Investigating the effect of enriched environment on Parkinson's disease by measuring the activity of detoxifying enzymes at the dopaminergic pathways in the mouse model with α-synucleinopathy

Jang Woo Lee

Department of Medicine

The Graduate School, Yonsei University

Investigating the effect of enriched environment on Parkinson's disease by measuring the activity of detoxifying enzymes at the dopaminergic pathways in the mouse model with α-synucleinopathy

Directed by Professor Seong-Woong Kang

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Jang Woo Lee

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This certifies that the Doctoral Dissertation of Jang Woo Lee is approved.

Thesis Supervisor: Seong-Woong Kang

Thesis Committee Member#1: Young-Chul Choi

Thesis Committee Member#2: Sung-Rae Cho

Thesis Committee Member#3: Sung Hoon Kim

Thesis Committee Member#4: Lark Kyun Kim

The Graduate School
Yonsei University

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Abstract

Investigating the effect of enriched environment on Parkinson's disease with α-synucleinopathy by measuring the activity of the detoxifying enzymes at the dopaminergic pathways in the mouse model

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Parkinson's disease (PD) is characterized by cardinal motor symptoms, including resting tremor, bradykinesia, rigidity, and postural instability, preceded by various non-specific non-motor symptoms. PD is caused by the loss of dopaminergic neurons in the substantia nigra pars compacta, and is known to involve Lewy bodies, which are associated with neuroinflammation and oxidative stress. Although the pathophysiology of PD remains to be fully elucidated, metabolic alternations in the detoxification of endogenous and xenobiotic compounds represent potential mechanism. In our preliminary study, enzymes involved in the toxin metabolism at the olfactory bulb (OB) of transgenic PD mice were found to be overexpressed, which was reversed in an enriched environment (EE), a rehabilitation treatment model using mice. Based on these findings, this study aims to further understand the role of detoxifying enzymes, especially those participating Phase I metabolism, as an initial stage of detoxification by investigating neuronal structures other than the OB,

including the frontal cortex, striatum, and brain stem. Eight-month aged transgenic PD mice with overexpression of human A53T α-synuclein were randomly allocated to standard cage and EE for 2 months. At age of 10 months, the expression of detoxifying Phase I enzymes was evaluated via quantitative polymerase chain reaction and compared with wild type mice of the same age raised in standard cage. A tendency to overexpress detoxifying enzymes was observed in the OB and brain stem of PD mice, which was offset by EE. In the striatum, the activity levels of some enzymes were highest in normal wild type mice. Moreover, distinct differences were not observed in the frontal cortex among the study groups. EE also was found to be related with reduced oxidative stress, prevention of apoptosis, and suppression of α-synuclein as well as induction of neuronal proliferation in the brain tissues. These results suggest that the strategy against PD pathogenesis differs according to the brain region, and rehabilitation has a protective effect against neuronal deterioration with subsequent decreased detoxifying burden in PD.

Key words : parkinson's disease, dopaminergic pathway, detoxifying enzymes, phase I metabolism, rehabilitation, enriched environment

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I. INTRODUCTION

1. Overview of Parkinson's disease

Parkinson's disease (PD) is the second most common neurodegenerative disease after Alzheimer disease, affecting about 1~2% of the population older than 60 years of age.[1,2] In Korea, more than 10,000 new cases occur each year and more than 80,000 individuals are affected.[3] The incidence and prevalence of this disease are gradually increasing, as the life expectancy of society as a whole increases. PD is considered an intrusive condition involving multiple functional systems. It is characterized by four cardinal motor symptoms: resting tremor, bradykinesia, rigidity, and postural instability.[4] Recently, diverse non-motor symptoms, such as autonomic dysfunction, gastrointestinal symptoms, cognitive dysfunction, sleep disturbance, and neuropsychiatric symptoms have been proposed as accompanying presentations in PD.[5] Therefore, PD is considered an intrusive condition involving multiple systems. Occasionally, these non-motor symptoms precede the cardinal motor symptoms

by up to several decades, and can become more problematic in the advanced stages.[6-8]

2. Pathophysiology of PD

Despite a wide range of studies on the pathophysiology of PD, the exact mechanisms of PD have not yet been elucidated. Various hypotheses have been suggested, including the alternation of metabolism.[9] Because various endogenous and environmental toxic materials are responsible for neuronal degeneration, detoxifying is believed to represent an important defensive mechanism against PD.[10,11] The detoxifying metabolism is largely divided into three phases: Phase I modifies toxins into active metabolites via hydrolysis, oxidation, and reduction; Phase II catalyzes the products derived from Phase I into hydrophilic products with transferase enzymes; and Phase III excretes the final products from cells via various transporter systems.[12]

Classically, it is thought that PD is caused by the loss of dopaminergic neurons in the substantia nigra pars compacta, with typical motor symptoms being induced by disruptions of the basal ganglion. Lewy bodies are α-synuclein intracellular inclusions that are known to cause neuroinflammation and mitochondrial dysfunction, as well as subsequent oxidative stress. They are believed to play significant roles in various neurodegenerative diseases.[9] In more recent research studies, symptoms related to olfactory structures and the gastrointestinal tract have been proposed as induction sites of PD.[6,13,14] Non-motor symptoms are known to occur when these regions are affected.[6] However, because non-motor symptoms are non-specific and poorly recognized, especially in the elderly population, it is difficult to diagnose PD during the premotor period, that is, before the presentation of the typical motor symptoms.[4]

3. The olfactory bulb (OB)

The OB acts as an entry site for external materials, delivering signals from

the olfactory mucosa, which is directly exposed to the external environment, into the olfactory cortex.[15] In addition, it provides protecting against harmful substances by secreting antioxidant and detoxifying enzymes.[16] The detoxifying properties of the OB play an important role against various neurodegenerative diseases associated with xenobiotics and environmental toxins.[17] The non-motor symptoms of PD, such as hyposmia, a representative non-motor symptom of PD that develops in the premotor period, can precede the development of typical motor symptoms by up to several decades.[18] Therefore, the OB is considered an induction site of PD pathogenesis.[6]

4. Enriched environment (EE) as a model of rehabilitation in animal study

In combination with physical, cognitive, and social interactions, EE is generally accepted as a typical model of rehabilitation in animal models.[19] Functional improvements in neurological diseases have been proven by EE in several studies.[20] Moreover, various neurochemical and histological hypotheses have been suggested for the underlying mechanisms of this improvement, including reduced oxidative stress, anti-neuroinflammation, neurogenesis, synaptic plasticity, and neuroprotection via the induction of neurotrophic factors.[21-24]

5. Preliminary study evaluating the effects of EE in the OB in adult normal mice

In a preliminary study investigating the OB of normal adult mice, exposure to EE for 2 months resulted in a significant up-regulation of several enzymes of Phase I and II compared with the control group. A reduction in oxidative stress, neuroinflammation, apoptosis, and induced neurogenesis were also observed in the EE group.[16]

6. Followed preliminary study in transgenic PD mice

In the followed research using transgenic PD mice that overexpress human A53T α-synuclein, several detoxifying enzymes were found to be significantly enhanced in the OB of PD mice compared to wild-type mice at the early stages of disease, which were less enhanced in the later stages. However, EE was found to offset this up-regulation, resulting in normalized levels of detoxifying enzymes.[25]

7. Dopaminergic pathways

Various dopaminergic pathways are known to be related to the pathogenesis of PD and its symptoms. The ventral pathway, including both the mesolimbic and mesocortical pathways, is responsible for hypokinesia and bradyphrenia, also known as psychic akinesia or slowness of thinking. The ventral pathway projects from the ventral tegmental area in midbrain to the ventral (or limbic) striatum, which includes the nucleus accumbens, olfactory tubercle (mesolimbic pathway), and prefrontal cortex (mesocortical striatum). The dorsal striatal, or nigrostriatal pathway, is responsible for motor-specific symptoms, such as motor control speed and dexterity. The dorsal striatal pathway projects from the substantia nigra pars compacta to the dorsal striatum (caudate nucleus and putamen).[26,27]

8. Aims of this study

This study aims to expand on preliminary findings of the activities of detoxifying enzymes in various dopaminergic structures, including the frontal cortex, striatum, and brain stem, in addition to the OB. In particular, we focused on the Phase I metabolism, an initial stage of detoxification. The effect of EE on enzymatic activity, oxidative stress, neurogenesis, neuroprotection, and functional ability were also investigated to determine their significance in PD rehabilitation.

Ⅱ. MATERIALS AND METHODS

1. Transgenic mouse model

To generate wild-type and transgenic mice, the human α-synuclin A53T transgenic line G2-3 (B6.Cg-Tg [Prnp-SNCA*A53T] 23 Mkle/J; Jackson Laboratories, stock no. 006823, Bar Harbor, ME, USA) was used. The transgenic mice used in this model were heterozygous offspring of an overexpressed copy of the A53T mutant mice (Figure 1A). All animals were raised in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care under alternate 12-h light and dark cycles, with free access to food and water. The experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC 2017-0039).

2. Genotyping of PD mice

For the genotyping of the mice, 1-2 mm samples of tail tissue were obtained. The genomic DNA was extracted from the samples using PDQeX *prep*GEM Universal Kits (MicroGEM, Cat. number; XPA0500). The samples were soaked in the extraction mixture (1 μL of *prep*GEM, 10 μL of *Histosolv*, 10× ORANGE+ Buffer, and 78 μL of autoclaved 3’ distilled water), followed by incubation at 72℃ for 5 minutes, 75℃ for 10 minutes, and 95℃ for 2 minutes. The following primers were used for the polymerase chain reaction (PCR): transgene forward, 5’-TCA TGA AAG GAC TTT CAA AGG C-3’; transgene reverse, 5’-CCT CCC CCA GCC TAG ACC-3’ (transgene = ~500 bp); internal positive control forward, 5’-CTA GGC CAC AGA ATT GAA AGA TCT-3’; and internal positive control reverse, 5’-GTA GGT GGA AAT TCT AGC ATC C-3’ (internal positive control = 324 bp). The genotyping results were confirmed by electrophoresis using 10 μL of each PCR product in a 1.5% agarose gel (Figure 1B).

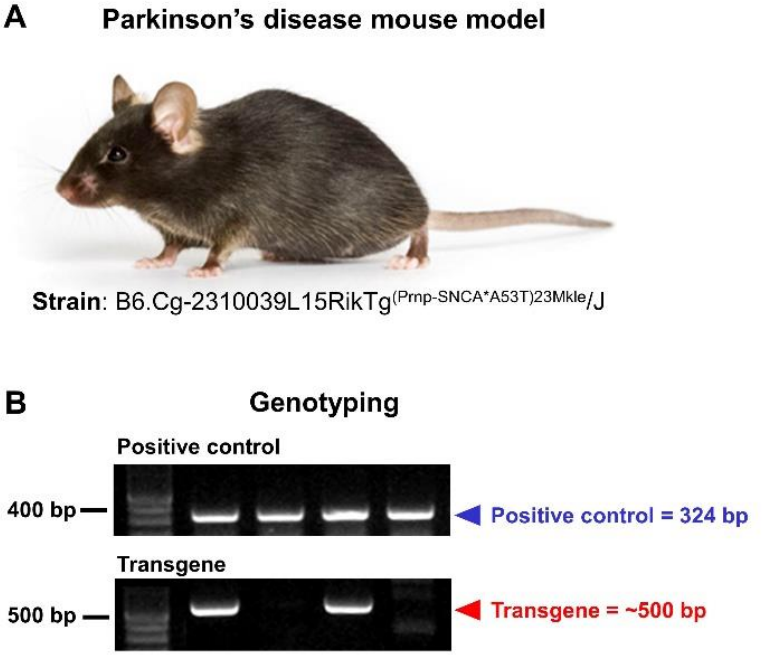

Figure 1. Parkinson's disease mouse model and genotyping. (A) The human A53T α-synuclein transgenic line G2-3 (B6.Cg-Tg [Prnp-SNCA*A53T] 23 Mkle/J; Jackson Laboratories, stock no. 006823, Bar Harbor, ME, USA) was used. (B) Mutation of human α-synuclin in transgenic mice was confirmed by PCR.

3. Mice rearing and sacrifice

EE was established by placing the mice in large cages (86 × 76 × 31 cm^3) with various equipment, such as tunnels, shelters, toys, and running wheels.[19] These objects were provided for voluntary exercise and social interaction. Only 12 to 15 mice were reared in each EE cage. In contrast, the control mice were housed in 27 × 22.5 × 14 cm^3 standard cages (SC) without social interaction (3 to 5 mice/cage). All transgenic and wild-type mice were reared in either the EE or SC cages from the age of 8 months to 10 months (Figure 2). The three experimental groups were as follows: wild-type mice reared in SC (WT),

transgenic mice reared in SC (PD-SC), and transgenic mice reared in EE (PD-EE) (N = 12). The mice were sacrificed at 10 months of age. In addition to the OB investigated in our preliminary study, the frontal cortex, striatum, and brain stem were used for analysis.

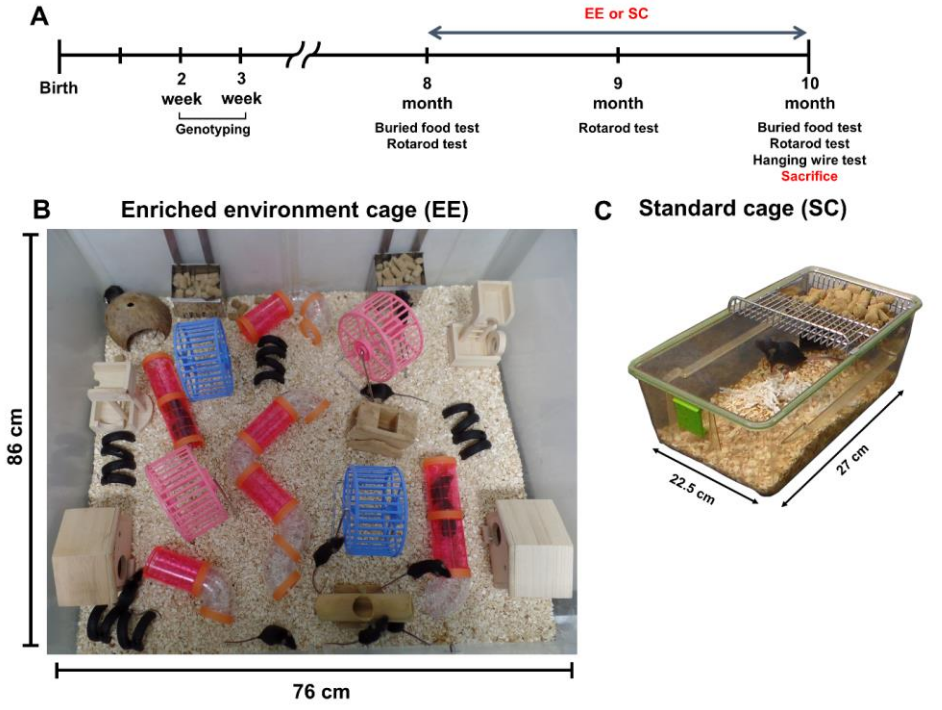

Figure 2. Experimental design of an enriched environment vs. standard cage. (A) Schematic timeline of the experiment in a mouse model of PD. All subjects were sacrificed at the age of 10 months. (B and C) Images of enriched environment (B) and standard cage (C).

4. Quantitative real-time polymerase chain reaction (qRT-PCR)

Complementary DNA (cDNA) synthesis was performed using Prime Script™ Reverse Transcriptase (TAKARA). cDNA was subjected to RT-PCR using LightCycler 480 SYBR Green master mix (Roche Applied Science), and Gene Amp PCR system 9700 (Applied Biosystems/Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions. The quantitative expression of the eleven enzymes of Phase I metabolism, namely *cytochrome P450 family 1 subfamily A member 2* (*CYP1A2*), *CYP family 2 subfamily A member 5* (*CYP2A5*), *CYP family 2 subfamily F member 2* (*CYP2F2*), *carbonyl reductase* (*CBR*), *crystallin lambda 1* (*CRYL1*), *alcohol dehydrogenase 1* (*ADH1*), *aldehyde dehydrogenase 1A7* (*ALDH1A7*), *aldehyde oxidase 3L1* (*AOX3L1*), *carboxylesterase 1D* (*CES1D*), *dihydropyrimidinase* (*DPYS*), and *paraoxonase 1* (*PON1*), was evaluated by qRT-PCR. The amount of expressed human α-synuclein was also measured by qRT-PCR. The primer sequences used are listed in Table 1. The thermocycler conditions were as follows: denaturation for 5 minutes at 95℃, followed by 40 cycles at 95℃ for 5 seconds, at 62℃ for 20 seconds, and at 75℃ for 15 seconds. Melting curve analysis began at 95℃ for 15 seconds, followed by 1 minute at 60℃.

Table 1. List of primers used for qRT-PCR.

Genes	Primer sequences	
CYP1A2	Forward	GCTTCTCCATAGCCTCGGAC
	Reverse	TTAGCCACCGATTCCACCAC
CYP2A5	Forward	ATCGGGTGATTGGCAGGAAC
	Reverse	TACCCTTGGGGAGGAGGAAA
CYP2F2	Forward	ATGACCACACACAACCTGCT
	Reverse	GATGTACGGTCTTCCAGCGT
CBR	Forward	CCCCCTTCCACATTCAAGCA
	Reverse	CTCCTCTGTGATGGTCTCGC
CRYL1	Forward	GATTGACGGCTTCGTCCTGA
	Reverse	GCATAGTCTCCAAGGGTCCG
ADH1	Forward	GACATAGAAGTCGCACCCCC
	Reverse	CCAACGCTCTCAACAATGCC
ALDH1A7	Forward	GGTTTAGCAGCAGGAGTCTTCA
	Reverse	CAGCCAAATAGCAGTTCACCC
AOX3L1	Forward	CTCGGGGAGTCTGGGATGTT
	Reverse	GTTTTTGGGTCATCTCTCGGG
CES1D	Forward	CCCAAGCTTTGTATCGGCCA
	Reverse	CATTGGGGTTCCCATTCCGA
DPYS	Forward	AGACCCGAAAGTCTGCTGTG
	Reverse	CTGAGAGAAGGCCGCATACA
PON1	Forward	ATGACGCAGAGAATCCTCCC
	Reverse	TTTGTACACAGAGGCGACCG

CYP1A2, cytochrome P450 family 1 subfamily A member 2; *CYP2A5, CYP family 2 subfamily A member 5*; *CYP2F2, CYP family 2 subfamily F member 2*; *CBR, carbonyl reductase*; *CRYL1, crystallin lambda 1*; *ADH1, alcohol dehydrogenase 1*; *ALDH1A7, aldehyde dehydrogenase 1A7*; *AOX3L1, aldehyde oxidase 3L1*; *CES1D, carboxylesterase 1D*; *DPYS, dihydropyrimidinase*; PON1,

paraoxonase 1

5. Western blotting

The mice were euthanized and perfused with 0.9% saline. The brain tissues were extracted from the skulls and stored at -80 ℃ until further use. The mouse brain tissues were homogenized in RIPA lysis buffer (Thermo Fisher Scientific, Waltham, MA, USA) with protease inhibitor cocktail (Cell Signaling Technology, Beverly, MA, USA). The loading samples were prepared by solubilizing the brain lysates in NuPAGE® LDS sample buffer (Invitrogen), denatured for 10 min at 90℃. The samples were loaded into NuPAGE® 4-12% Bis-Tris gels and run in 1× NuPAGE® MES SDS running buffer (Invitrogen) at 120 V for 1 hour and 30 minutes. The resolved proteins were then transferred onto a 0.45 μm Invitrolon™ polyvinylidene difluoride (PVDF) (Invitrogen) membrane in 1× NuPAGE® Transfer buffer with 10% (vol/vol) methanol (EMSURE®) using an XCell IITM Blot Module (Invitrogen) at 4℃ for 1 hour.

The expression of various proteins, including human α-synuclein, phosphorylated α-synuclein, tyrosine hydroxylase (TH), glial fibrillary acidic protein (GFAP), and neuronal nuclei (NeuN), was measured by Western blotting. Phosphorylation at serine 129 of α-synuclein is known to result in the aggregation of Lewy bodies and Lewy neuritis.[28,29] GFAP is the main intermediate filament protein found in mature astrocytes. The expression of GFAP is generally increased, corresponding with the activity of various neuronal diseases, indicating reactive astrocytosis.[30,31] Furthermore, NeuN, a vertebrate neuron-specific nuclear protein, is widely used to label neurons, and is used for the quantification of neuronal cell numbers.[32]

6. Immunohistochemistry (IHC)

The animals were euthanized, perfused with 1× PBS, and re-perfused with 4% paraformaldehyde (PFA). After removing the brain tissues from the skulls, the brains were soaked in 6% sucrose for 1 day, followed by soaking in 30% sucrose until sinking completely. The tissues were then frozen in section compound (Leica, Wetzlar, Germany) and cryosectioned at 16-μm thickness along the sagittal or coronal plane using cryomicrotome (Cryostat Leica 1860; Leica Biosystem, MI, Italy).

For the IHC assays, the brain sections were stained with primary antibodies against nitrated α-synuclein (1:200, Novusbio), iNOS (1:200, Abcam), and Ki67 (1:200, Leica), as well as with the corresponding secondary antibodies, including Alexa Fluor® 488 goat anti-mouse (1:400, Invitrogen) and Alexa Fluor® 488 goat anti-Rabbit (1:400, Invitrogen). The primary antibodies for IHC are listed in Table 2. The sections were then mounted with a fluorescent mounting medium containing 4',6-diamidino-2-phenylindole (DAPI) (Vectorshield, Vector, Burlingame, CA) and covered with glass slides.

The nitrated α-synuclein and iNOS were used to determine the levels of oxidation in the OB. To evaluate cell proliferation, the sections were stained with Ki-67, a cell cycle-associated protein and a neurogenesis marker,[33] in the subventricular zone (SVZ), where neurogenesis is known to occur in the adult brain.[34]

For the analysis of apoptotic cells, the brain sections were stained using a fluorometric terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining, according to the manufacturer's instructions. The number of TUNEL-positive cells were counted in the brain sections, and the stained sections were analyzed using confocal microscopy (LSM 700, Zeiss).

Table 2. List of primary antibodies for Western blotting and IHC

Proteins	Category No.	Company	Dilution
Human α-synuclein	Ab138501	Abcam	WB, 1:1,000
pSer129 α-synuclein	Ab168381	Abcam	WB, 1:1,000
iNOS	Ab15323	Abcam	WB, 1:1,000; IHC, 1:200
Nitrated α-synuclein	NBP1-26380	Novusbio	WB, 1:1,000; IHC, 1:200
Ki-67	NCL-Ki67p	Leica	WB, 1:1,000; IHC, 1:200
TH	P40101-0	Pel-Freez	WB, 1:1,000
GFAP	RA-22101	Neuromics	WB, 1:2,000
NeuN	Mab377	Millipore	WB, 1:2,000
Actin	SC-47778	Santacruz	WB, 1:2,000

pSer129, phosphorylation at serine 129; iNOS, inducible nitric oxide synthase; TH, tyrosine hydroxylase; GFAP, glial fibrillary acidic protein; NeuN, neuronal nuclei

7. Glutathione activity assay using enzyme-linked immunosorbent assay (ELISA)

The ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG), indicating a reversal to oxidative stress, was measured using a glutathione detection kit ADI-900-160; Enzo Life Science, East Farmingdale, NY, USA), according to the manufacturer's instructions.

8. Neurobehavioral tests

Olfactory function was evaluated using buried food tests, while motor function was evaluated using handing wire and rotarod tests. Buried food tests were used to determine how quickly an overnight-fasted mouse could find a small palatable food, hidden underneath a layer of bedding (Figure 3A).[35]

The hanging wire test evaluated the neuromuscular strength of the paws of the experimental mice. To this end, mice were suspended on a horizontal rod (5 × 5 mm area, 35 cm long, between two 50-cm high poles), and the suspension latencies were measured for 5 minutes (Figure 3B).[36]

The rotarod (No. 47600; UGO Basile, Comerio, VA, Italy) test was used to evaluate the motor coordination and balance of the experimental mice using accelerating (4~80 RPM) speed paradigms. After the placing mice on the rotating rods, the time taken for the mice to fall from the rods was measured (Figure 3C).

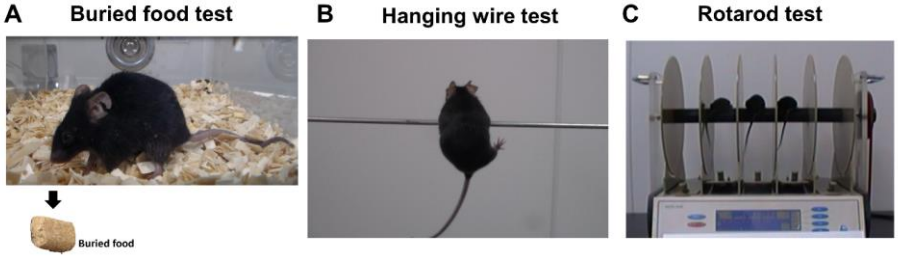

Figure 3. Neurobehavioral tests. (A) Buried food test, (B) hanging wire test and (C) rotarod test.

9. Statistical analysis

Statistical analyses were performed using Statistical Package for Social Sciences software version 23.0 (IBM Corporation, Armonk, NY, USA). For comparison among three experimental groups, one-way analysis of variance (ANOVA) was used with least significant difference for post-hoc analysis.

III. RESULTS

1. Expression of detoxifying enzymes measured by qRT-PCR

In the OB of the PD-SC mice, the expression of the all examined enzymes were up-regulated compared to the WT mice. Except *ADH1* and *DPYS*, the expression of enzymes was significantly lowered by EE. The levels of *CBR*, *CRYL1*, *ALDH1A7*, *AOX3L1*, and *PON1* in the PD-EE mice reached to those of the WT, on the other hand, the levels of *CYP*s of the PD-EE did not reach to those of the WT (Figure 4).

In the frontal cortex, the levels of *CYP1A2* and *CBR* were significantly higher in the PD-SC compared to the WT and also higher than those of PD-EE. *CYP2A5*, and *AOX3L1* were found to decrease in the PD-EE compared to the PD-SC mice. The level of *CYP2F2* was higher in the WT compared to the PD-EE. And the level of *CES1D* was higher in the WT compared to both PD-SC and PD-EE (Figure 5).

In the striatum of the WT mice, *ADH1*, *AOX3L1*, and *PON1* were expressed more highly than in both PD-SC and PD-EE. The expression of *CYP2F2* of the WT was higher compared to the PD-SC. Conversely, the levels of *CRYL1* and *CES1D* were higher in the PD-SC compared to the WT. And *CBR* was expressed higher in the PD-EE compared to both WT and PD-SC (Figure 6).

Lastly, in the brain stem, *CYP1A2*, *CBR*, *CRYL1*, *ADH1*, *ALDH1A7*, CES1D, PON1 were up-regulated in the PD-SC and ameliorated in the PD-EE to the levels of the WT. The level of *DYPS* was higher in the WT compared to both PD-SC and PD-EE mice. No significant difference of *CYP2A5* and *CYP2F2* was observed among the groups (Figure 7). *AOX3L1* was not exclusively expressed in any of the experimental groups.

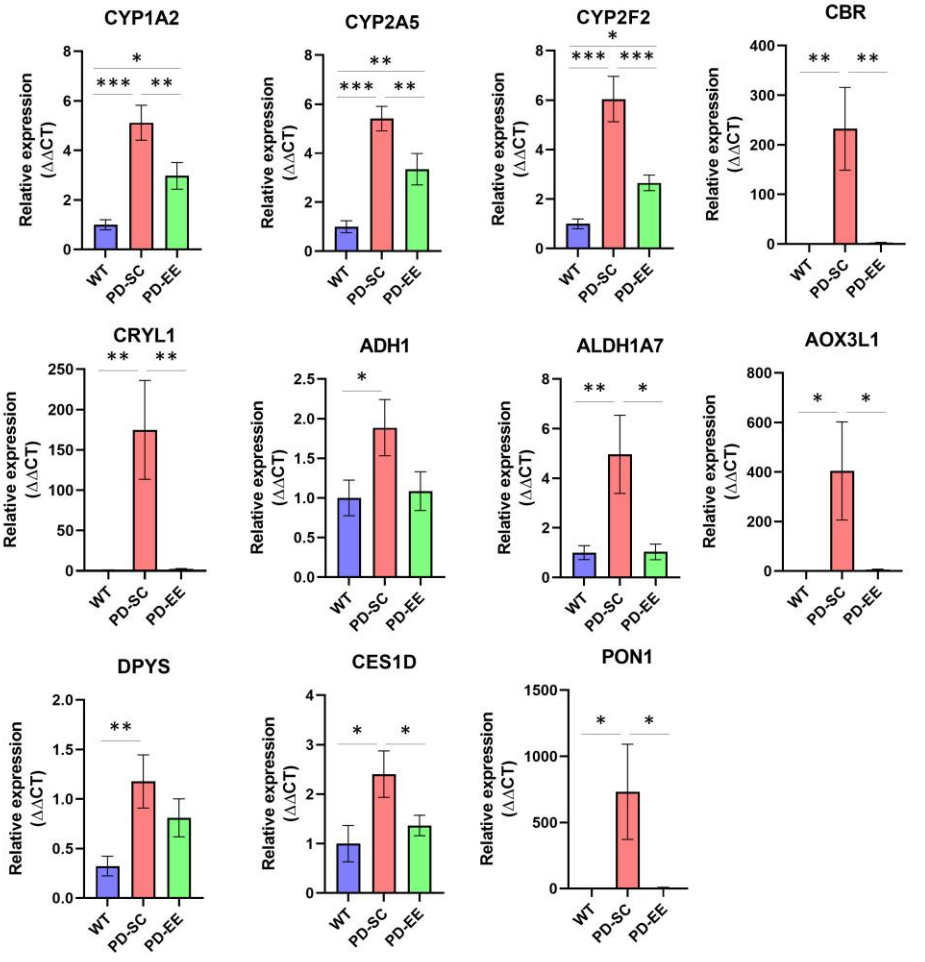

Figure 4. qRT-PCR of detoxifying enzymes at the OB. Significant overexpression of all examined enzymes was observed in the PD-SC. EE significantly offset the overexpression of all enzymes except *ADH1 and DPYS*. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; one-way ANOVA followed by least significant difference for post-hoc analysis.

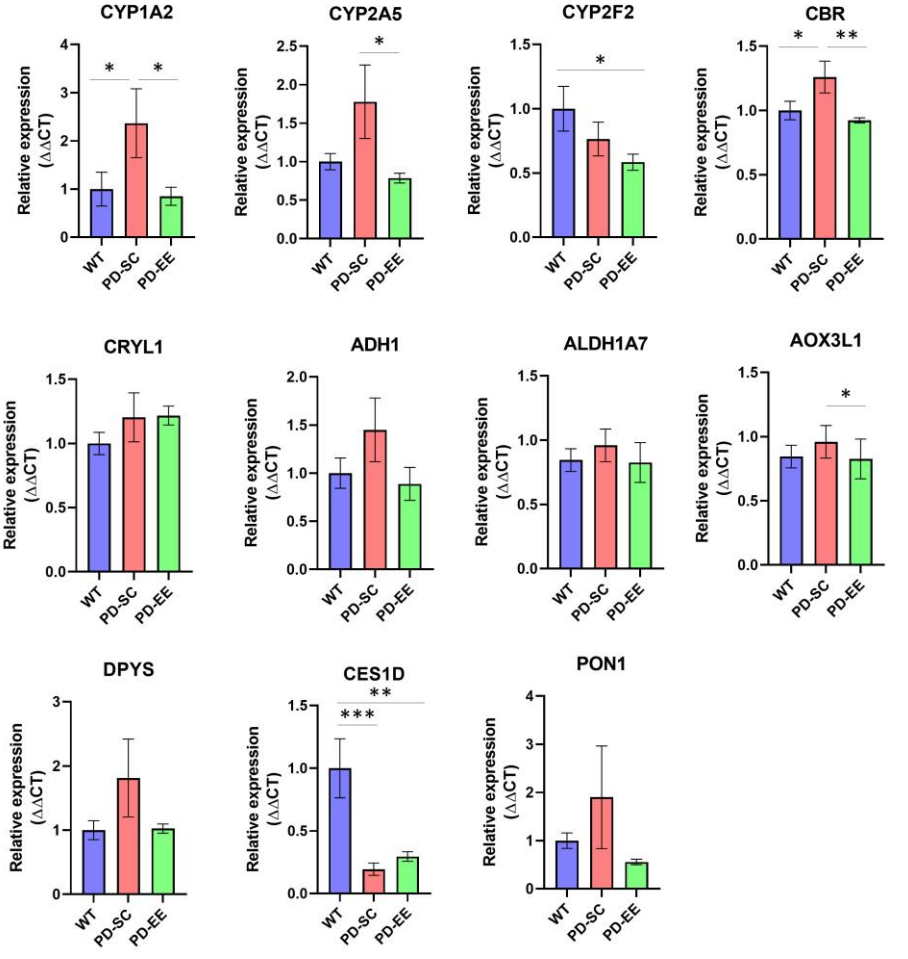

Figure 5. qRT-PCR of detoxifying enzymes at the frontal cortex. Significant increased levels of *CYP1A2* and *CBR* was observed in the PD-SC compared to both WT and PD-EE. *CYP2A5* and *AOX3L1* were found to decrease in the PD-EE compared to the PD-SC. The level of *CES1D* was higher in the WT compared to both PD-SC and PD-EE. And the expression of *CYP2F2* was higher in the WT compared to the PD-EE. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; one-way ANOVA followed by least significant difference for post-hoc analysis.

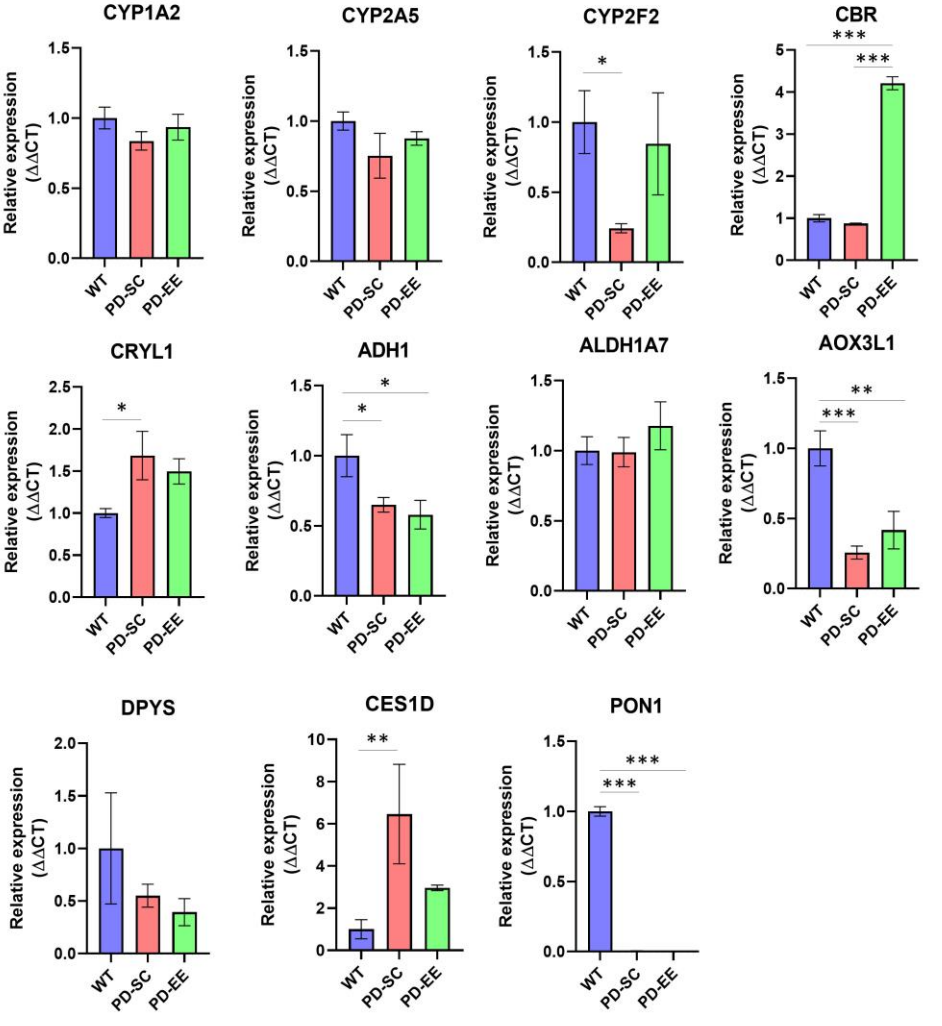

Figure 6. qRT-PCR of detoxifying enzymes at the striatum. The higher levels of *AHD1*, *AOX3L1*, and *PON1* were observed in the WT compared to both PD-SC and PD-EE mice. The expression of *CYP2F2* was higher in the WT compared with the PD-SC. On the other hand, *CRYL1* and *CES1D* were highly expressed in the PD-SC compared to the WT. And *CBR* expression was higher in the PD-EE compared to both WT and PD-SC. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; one-way ANOVA followed by least significant difference for post-hoc analysis.

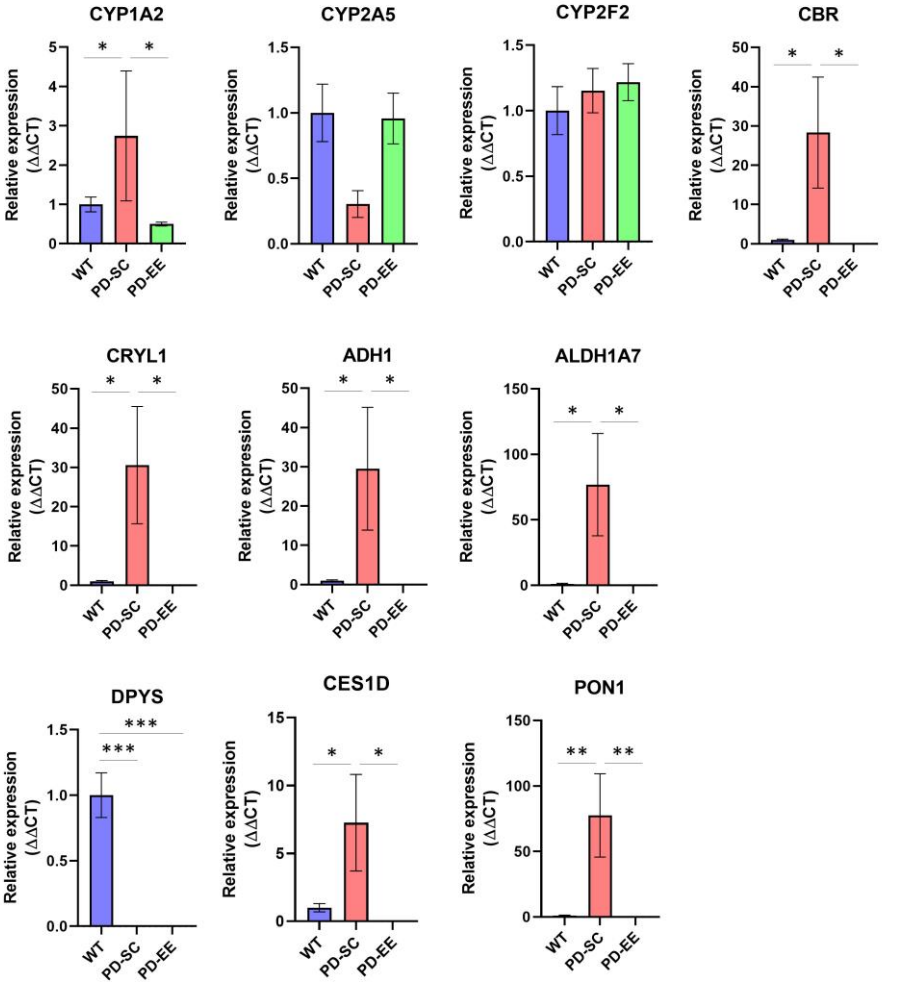

Figure 7. qRT-PCR of detoxifying enzymes at the brain stem. The expression of *CYP1A2*, *CBR*, *CRYL1*, *ADH1*, *ALDH1A7*, *CES1D*, and *PON1* was significantly higher in the PD-SC compared to both WT and PD-EE. The level of *DPYS* was higher in the WT compared to both PD-SC and PD-EE, **P* < 0.05; ***P* < 0.01; ****P* < 0.001; one-way ANOVA followed by least significant difference for post-hoc analysis.

2. Expression of human α-synuclein

The phosphorylation (at serine 129) and nitration of α-synuclein via post-translational modifications tends to result in the formation of aggregates, which is considered a feature of PD.[28] Nitrated α-synuclein is a common effect of increased oxidative stress.[29]

Nitrated α-synuclein measured by IHC was only observed in the OB of all of the regions studied. The density of nitrated α-synuclein was found to be highest in the PD-SC mice and lowest in the WT mice. The differences among the three groups were statistically significant (Figure 8).

Significant increased levels of human A53T α-synuclein mRNA were observed in both PD-SC and PD-EE mice at all experimental regions. Moreover, in the striatum, the expression in PD-EE mice was found to be lower compared with the PD-SC mice (Figure 9A). A significant increase in α-synuclein protein was observed in the PD-SC mice compared with the WT mice (Figure 9B).

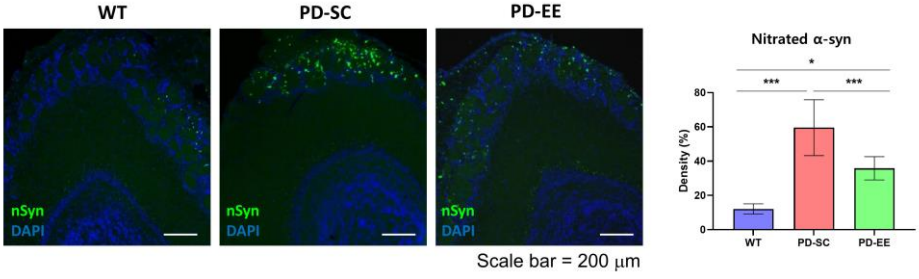

Figure 8. Expression of nitrated α-synuclein at the OB measured by IHC. The density of nitrated α-synuclein was lowest in the WT mice and highest in the PD-SC mice among the three experimental groups. Scale bar = 200 μm. **P* < 0.05; ***P* < 0.01; ****P* < 0.001; one-way ANOVA followed by least significant difference for post-hoc analysis.

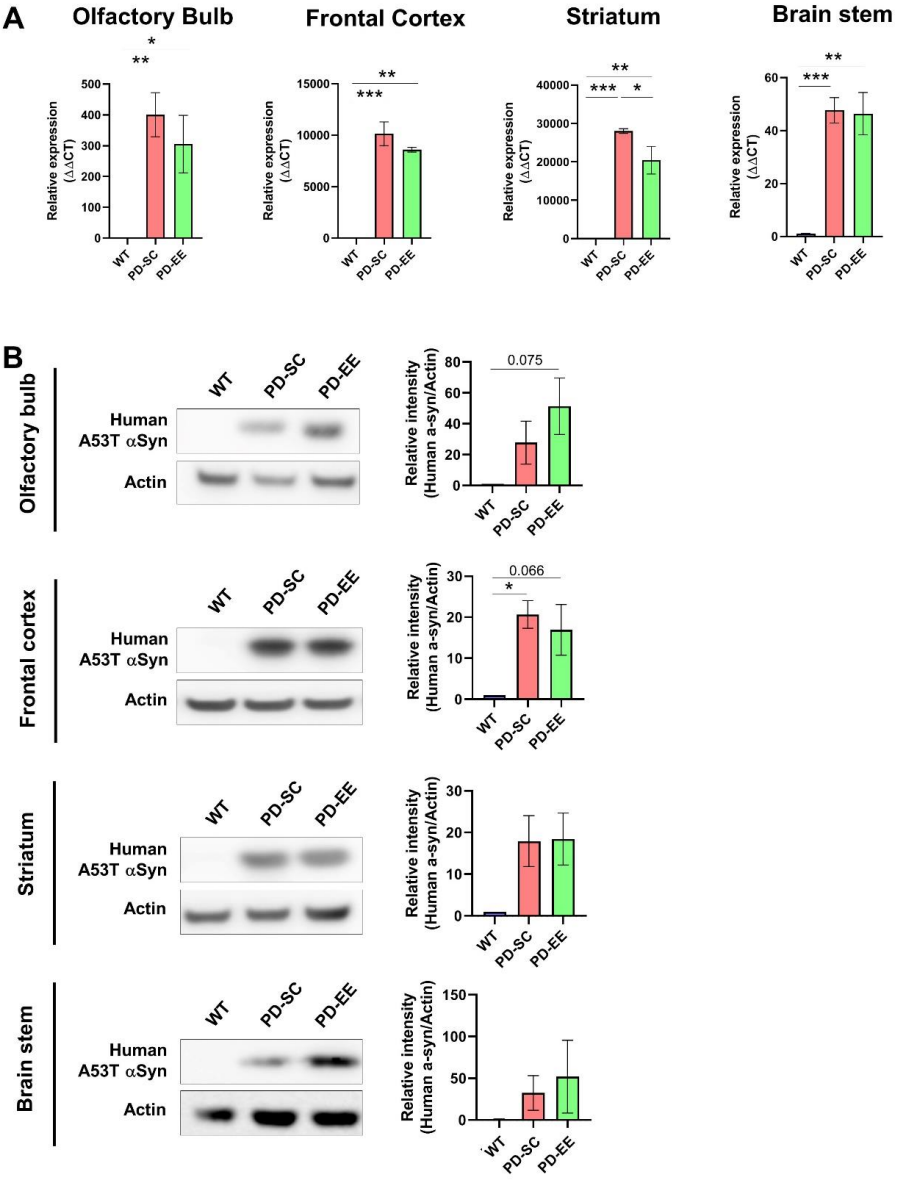

Figure 9. Expression of human A53T α-synuclein measured by (A) qRT-PCR and (B) Western blotting. (A) Human A53T α-synuclein was overexpressed in both the PD-SC and PD-EE mice compared to the WT. Significant differences were observed between PD-SC and PD-EE at the striatum, with decreased expression in the PD-EE mice. (B) Significant

differences of human A53T α-synuclein protein were observed at the frontal cortex between the WT and PD-SC mice. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; one-way ANOVA followed by least significant difference for post-hoc analysis.

3. Expression of mouse α-synuclein

Although low levels of mouse α-synuclein monomer expression were observed in all the regions of the WT mice, significant levels of expression were observed in the PD mice, except at the OB of PD-EE mice. Oligomers of α-synuclein were expressed at the OB, striatum, and brain stem of the PD-SC mice, while those were little seen at the frontal cortex even of the PD-SC mice (Figure 10).

Quantification analysis of Western blotting showed that the expression of α-synuclein had a tendency to increase in both the PD-SC and PD-EE compared to the WT, showing significant difference between the WT and PD-EE at the frontal cortex and striatum. Although more high expression of phosphorylated α-synuclein was observed in PD-SC and PD-EE also, statistical differences were not found among the experimental groups (Figure 11).

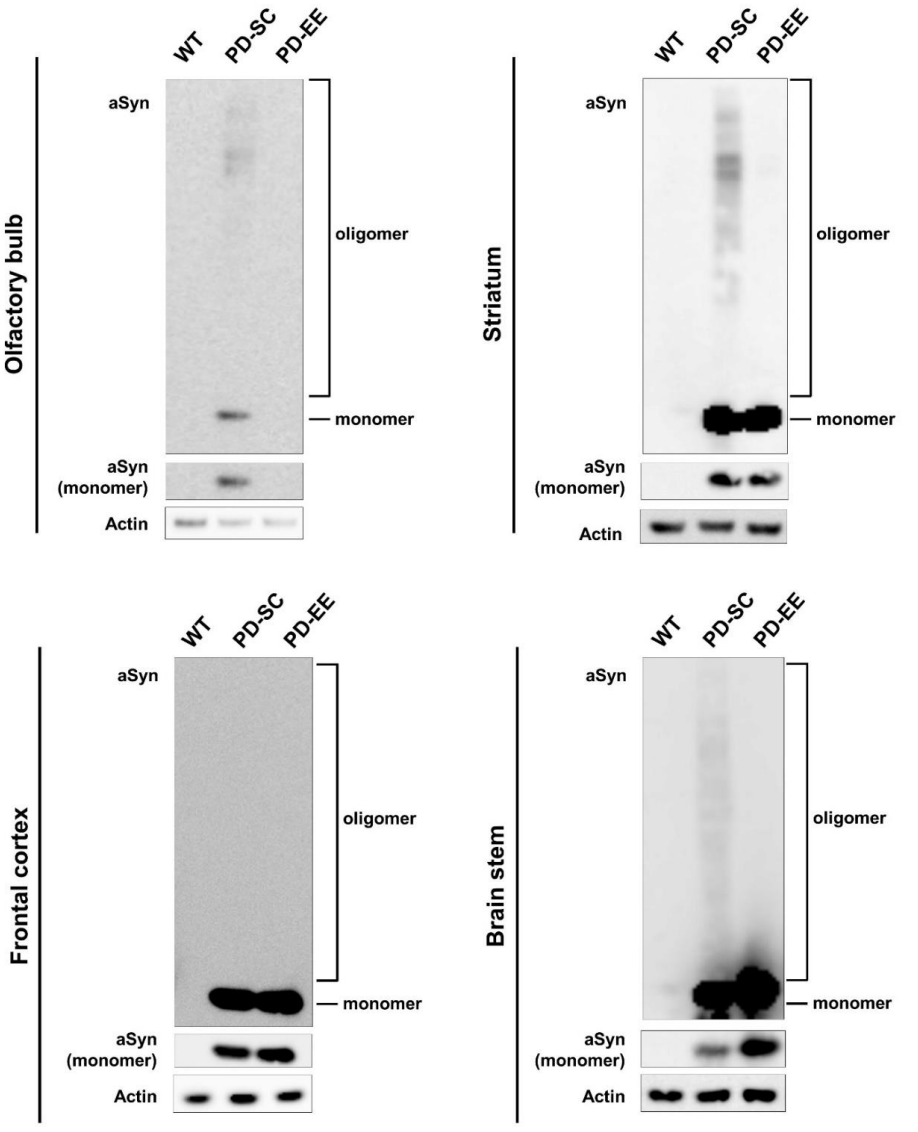

Figure 10. Expression of mouse α-synuclein monomers and oligomers. The expression of mouse α-synuclein protein was low across all the studied regions in the WT mice. Except for the scanty levels mouse α-synuclein protein oligomer expression in the frontal cortex of the experimental groups, oligomers were found to be significantly expressed in PD-SC mice. However, oligomers were almost not observed in the PD-EE or WT mice.

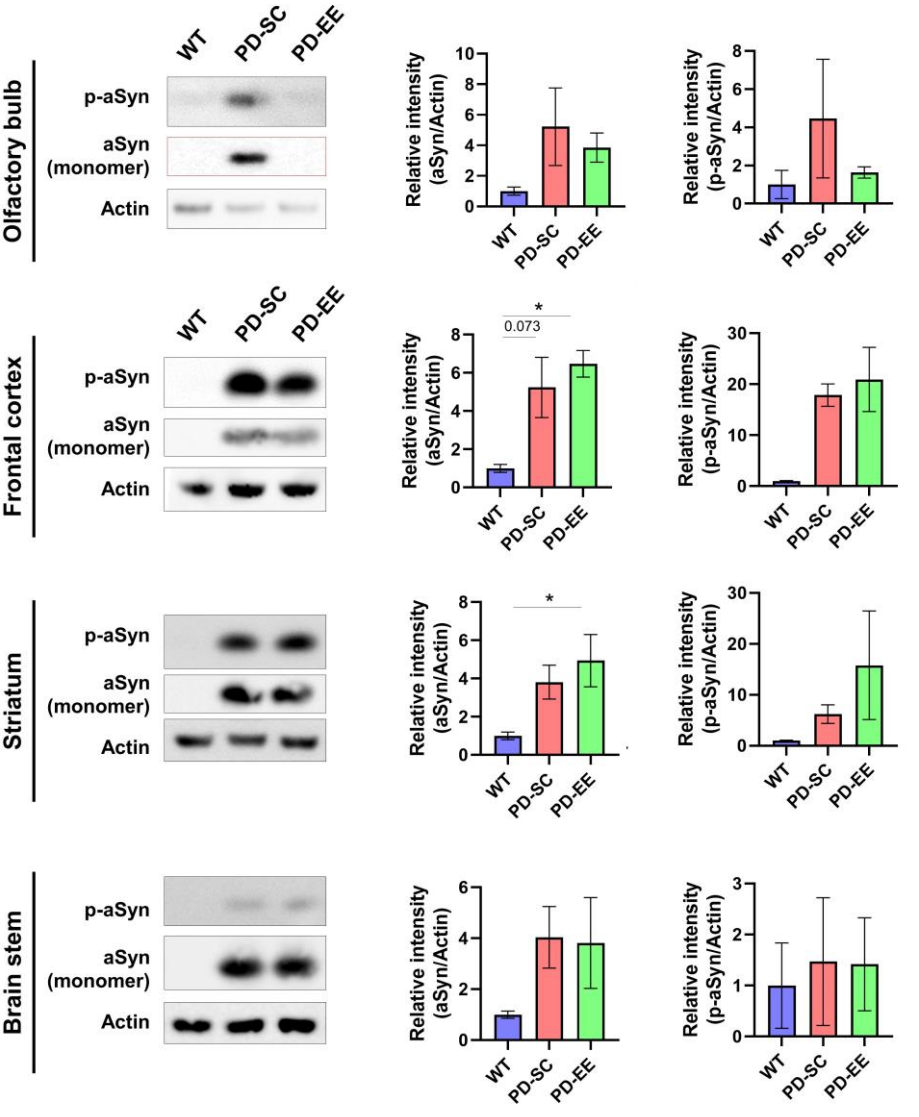

Figure 11. Expression of mouse α-synuclein monomer and phosphorylated α-synuclein. Significant higher expression of mouse α-synuclein was observed in the PD-EE compared to the WT at the frontal cortex and brain stem.

4. Measurement of iNOS at the OB

iNOS, a representative indicator of oxidative stress, was only observed in the OB, and showed similar results to the expression of nitrated α-synuclein. The density of iNOS staining was higher in the PD-SC mice than in both the WT and PD-EE groups. There was no significant difference in density between the WT and PD-EE mice (Figure 12).

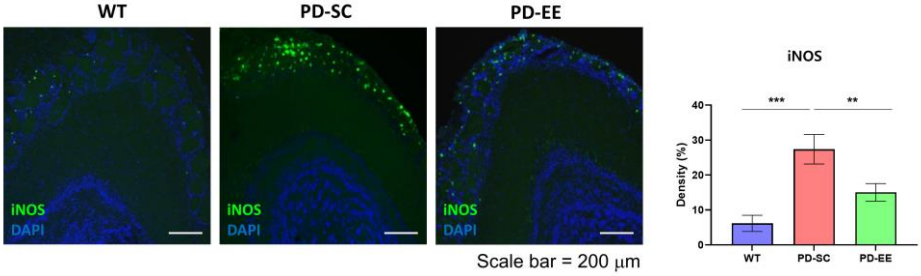

Figure 12. iNOS evaluated at the OB by IHC. The density of iNOS was higher in PD-SC mice compared with both WT and PD-EE mice. There was no significant difference between WT and PD-EE mice. Scale bar = 200 μm. *P < 0.05; **P < 0.01; ***P < 0.001; one-way ANOVA followed by least significant difference for post-hoc analysis.

5. The ratio of GSH/GSSG

The GSH/GSSG ratio is inversely correlated with oxidative stress and was found to be significantly lower in the PD-SC mice than the WT mice at the OB. The ratio was significantly highest in the PD-EE and lowest in the PD-SC among the groups. In the brain stem, the ratio was lower in the PD-SC compared to both WT and PD-EE. However, difference in GSH/GSSG was not observed in the striatum among the groups (Figure 13).

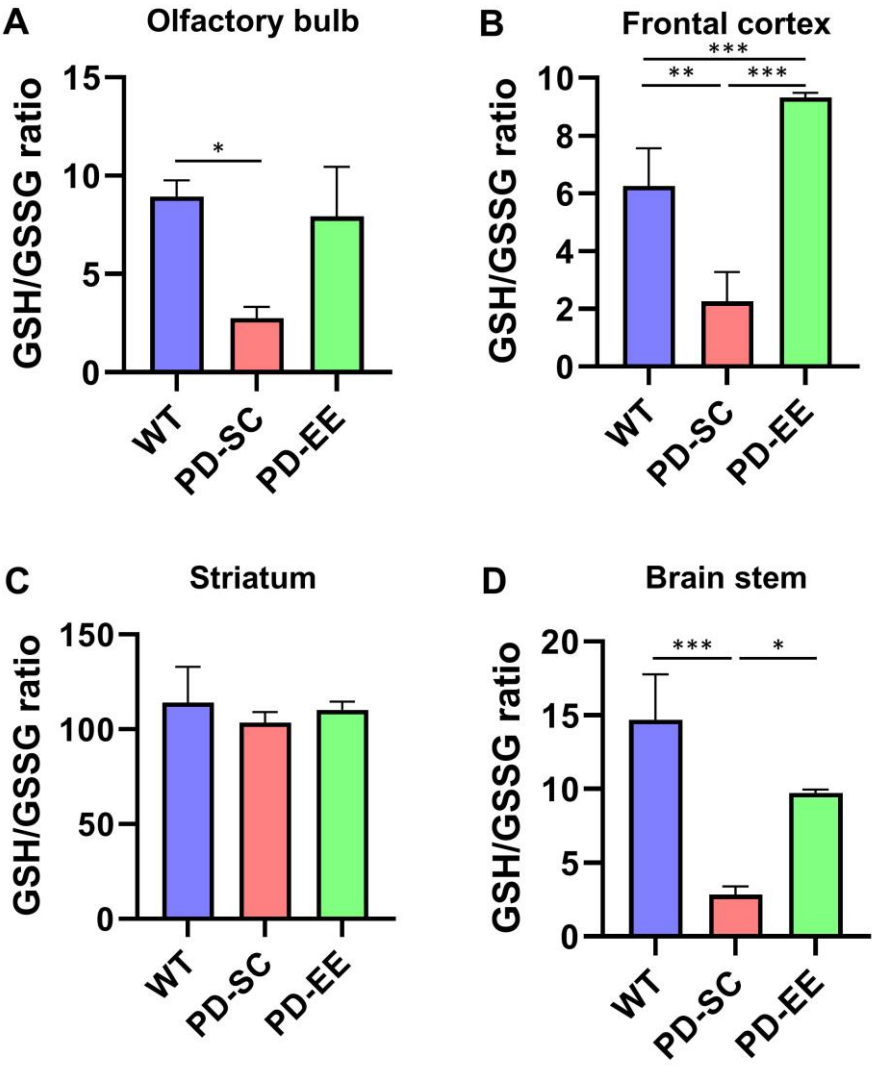

Figure 13. The ratio of GSH/GSSG. (A) GSH/GSSG was lower in the PD-SC mice than the WT mice in the OB. (B) GSH/GSSG was lowest in the PD-SC and highest in the PD-EE. (C) In the striatum, no significant difference in GSH/GSSG ratio was observed among the experimental group. And (D) the ratio was lower in the PD-SC compared to the WT and PD-EE in the brain stem. *P < 0.05, **P < 0.01; ***P < 0.001; one-way ANOVA followed by least significant difference for post-hoc analysis.

6. Expression of TH

The expression of TH, the enzyme responsible for catalyzing tyrosine into 3,4-dihydroxyphenylamine (L-DOPA), was not found to be significantly difference among the brain regions of the experimental groups (Figure 14).

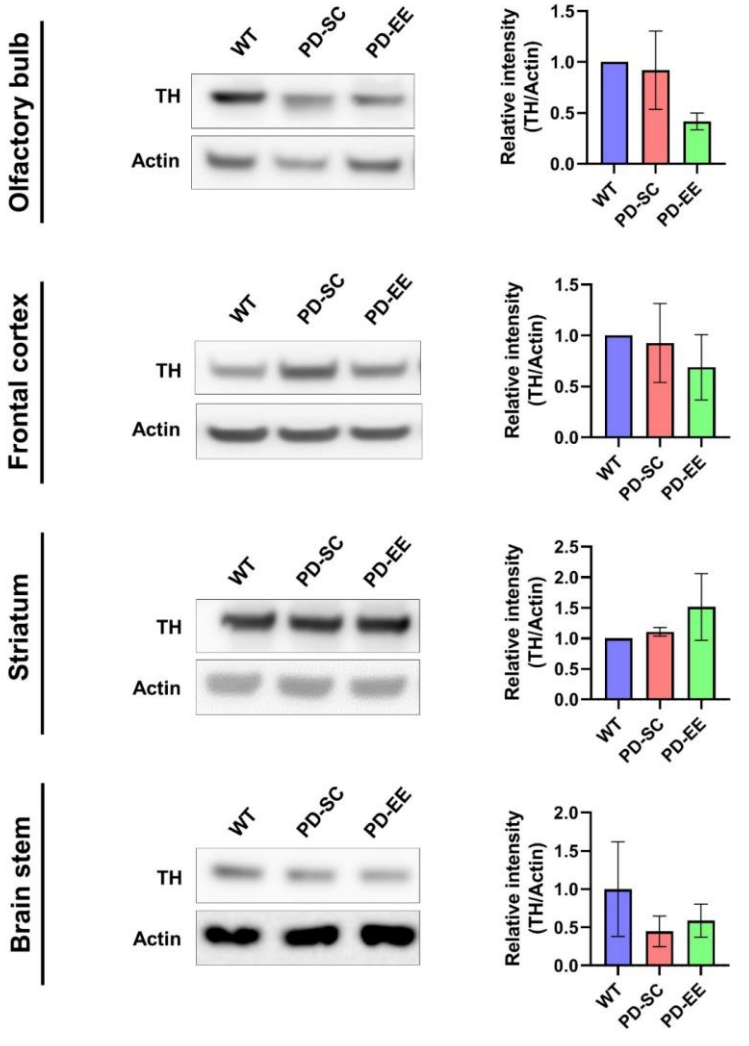

Figure 14. The expression of TH measured by Western blotting. No significant differences were observed in the brain regions of the experimental groups.

7. Expression of GFAP and NeuN

At the OB and striatum, GFAP expression was highly expressed in the PD-SC group, and decreased in the PD-EE group. The expression of NeuN was lowest in PD-SC at the OB and brain stem; however, this was not statistically significant (Figure 15).

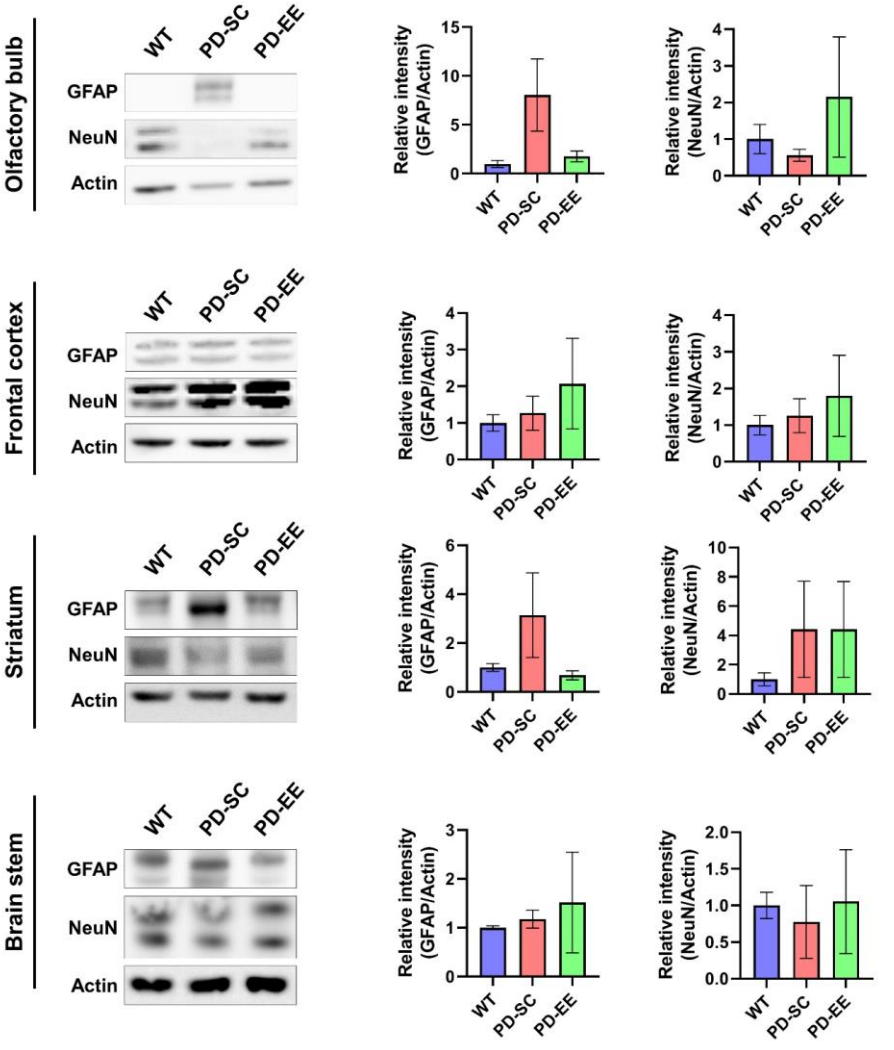

Figure 15. GFAP and NeuN expression measured by Western blotting. No significant differences were observed in the brain regions of the experimental groups.

8. Expression of Ki-67 at the SVZ

The concentration of Ki-67 positive cells was significantly lower in the PD-SC mice. EE was found to ameliorate this deterioration (Figure 16).

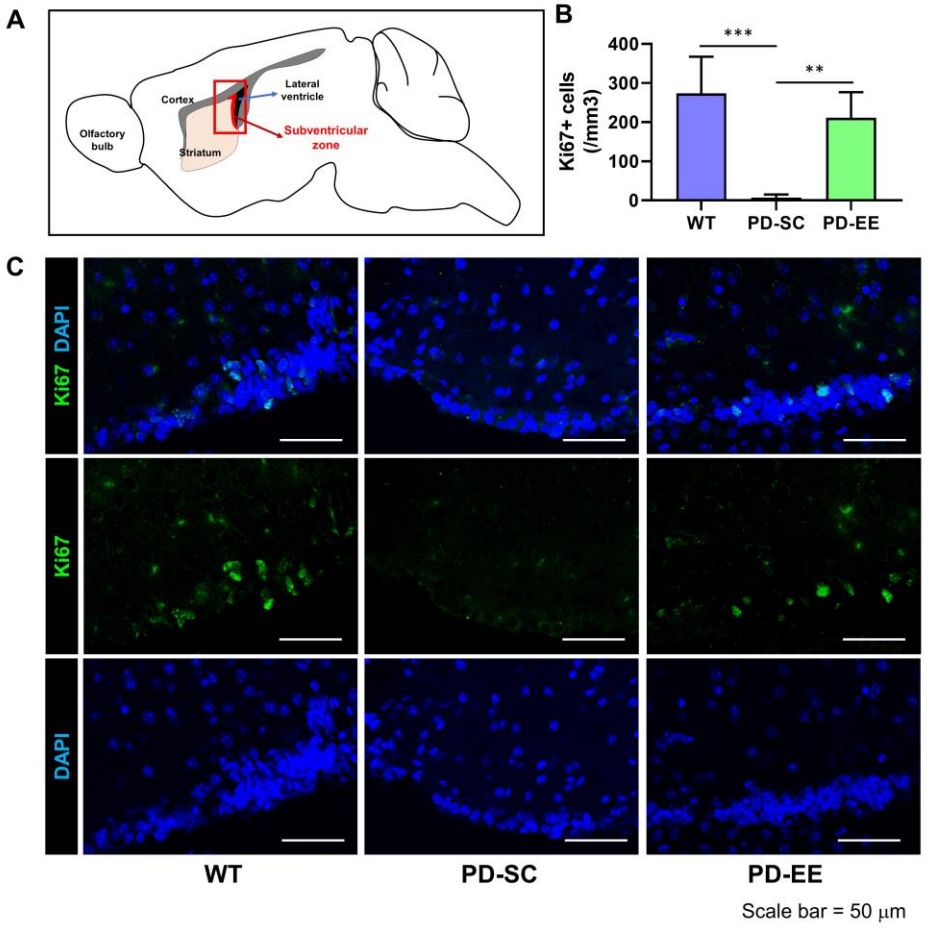

Figure 16. IHC of Ki-67 at the SVZ. (A) The density of Ki-67 was evaluated at the SVZ. (B) The density of Ki-67 was significantly lower in the PD-SC mice compared with both the WT and PD-EE mice. No significant differences were observed between the WT and PD-EE mice. (C) Representative confocal microscopic images of Ki-67^{+} cells at the SVZ of sagittal sections. *P < 0.05; **P < 0.01; ***P < 0.001; one-way ANOVA followed by least significant difference for post-hoc analysis.

9. Expression of TUNEL at the SVZ

The concentration of TUNEL positive cells was significantly lower in the PD-EE mice than the PD-SC mice. No significant differences between the WT and PD-SC mice and between the WT and PD-EE mice were observed (Figure 17).

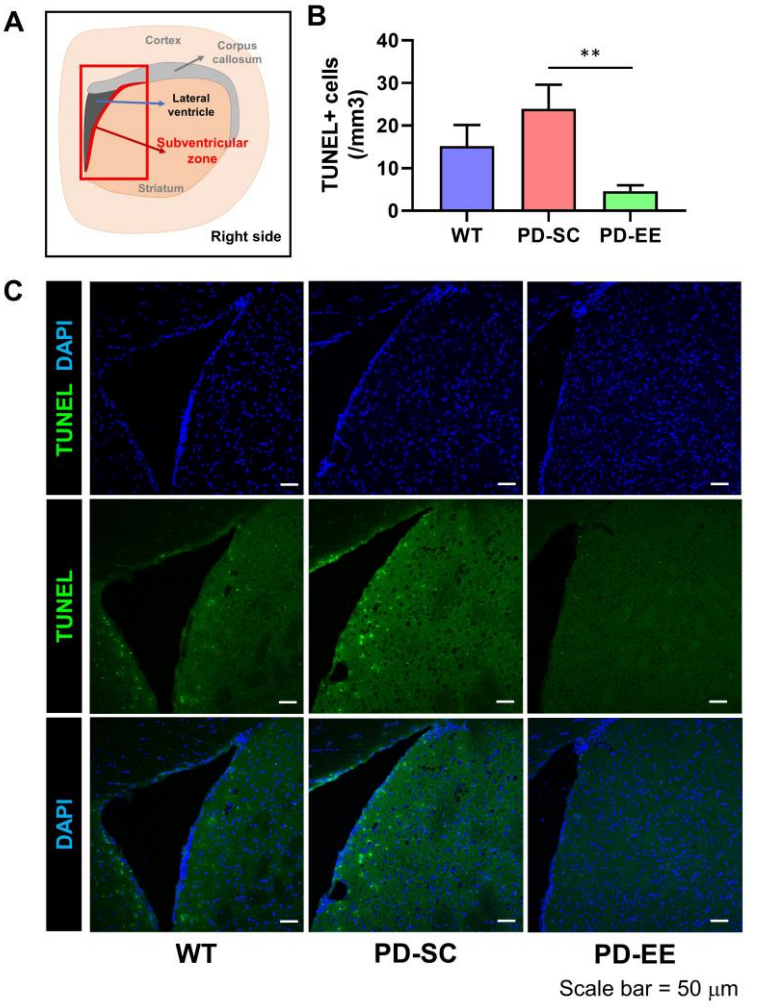

Figure 17. IHC of TUNEL at the SVZ. (A) The density of TUNEL was evaluated at the SVZ. (B) The number of TUNEL$^+$ cells were significantly decreased in the PD-EE mice compared to the PD-SC mice. (C) Representative confocal microscopic images of TUNEL$^+$ cells in the SVZ of coronal sections.

*P < 0.05; **P < 0.01; ***P < 0.001; one-way ANOVA followed by least significant difference for post-hoc analysis.

10. **Neurobehavioral tests**

At the age of 8 months and before EE intervention, the olfactory function of the experimental mice was evaluated using buried food tests, and the best results were observed in the WT mice. However, EE for 2 months ameliorated the deterioration of olfactory function. The hanging wire tests showed similar results, in which EE was found to improve the motor function to a similar level as the WT mice. In the rotarod test, the best outcome was observed in the PD-EE mice compared to the PD-SC mice (Figure 18).

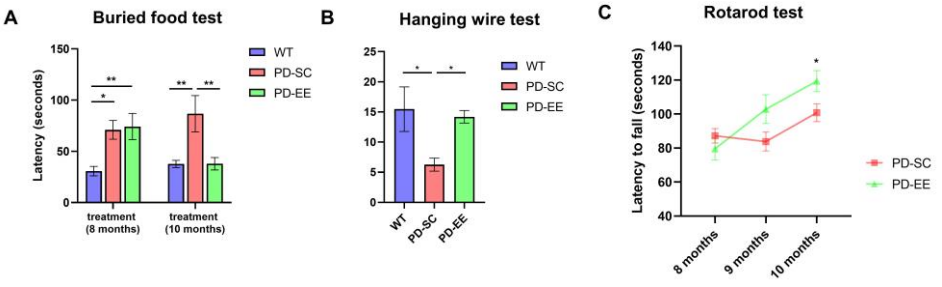

Figure 18. Results of neurobehavioral tests. (A) Buried food test result. The latency time of finding food in PD mice at 8 months of age. However, EE for 2 months significantly reduced the latency time. (B) Hanging wire tests evaluated at 10 months of age showed similar results. The PD-SC mice showed reduced motor function, while the PD-EE mice showed a similar level as the WT mice. (C) At 8 months of age, no significant differences were observed between the PD-SC and PD-EE mice. However, at 10 months of age, a better outcome was obtained in the PD-EE mice compared to the PD-SC mice. *P < 0.05; **P < 0.01; ***P < 0.001; one-way ANOVA followed by least significant difference for post-hoc analysis.

IV. DISCUSSION

Various xenobiotic and environmental toxins are associated with the pathogenesis of PD, including 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), pesticides, solvents, and metals, and endogenous toxins, including 4-phenylpyridine, *N*-methyl nicotinamide, and *N*-methyl-(R)-salsolinol.[37-43] As such, an altered capacity for detoxification is a potential risk factor for PD. Moreover, the abnormal function of detoxifying enzymes can result in the induction of mitochondrial dysfunction and reactive oxygen species.[10,11]

The *CYP* superfamily of enzymes are membrane-bound proteins containing heme as a cofactor. Although they are mainly expressed in the liver, a major site of detoxification, they are also found universally around the body, including in neuronal structures.[44] As catalytic enzymes, they most commonly act as mono-oxygenases, inserting one atom of oxygen into substrates, and may also act as oxidases and reductases in the Phase I metabolism.[45] Because *CYP* enzymes are responsible for the metabolism of a huge range of endogenous and xenobiotic compounds, they are widely associated with not only the drug metabolism, but also the pathogenesis of various diseases, including metabolic diseases, cardiovascular diseases, malignancies, and neuroinflammatory diseases.[44,46-48] Owing to the active function of *CYP* enzymes in neuronal tissues, their roles in various brain disorders are becoming increasingly evident.[49] *CYP1A2* exerts detoxification properties against xenobiotics, in particular MPTP, a well-known toxic material that has been found to induce PD in both animals and humans.[50-52] Caffeine consumption has been reported to provide neuroprotection against PD in epidemiological and meta-analysis studies.[53,54] Surprisingly, cigarette smoking is also known to lower the risk of PD.[55,56] It is thought that the induction of *CYP1A2* via caffeine and cigarettes occurs as a mechanism of neuroprotection against the pathogenesis of PD.[57,58] Another two

enzymes, *CYP2F2* and *CYP2A5*, are homologues of human *CYP2F1* and *CYP2A6*, respectively, and are mainly expressed in the respiratory tract. These enzymes participate in the activation of toxic materials, resulting carcinogenesis, especially lung cancer.[59,60] However, the roles of these enzymes in the central nervous system (CNS) have not yet been fully elucidated.

Carbonyl compounds are formed during the peroxidation of lipids and lipoproteins, as well as food poisoning, and are frequently found in various endogenous and xenobiotic compounds. Because reactive carbonyl groups modify proteins and DNA, they are closely associated with various neurodegenerative diseases.[61-64] Dicarbonyl compounds, which have dual carbonyl groups, are also generated by various oxidative processes, such as oxidative glycation, sugar autoxidation, and lipid peroxidation. The advanced glycation end (AGE) products, derived from modification of proteins by carbonyl and dicarbonyl compounds, induce the release of cytokines, free radicals, and the direct modification of the extracellular matrix and hormonal actions, as well as protein aggregation by oxidative stress. Therefore, AGE products are known to exert a wide range of pathological properties in various diseases, including connective tissue disease, end-stage renal disease, and neurodegenerative diseases.[65,66] *CBR* detoxifies reactive aldehyde, as one of carbonyls derived from lipid peroxidation, and is responsible for neuronal survival and protection against oxidative stress.[62] Because *CRYL1* also catalyzes the detoxification of α-dicarbonyls,[67] both enzymes may be associated with the pathogenesis of PD.

Aldehydes, one of carbonyl compounds, exist ubiquitously in all body tissues. Because they are a very reactive species, various enzymes, such as *AOX*, *CYP*, *aldo-ketoreductases, ADH*, *short-chain dehydrogenases/reductases*, and *ALDH*, are involved in the metabolism of aldehydes.[68] Aldehydes are catalyzed to the corresponding carboxylic acids via *ALDH* (via NAD(P)+ dependent oxidation) and *AOX* (in the presence of water and oxygen). *ADH*, *ALDH*, and

AOX are involved in neuroprotection and antioxidation via the metabolism of vitamin A (retinol) into retinoic acid, the most active form of retinoids. Retinoic acid participates in neuronal differentiation and neural tube patterning via signaling pathways that regulate the gene expression of retinol-binding proteins.[69,70] The dopaminergic system of the CNS is a target of the action by retinoic acid.[71] The *Aldh1a7* gene is expressed in the mouse, but in not humans, and shares identical residues of 84% and 91% similarity with *Aldh1a1* in humans and mice, respectively.[72] *ALDH1A1* degrades dopamine-3,4-dihydroxyphenylacetaldehyde (DOPAL) into 3,4-dihydrophenylacetic acid (DOPAC), blocking the α-synclein-related cytotoxicity of DOPAL.[73]

Thymine and uracil are catalyzed into β-alanine and β-aminoisobutyric acid, respectively, via three sequential reactions. *DPYS* is known to participate in the second stage.[74] β-alanine is a structural analogue with major inhibitory neurotransmitters, γ-aminobutyric acid (GABA), and glycine, and regulates synaptic transmission and dopamine levels.[74] β-aminoisobutyric acid is partial agonist of the glycine receptor and increases the excretion of leptin,[75,76] an important neurotrophic factor associated with brain-derived neurotrophic factor (BDNF) and reversal against dopaminergic cell loss.[77,78] *DYPS* is also involved in the detoxification of endogenous compounds and xenobiotics,[79-82] and is highly homologous with some non-catalytic proteins by itself.[83]

Although the relationship between *CES1D* and PD has not yet been clearly elucidated, it is thought that *CES1D*, as a triacylglycerol (TAG) hydrolase is related with PD pathogenesis via lipid metabolism.[84] TAG, a phospholipid, forms lipid droplets by combining with α-synyclein.[85] It has been reported that the levels of TAG decrease in PD patients, while high levels of TAG decrease the risk of PD.[86-88] The overexpression of α-synyclein increases the intracellular deposits of TAG and decreases the serum levels of TAG.[89,90] Given the decreased turnover of stored TAG and increased α-synuclein aggregation in

both the xenobiotic and genetic mutant models of PD,[91,92] it is believed that the intracellular deposition and reduced turnover of TAG are involved in the pathogenesis of PD.[85]

PON1 catalyzes the hydrolysis of oxons, which are toxic metabolites of organophosphates.[93] It exerts a neuroprotective action in addition to the detoxification of pesticides, including antioxidation, anti-inflammation, and protection against cardiovascular diseases via an inverse correlation with atherosclerosis.[93-95] As a result, mutated *PON1* is known as a risk of PD pathogenesis.[95-97]

In this study, higher levels detoxifying enzyme activity in the early stage of PD suggested reactive and compensatory increased responses. To counteract oxidative stress and restore the redox balance, cells are equipped with endogenous mechanisms, consisting of antioxidant enzymes.[98] The results of several previous studies are consistent with the findings of this concept. In a study by Mann et al.,[99] the activity of *CYP2D6*, which participates in the synthesis of tyramine and metabolizes tyramine to dopamine, was decreased in the frontal cortex, cerebellum, and hippocampus. However, in PD-affected areas, such as the substantia nigra and caudate, the activity of *CYP2D6* was similar to that in the control group.[99] Although the loss of dopaminergic cells is a major pathological element of PD, animal studies have showed increased numbers of dopaminergic cells at the striatum and OB in the early stages of PD.[100-102] This may represent compensatory mechanisms for the dopaminergic deficit and phenotypical changes, rather than true neurogenesis.[103] According to a human study by Godau el al.,[104] insulin-like growth factor 1 (IGF-1) levels, shown to be protective against the loss of dopaminergic cells, were found to be higher in patients with PD than in healthy controls, with higher levels in diseases of shorter durations. In another study, Johannsen et al.[105] also found a negative correlation between PD duration and the activity of erythrocyte glutathione peroxidase, a representative antioxidant enzyme. The hypothesis that metabolic

reactions are relatively increased in the brain in the early stages of PD is reinforced by our research tracking sequential changes.

In our preliminary study,[25] we chose to examine the OB as an induction site of PD, along with the enteric nerve cell plexus. We expanded the areas investigated to other dopaminergic structures, including the frontal cortex, striatum, and brain stem. Activity was found to be higher in the PD-SC mice and ameliorated by EE in the OB and similar tendency was observed in the brain stem. On the other hand, these tendencies were not significantly apparent in the frontal cortex and striatum. And in the striatum, it showed a tendency that activities of some enzymes were higher in the WT compared to other two experimental PD groups. These results suggest that the activity of detoxifying enzymes varies according to the brain regions.

First of all, it will be necessary to consider the characteristics of the OB further to determine why the results vary between the different regions. The OB acts as an inflow route of exogenous compounds due to its direct exposure to the external environment, and directly relays biological information to the CNS.[15,106] This characteristic of the OB is believed to allow it to function as an induction site of PD and is associated with the non-motor olfactory symptoms in the premotor period, which can precede motor symptoms by up to several decades.[6] The OB secretes antioxidants and detoxifying enzymes to provide protection against harmful substances.[12] Therefore, the OB has a high burden and dependence on detoxifying enzymes. Because the enteric nervous system (ENS), regulating GI motility, mucosal blood flow, and transport and resorption of ions and water has similar neural activities with the CNS, it is also known as "the little brain of the gut."[107,108] The ENS is very similar to the OB in terms of the putative entry of exogenous materials and its direct connection with the CNS via the vagal nerve.[6] Similar finding showing compensatory mechanisms in the ENS of the PD were reported by Barrenschee et al.[109] They found enhanced activity in vasoactive intestinal peptide (VIP), which counteracted

dopaminergic cell loss and neuroinflammation, in the ENS of PD patients.

In the same course of the disease, and even in the same subject, the disease stages of PD differs among the brain regions. Generally, the olfactory system and lower brain system are affected first. Then, the pathogenesis propagates to other sites, reaching the neocortex last.[6] Thus, we expect that the oxidative stress burden will vary depending on the brain site.

In addition, the vulnerability against oxidative stress is different for each brain region. A high utilization of oxygen and an enriched content of polyunsaturated lipids contribute to the vulnerability of the central nervous system to oxidative stress compared to other tissues.[62] Within the brain, the resiliency to oxidative stress varies by region,[110] with the substantia nigra (SN) being the more vulnerable to oxidative stress compared to the other brain regions.[111] There are several reasons why the SN is highly sensitive to oxidative stress: (1) The concentration of dopaminergic cells in the SN is high, and the dopamine itself is break down into oxidative metabolites through auto-oxidation.[112] (2) There is a high content of iron, which accelerate the auto-oxidation of dopamine and are involved in α-synuclein aggregation.[113] (3) The levels of monoamine oxidase activity are low.[114] (4) The level of melanin acting as a support matrix upon which ionized iron can catalyze oxyradical generation is high.[114] (5) The content of glutathione, a cofactor of peroxidases, is lower than in the other brain areas.[115] Cardoso et al. reported differences in the response of the SN and striatum to oxidative stress using sequential studies on rats. When the dietary provision of essential fatty acids, which are crucial for regulating oxidative stress, cell signaling, and apoptosis, was restricted, lipid peroxidation was found to increase in the SN but not the striatum in young animals (30~42 days). The activity of superoxide disputase (SOD) increased in the striatum, while that of catalase decreased in the SN, indicating that the SN was more vulnerable to oxidative stress than the striatum.[116] In a followed subsequent study using older animals (90~110 days), resilience of the striatum

against oxidative stress was found to decrease with age, based on the findings of increased lipid peroxidation in both the SN and striatum and decreased *SOD* activity in the striatum.[117] The striatum is thought to have a different defensive mechanism against dopaminergic cell loss than other regions of the CNS.[110]

Dopaminergic neurons are also referred to as bienzymatic neurons because they possess the ability to synthesize dopamine from L-tyrosine via subsequent reactions mediated by both tyrosine hydroxylase (TH) and aromatic L-amino acid decarboxylase (AADC). Non-dopaminergic monoenzymatic neurons, which have only one of the two enzymes, have been observed in various areas of the CNS.[118] Although their exact function has not yet been clearly elucidated, under conditions of dopaminergic cell loss, as observed in PD, TH-monoenzymatic neurons and AADC-monoenzymatic neurons cooperate to synthesize dopamine as an alternative mechanism of dopamine synthesis.[119] Both TH- and AADC monoenzymatic neurons are found in the striatum and substantia nigra. On the other hand, only TH-monoenzymatic neurons are found in OB and frontal cortex.[118] In other studies, biological markers related to neurogenesis and axonal growth were observed in the striatum, but not in the cerebellum or frontal cortex.[120,121]

Both clinical and experimental evidence of the impact of physical exercise on PD is increasing, whereby exercise has been found to induce various neuroprotection mechanisms.[122,123] Physical activity not only improves motor function, but also non-motor symptoms and cognitive function in PD.[124,125] Evidence is increasingly recommending the rehabilitation of PD patients as a complementary and indispensable treatment, not merely as an ancillary modality to pharmacological and surgical treatments.[126] Although the exact mechanism still needs to be clarified, exercise induces neuroplasticity. The best-known effect of exercise on brain plasticity is via neurotrophic factors. Various endogenous neurotrophins, such as BDNF, glial cell line-derived neurotrophic factor (GDNF), IGF-1, and vascular endothelial growth factor

(VEGF), are upregulated by exercise.[127] These substances prolong the survival of dopaminergic neurons and increase dopamine levels by stabilizing the intracellular concentration of calcium, reducing oxidative stress, suppressing neuroinflammation, inducing mitochondrial function, and consequently promoting neurogenesis.[123] Moreover, in human studies, increases in the binding potential of dopamine receptors and structural changes via rehabilitation have been observed in patients with PD.[128-130] Our study found that EE, a representative rehabilitation model in mice, reduced oxidative stress, lessened apoptosis, and suppressed expression of α-synuclein, on the other hand, induced neuronal proliferation. The normalized levels of detoxifying enzymes by EE suggests that exercise can reduce metabolic burden of detoxification, being resulted from neuroprotection and reduction of oxidative stress.

V. CONCLUSION

In a PD mouse model, the activity of detoxifying enzymes increased in the OB and brain stem during the early stage of disease and was stabilized by EE. However, in the other dopaminergic structures, namely the frontal cortex and striatum, enzymatic activity was not corresponded to the responses in the OB and brain stem. Thus, strategies for treating the pathogenesis of PD may vary from region to region in the brain. Enzymatic activity and motor functions, as well as olfactory function, were improved by EE. Our results indicate that rehabilitation has a protective effect against neuronal deterioration in PD and subsequent decreased detoxifying burden.

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ABSTRACT (IN KOREAN)

알파시누클레인병증 백서 모델에서 도파민 회로의 해독효소 활성도 측정을 통한 부유 환경의 파킨슨병에 대한 효과 조사

<지도교수 강성웅>

연세대학교 대학원 의학과

이 장 우

파킨슨병은 안정시 떨림, 운동 완서, 진전, 자세 불안정과 같은 주요운동증상을 특징으로 하는 신경퇴행성 질환으로 운동증상이 발생하기 전에 다양한 비운동성 증상들이 발생한다. 파킨슨병의 발병은 흑질 치밀부에서 도파민 신경세포의 소실 및 신경염증 및 산화스트레스와 관련된 루이소체가 연루되어 있는 것으로 알려져 있다. 완전한 병태생리가 아직 밝혀지지는 않았지만, 내인성 독소 및 외래 물질의 해독작용에 대한 대사의 변화가 가능성 있는 기전으로 생각된다. 우리는 선행 연구를 통해 형질전환 백서의 후구에서 독성 물질 대사에 참여하는 일부 효소들이 과발현되는 것을 관찰할 수 있었다. 또한 동물실험에서 재활치료의 모델로 여겨지는 부유 환경에 노출되었을 때에 이러한 과발현이 오히려 상쇄되는 현상을 보였다. 이러한 선행연구결과를 바탕으로, 본 연구에서는 후구 외에 전두엽, 선조체, 뇌간과 같은 다른 도파민 회로의 신경세포들에서 해독과정의 첫번째 단계인 1상 대사에 참여하는 효소들의 활성도를 조사하였다. 인간 A53T 알파-시누클레인을 과발현한 형질전환 백서를 8개월 째에 무작위로 두 군으로 나눠, 한 군은 표준 케이지에서, 다른 한 군은 부유 환경에서 2개월 간 사육하였다. 생후 10개월이 되었을 때 1상 대사 효소의 활성을 정량중합효소연쇄반응을 통해 측정하였고, 대조군으로는

10개월 간 표준 케이지에서 사육된 야생형 백서가 사용되었다. 후구와 뇌간에서는 파킨슨병 모델에서 대체로 해독효소가 과발현되고 이는 부유 환경을 통해 상쇄되는 경향성을 보였다. 선조체에서는 일부 효소의 활성도가 정상 대조군에서 제일 높은 활성도를 보였고, 전두엽에서는 실험군 간에 해독효소의 활성도에서 뚜렷한 차이가 관찰되지 않았다. 부유 환경은 뇌 조직에서 산화스트레스와 세포자연사의 감소, 알파시누클레인 발현 억제와 신경세포 증식과 연관되었다. 본 연구의 결과는 파킨슨병의 병태생리에 대해 대응하는 기전이 뇌의 부위별로 다르다는 점과 재활치료가 파킨슨병에서 신경세포의 손상을 줄임으로써 독성물질에 대한 부담을 감소시킨다는 점을 시사한다.

핵심되는 말 : 파킨슨병, 도파민 경로, 해독효소, 1상 대사, 재활, 부유환경

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