous environmental factor to induce acute PD, not only because it is a well-established chemical model of PD, but also its metabolite, MPP⁺, structurally resembles paraquat and diquat (Blesa *et al.*, 2012; Karuppagounder *et al.*, 2012). Paraquat and diquat are pesticides widely used around the world, the usage of these environmental toxins in global food production is concerning.

The major finding of this study is that injecting MPTP into LRRK2 G2019S mice augments TH neuronal death in the SNpc when compared to MPTP injected into LRRK2 WT mice. Accompanying the loss of TH neurons in LRRK2 G2019S injected with MPTP we observed a loss of DA in the striatum. We also observed a difference in GFAP immunoreactivity among the different genotypes (Di Monte *et al.*, 1992). The reduction of TH neurons induced by MPTP impairs DA synthesis in the SNpc, thereby resulting in significant decreases in DA storage at the terminal, and the loss of DA and its metabolites in the striatum. A greater reduction of DA levels was observed in LRRK2 G2019S mice when compared with LRRK2 WT mice. PDGF is known to drive expression in macrophages (Demoulin and Montano-Almendras, 2012; Morelli *et al.*, 2006) and there is a significant inflammatory and brain macrophage response to MPTP intoxication, and probable breakdown of the blood-brain barrier. Since LRRK2 G2019 rats are more susceptible to neurodegeneration caused by LPS inflammation it is possible that toxicity is due LRRK2 G2019S expression in non-neuronal cells (Moehle *et al.*, 2015).

The enhanced loss of DA neurons and reduction in striatal DA was observed in the LRRK2 G2019S mice did not translate into a significantly worse behavioral phenotype as determined by the pole test. Although there was a trend toward a worse behavioral phenotype, the severity of the MPTP lesion may account for the lack of significance. In addition, the sensitivity of the behavioral test or small differences in MPTP-induced DA depletion in LRRK2 G2019S versus LRRK2 WT mice may account for the lack of significance

The enhanced susceptibility to MPTP in the LRRK2 G2019S mice, but not the LRRK2 WT mice. The difference in the susceptibility to MPTP in the LRRK2 G2019S versus LRRK2 WT is known. However, the enhanced toxicity is likely due to a gain of function specifically due to expression of LRRK2 G2019S. Consistent with this notion is the observation that LRRK2 knockout mice do not show any significant differences in the sensitivity to MPTP-induced neurodegeneration (Andres-Mateos *et al.*, 2009). DA neurons in *C. Elegans* expressing LRRK2 G2019S have enhanced susceptibility to the mitochondrial complex I inhibitor rotenone (Saha *et al.*, 2009). In addition, DA neurons in *C. Elegans* expressing LRRK2 G2019S have enhanced susceptibility to the putative environmental toxin from S. Venezuela, in part through mitochondrial dysfunction and oxidative stress (Ray *et al.*, 2014). LRRK2 mutant *Drosophila* also show increased sensitivity to rotenone (Ng *et al.*, 2009). LRRK2 G2019S knockin mice exhibit striatal mitochondrial abnormalities late in life (Yue

et al., 2015). DA neurons derived from human inducible pluripotent stem cells (iPSCs) also exhibit mitochondrial defects (Sanders *et al.*, 2014) and enhanced sensitivity to mitochondrial toxins, including rotenone (Cooper *et al.*, 2012). How LRRK2 G2019S leads to mitochondrial dysfunction is not known, but the mitochondrial dysfunction is likely to contribute to the enhanced MPTP neurotoxicity and sensitivity to other mitochondrial stressors.

LRRK2 mediated toxicity is mainly due to upregulation of kinase activity (Smith *et al.*, 2006; West *et al.*, 2005) and downregulation of its LRRK2 GTPase activity (Xiong *et al.*, 2010; Xiong *et al.*, 2012). Recently, it was reported that an increase in LRRK2 G2019S kinase activity can phosphorylate ribosomal protein 15 (Rps15), leading to an increase in protein translation, which contributes to neurotoxicity (Martin *et al.*, 2014a; Martin *et al.*, 2014c). It will be important to determine whether the increased protein translation leads to mitochondrial defects.

5. Conclusion

In summary, we find that the overexpression of LRRK2 G2019S in mice increases the susceptibility of dopaminergic neurons to degeneration in the context of exposing to MPTP (environmental risk factor). These finding further support the notion that genetic and environmental factors contribute to aggravate and advance the disease pathology in PD, particularly due to LRRK2 G2019S mutation.

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Highlights

- Parkinson's disease is a complex-multifactorial disorder, which involves genetic and environmental factors.
- Stereological counting demonstrates that LRRK2 G2019S mice are more susceptible to the loss of TH-positive neurons in SNpc and reduction of striatal TH-positive fiber density in the striatum in response to MPTP intoxication.
- LRRK2 G2019S mice injected with MPTP show reduction of dopamine levels in striatum but no change in the behavioral performance.
- LRRK2 G2019S genetic mutation of PD increases the susceptibility to MPTP induced degeneration of nigrostriatal pathway neurons.

Fig 1. Validation of LRRK2 transgenic mice and experimental plan

(A) Schematic illustration shows the positions of G2019S mutations in the LRRK2 gene and the expression CMVE-PDGFβ vector. (B) Quantitative PCR analysis was performed to identify LRRK2 mRNA expression and primers were design in CMVe region. GAPDH used as loading controls. (C) Immunoblots of ventral midbrain lysate from non-Tg, LRRK2 WT Tg and LRRK2 G2019S Tg were immunoblotted with anti-LRRK2 antibodies. β-actin serves as a loading control. LRRK2 proteins migrated at ≈280 kDa. The overexpression of LRRK2 shows two fold increase compared to endogenous LRRK2 levels. Relative LRRK2 levels were normalized to β-actin as indicated. The error bars represent the mean ± SEM. (n=3 mice per group). Statistical significance was determined by performing one-way ANOVA followed by Tukey's multiple comparisons test. ***p < 0.001, LRRK2 WT or G2019S compared with non-Tg group. (E) A schematic diagram depicts the experimental design of present study. Numbers represent the days when experiments conducted. On day zero, we injected saline or MPTP (18 mg/kg free base, 2 h interval, 4 times) in the respective treatment groups. On 5th day the pole test was performed. On 7th day, mice were sacrificed for indicated studies. Animal numbers used for these studies are as follows: behavioral (n=6-10), neurochemical (n=5), and immunohistochemistry (n=6-8).

Fig. 2. Overexpression of LRRK2 G2019S increases MPTP-induced dopaminergic neurodegeneration

On seventh day following the last MPTP injection, in the saline or MPTP group the number of tyrosine hydroxylase (TH)-positive neurons were counted by unbiased stereology in the substantia nigra pars compacta (SNpc) region of non-Tg, LRRK2 WT Tg and LRRK2 G2019S Tg. (A) Representative photomicrographs from MPTP and saline treated non-Tg, LRRK2 WT Tg and LRRK2 G2019S Tg stained for TH immunoreactivity. (B) Stereological quantification of TH and (C) Nissl-positive neurons in the SNpc. (D) Representative photomicrographs of striatal sections stained for TH immunoreactivity. Non-Tg, LRRK2

WT Tg and LRRK2 G2019S Tg mice injected with saline (top panel) and injected with MPTP (bottom panel). (E) Quantification of dopaminergic fiber densities in the striatum using Image J software (NIH). (F) Representative photomicrograph of striatal sections stained for GFAP and (G) Stereological quantification of GFAP positive cells in the striatum. Error bars represent the mean $\pm$ SEM, n=6-8 mice per group for striatal TH stereology, and TH-fiber density. One-way ANOVA was used to test significance and followed with Turkey's multiple comparisons test. ***p 0.001 and ****p 0.001 for MPTP compared to saline injected mice, #p 0.05, ##p 0.01, ###p 0.001 for LRRK2 WT Tg MPTP compared to LRRK2 G2019S MPTP injected mice. (ns: not significant)

Fig 3. LRRK2 G2019S mutation leads to MPTP-induced dopamine depletion

Non-Tg, LRRK2 WT Tg and LRRK2 G2019S Tg mice were subjected to acute MPTP injections (18 mg/kg, MPTP free base, 2 h interval, and 4 times). Striatal dopamine (DA) and its metabolites levels were analyzed on day 7 after the last MPTP injection by HPLC-ECD analysis. (A) DA levels and (B) DOPAC (C) HVA and (D) 3MT. (E) DA turnover [(DOPAC+HVA/DA) and (F) (DOPAC+3MT/DA)] in the striatum. (G) Five days after the last MPTP injection, the pole test was performed in non-Tg, LRRK2 WT-Tg and LRRK2 G2019S Tg mice. Error bars represent the mean ± SEM, n=5 mice per group for striatal

catecholamine analysis. One-way ANOVA was used to test significance and followed with turkey's multiple comparisons test to compare the groups. *p 0.05, **p 0.01, ***p 0.001, ****p 0.0001 for MPTP compared with saline group, # p<0.05 for LRRK2 G2019S MPTP compared with LRRK2 WT MPTP group. (ns: not significant)

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