# Induction of synaptic plasticity by rehabilitation environment in Parkinson's disease with α-synucleinopathy

Soohyun Wi

Department of Medical Science

The Graduate School, Yonsei University

# Induction of synaptic plasticity by rehabilitation environment in Parkinson's disease with α-synucleinopathy

Soohyun Wi
Department of Medical Science
The Graduate School, Yonsei University

# Induction of synaptic plasticity by rehabilitation environment in Parkinson's disease with α-synucleinopathy

Directed by Professor Sung-Rae Cho

The Doctoral Dissertation
submitted to the Department of Medical Science
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy of Medical Science

Soohyun Wi

June 2018

This certifies that the Doctoral Dissertation of Soohyun Wi is approved.

---

Thesis Supervisor : Sung-Rae Cho

---

Thesis Committee Member #1: Jin Woo Chang

---

Thesis Committee Member #2: Hyongbum Kim

---

Thesis Committee Member #3: Philhyu Lee

---

Thesis Committee Member #4: Sung Hoon Kim

The Graduate school
Yonsei University

June 2018

# ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Prof. Sung-Rae Cho, my professor who made all the research possible with generous advice. I was interested in neuroscience because Sung-Rae Cho guided me to be interested in discovering and exploring during neuroscience research. I could not have imagined having a better advisor and mentor for my Ph.D study.

I would also like to show my appreciation to the member of my thesis committee: Prof. Jin Woo Chang, Prof. Hyongbum Kim, Prof. Philhyu Lee and Prof. Sung Hoon Kim for their insightful comments and encouragements.

My sincere thanks also goes to Ph.D Ji Hea Yu for helping me to be comfortable with my age and helping me to adjust to my life in the laboratory. And also thank you for giving me serious advice whenever I'm having a hard time.

And MinGi Kim! Thank you for filling my shortage of English every time I write my thesis. I am happy to eventually overcome hard times and graduate together with MinGi Kim and Jung Hwa Seo. I thank my all fellow labmates : Yoon-Kyum Shin, Suk-Young Song, Ahreum Beak, Soonil Pyo, Bae-Geun Nam and Eunju Cho in for the stimulating discussions, and for helping me to live a happy lab life.

I can not name all of them there are so many friends who supported me, but I thank all of my friends who have supported me silently.

I would like to thank my family: my parents and to my younger brother Dong Hee Wi for supporting me spiritually. I seem to have been able to walk without shaking because there are you.

June 2018

Soohyun Wi

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLE

**ABSTRACT**

Induction of synaptic plasticity by rehabilitation environment

in Parkinson's disease with α-synucleinopathy

Soohyun Wi

*Department of Medical Science*

*The Graduate School, Yonsei University*

(Directed by Professor Sung-Rae Cho)

Parkinson's dis ease (PD) is a neurodegenerative disease characterized by motor symptoms with accompanying non-motor symptoms such as olfactory loss, cognitive decline, depression and anxiety, which frequently appear in the early stage of the disease, including the pre-motor phase. Environmental enrichment (EE) which provides a complex combination of physical, cognitive and social

stimulations enhances synaptic plasticity and behavioral functions. However, there is no research on the effect of EE on non-motor symptoms in human A53T overexpressing transgenic mice. This study investigated the influence of an EE on change of synaptic plasticity in striatum and nucleus accumbens during the initial phase of PD. Eight-month-old human A53T overexpressing mice were randomly allocated to EE or standard conditions (SC) for two months. EE significantly ameliorated hyperactivity and anxiety in A53T mice which was demonstrated by an open field test. Dopaminergic neurons in the substantia nigra pars compata (SNpc) and ventral tegmental area (VTA) had not degenerated at 10 months of age. Dopaminergic nerve terminals of the striatum significantly decreased about 20% in A53T SC and A53T EE compared to wild-type (WT). Those of the nucleus accumbens significantly decreased in A53T SC compared to WT group and tended to increase in A53T EE compared to A53T SC. By alteration of dopamine transporter (DAT) and dopamine receptor 1 (DRD1), EE exposure may normalize dopamine level in striatal dopaminergic nerve terminals and decrease phosphorylated α-Synuclein (aSyn). Also, EE exposure improves the expression of SNARE complex forming genes such as SNAP-25, Syntaxin1 and VAMP-2 downregulated in the striatum of A53T mice. In conclusion, this study suggests that EE induces synaptic plasticity in dopaminergic nerve terminals in striatum and nucleus accumbens and ameliorates non-motor symptoms in pre-motor phase of PD.

Key words: Parkinson's disease, pre-motor phase, environmental enrichment, A53T transgenic mouse model, dopamine, phosphorylation α-synuclein, SNARE complex

# Induction of synaptic plasticity by rehabilitation environment in Parkinson's disease with α-synucleinopathy

Soohyun Wi

*Department of Medical Science*

*The Graduate School, Yonsei University*

(Directed by Professor Sung-Rae Cho)

## I. INTRODUCTION

α-Synuclein (aSyn) is the main constituent of the neuropathological lesions found in patients with Parkinson's disease (PD), dementia with Lewy bodies (DLB), multiple system atrophy (MSA), and other disorders collectively known as α-synucleinopathies.[1-3] Point mutation of A53T of the aSyn has been identified the in familiar form of PD and A53T mutant aSyn (A53T) is associated with early onset Parkinson's disease (PD) and Lewy bodies with highly phosphorylated aSyn. [4,5] Parkinson's disease is classically defined by progressive degeneration of the

dopaminergic nigrostriatal pathway and by the emergence of rigidity, tremor, and bradykinesia.[6] However, PD also accompanied by non-motor symptoms, including olfactory loss, cognitive decline, sleep disorders, gastrointestinal disorders, sensory disorders, depression and anxiety, which frequently appear in the early stage including the pre-motor phase.[7] Non-motor symptoms are increasingly recognized as major challenges in the treatment of PD, because theses affect many patients and are only partly resolved by conventional antiparkinsonian medications.

Environmental enrichment (EE) is a method of raising animals in a huge cage containing novel objects, running wheels, and providing social interaction with a complex combination of physical, cognitive, and social stimulations.[8] In clinical study, EE used as a rehabilitation therapy for human patients.[9,10] Long-term EE treatment is compatible with rehabilitation therapies for human patients with PD. Epidemiological studies support a link between strenuous exercise and reduced risk for PD.[11,12] Additionally, many studies on exercise in normal aging and in PD supports the benefits of exercise, physical activity, and EE.[13-15] Therapeutic effect of an EE in behavioral recovery of motor function by the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydrophyridine (MPTP) in a mouse model of PD pathology have been reported.[16,17] However, there is no research on the effect of an EE on non-motor symptoms of the pre-motor phase in human A53T overexpressing PD mice. Through this transgenic mouse model of PD, we investigated the influence of an EE

on the induction of synaptic plasticity in striatum and nucleus accumbens in the initial phase of PD.

## II. MATERIALS AND METHODS

### 1. Transgenic mouse model

The human alpha-synuclein (A53T) transgenic line G2-3 (B6.Cg-Tg (Prnp-SNCA*A53T) 23Mkle/J; Jackson Laboratories, stock no. 006823, Bar Harbor, ME, USA) was used to generate both wild-type (WT) and transgenic (Tg) mice (Figure 1A). The Tg mice produced heterozygous offspring that overexpressed one copy of A53T mutant human alpha-synuclein. All animals were housed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) and provided food and water *ad libitum* with alternating 12-hour light/dark cycles, according to animal protection regulations. The experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC 2017-0039).

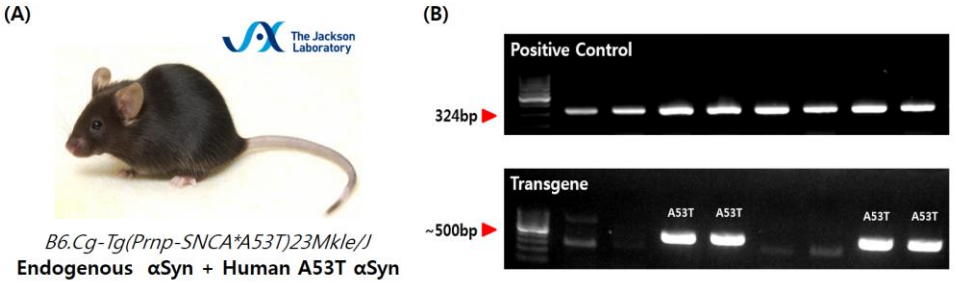


**Figure 1. Transgenic mouse model of Parkinson's disease and genotyping. (**A) Human A53T aSyn overexpressing mouse from the Jackson laboratory. (B) Separeted PCR. Internal positive control band shows in 324 bp and transgene band shows in about 500 bp.

**2. Genotyping**

Genotyping of mice was performed based on a protocol from Jackson Laboratories (Figure 1B). Genomic DNA (gDNA) was extracted from a 2-mm piece of each mouse tail using the standard procedure of the *prep*GEM Tissue Kit (ZyGEM, New Zealand). The mouse tail tissue was incubated with 1 ul of *prep*GEM, 10 ul of Buffer Gold, and 89 ul of autoclaved 3' distilled water at 75°C for 15 minutes and 95°C for 5 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). Electrophoresis was performed by loading 10 ul of each PCR product on 1.5% agarose gel.

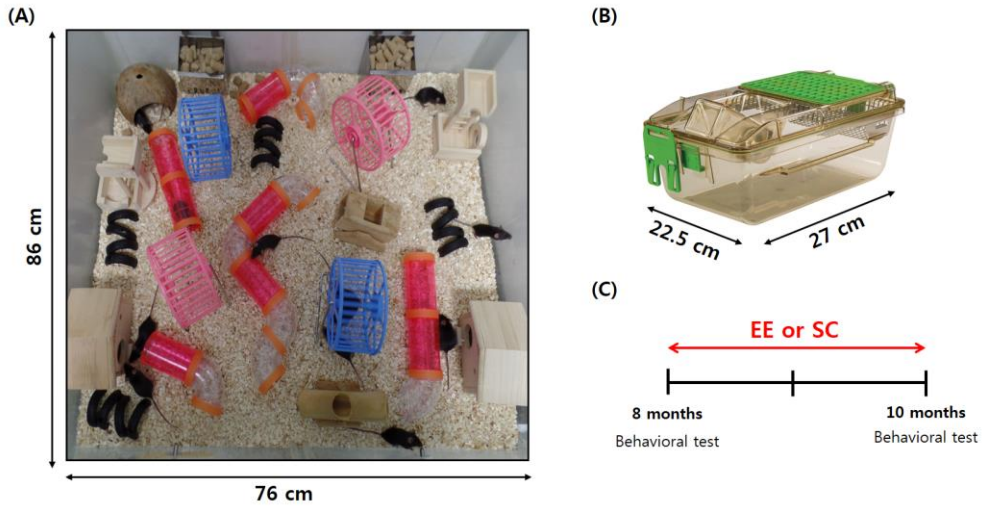


**Figure 2. Experimental design.** (A) Environmental enrichment (86 × 76 × 31 $cm^3$). (B) Standard cage (27 × 22.5 × 14 $cm^3$). (C) Schematic schedule of the study.

### 3. Enriched Environment

The EE mice were housed in a huge cage (86 × 76 × 31 $cm^3$) containing novel objects, such as tunnels, shelters, toys, and running wheels for voluntary exercise, and conditions allowing for social interaction (10 mice/cage) for two months, whereas the control mice were housed for the same duration in standard cages (27 × 22.5 × 14 $cm^3$) (Figure 2A-C).


**Figure 3. Behavioral tests for assessment of motor function.** (A) Cylinder rearing test. (B) Grip strength test. (C) Hanging wire test.

**4. Behavioral assessment**

*A. Cylinder rearing test*

When a mouse is placed in the cylinder it will spontaneously rear and use its forepaws for support (Figure 3A). For this test, the number of times each forelimb contacted the cylinder wall (Jeung Do B&P, Seoul, Korea) while the mouse was rearing, was counted over a period of five minutes.[18]

*B. Grip strength test*

A grip strength test was performed using the SDI Grip Strength System (San Diego Instruments Inc., San Diego, CA), which includes a push-pull strain gauge (Figure 3B). A triangular piece of metal wire 2 mm in diameter was used as the grip bar. Each animal was held near the base of its tail and was moved in close proximity to the bar until the animal could grip the bar with its forepaws. The apparatus

automatically registered peak force in gram-force. The mean peak force of three trials was used for analysis.[19]

*C. Hanging wire test*

Mice suspended by their forelimbs from a horizontal rod (5 mm × 5 mm area, 35 cm long, between two poles 50 cm high) tend to support themselves with their hind limbs to prevent themselves from falling and to aid their progression along the rod. For this test, suspension latencies were recorded for five minutes (Figure 3C).[20]

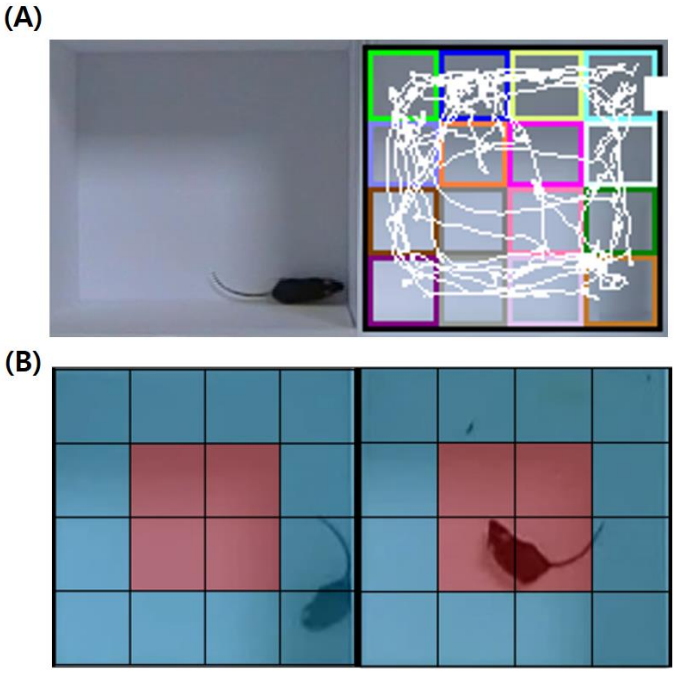


**Figure 4. Open field test.** (A) Activity monitoring was conducted in a square area (30 × 30 × 30 $cm^3$). (B) The floor was divided into 16 sectors. The four red sectors were the inner zone, and the 12 blue sectors were the outer zone. First, each mouse was placed into the outer zone and allowed to explore freely for 25 minutes while a video camera recorded. The resulting data was analyzed using video tracking system software.

*D. Open field test*

The open field test is generally used to evaluate locomotor activity and spontaneous exploration in a novel environment (Figure 4A).[21,22] Activity monitoring was conducted in a square area measuring 30 × 30 × 30 $cm^3$. The area's floor was divided into 16 sectors (Figure 4B). The four inner sectors represented the center, while the 12 outer sectors were defined as the periphery. Total time spent in the center was recorded as an index of anxiety.[23,24] Mice were placed individually into the periphery of the area and were allowed to explore freely for 25 minutes while being monitored with a video camera. The resulting data were analyzed using the Smart Vision 2.5.21 (Panlab, Barcelona, Spain) video tracking system.

**5. RNA preparation**

Total RNA was extracted from the mouse striatum and nucleus accumbens using Trizol (Invitrogen Life Technologies, Carlsbad, CA). After DNase digestion and clean-up procedures, RNA samples were quantified, aliquoted, and stored at -80°C until further use. The purity of the RNA was evaluated by the A260/A280 ratio and analyzed with an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA).

**6. Reverse transcription polymerase chain reaction (RT-PCR)**

Complementary DNAs (cDNAs) were synthesized from sample RNAs with the ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO, Osaka, Japan). Then, 1 $\mu$L of cDNA in a total volume of 20 $\mu$L was subjected to PCR using a RT-PCR analysis kit (Bioneer, Daejeon, South Korea) according to the manufacturer's instructions and a Gene Amp PCR system 9700 (Applied Biosystems/Life Technologies, Carlsbad, CA).

**7. Quantitative real time polymerase chain reaction (RT-qPCR)**

Complementary DNAs (cDNAs) were synthesized from sample RNAs with the ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO, Osaka, Japan). The following reaction used 1 $\mu$L of cDNA in a total volume of 20 $\mu$L. Real time quantitative polymerase chain reaction was performed in triplicate on a LightCycler 480 (Roche Applied Science, Mannheim, Germany) using the LightCycler 480 SYBR Green master mix (Roche Applied Science), and the thermocycler conditions were as follows: amplifications were performed starting with a 5-minute template preincubation step at 95°C, followed by 40 cycles at 95°C for 20 seconds, 62°C for 20 seconds, and 72°C for 15 seconds. Melting curve analysis began at 95°C for five seconds, followed by one minute at 60°C. The specificity of the produced amplification product was confirmed by melting curve analysis which showed a distinct single sharp peak with the expected *Tm* for all samples. The *GAPDH* gene was used as the internal control. The expression level of each gene of interest was obtained using the $2^{-\Delta\Delta Ct}$ method.

**Table 1. List of primers used for RT-PCR and RT-qPCR quantification**

| Gene | | Primer sequences |
|---|---|---|
| *VAMP-2* | Forward | 5′- GAGCGGGACCAGAAGTTGTC-3′ |
| | Reverse | 5′-GCGCAGATCACTCCCAAGATG-3′ |
| *SNAP-25* | Forward | 5′- GGATGAGCAAGGCGAACAAC-3′ |
| | Reverse | 5′-TCCTGATTATTGCCCCAGGC-3′ |
| *Syntaxin1* | Forward | 5′- AGGATCGGACTCAGGAGCTG-3′ |
| | Reverse | 5′-CTCACCCTGGCTCTCTACGA-3′ |
| *DRD1* | Forward | 5′- ACCCAGCCATTCTGCATTGA-3′ |
| | Reverse | 5′-CAGCCCCGTTGTTGTTGATG-3′ |
| *DRD2* | Forward | 5′- CTACATCGTTCTCCGCAAGC-3′ |
| | Reverse | 5′-CCATTCTCCGCCTGTTCACT-3′ |
| *DAT* | Forward | 5′- CAACAACACCTGGAACAGCC-3′ |
| | Reverse | 5′-AATGCCACGACTCTGATGGA-3′ |
| human *A53T aSyn* | Forward | 5′- AGGCTCCAAAACCAAGGAGG-3′ |
| | Reverse | 5′- TTCTTGCCCAACTGGTCCTT-3′ |
| *GAPDH* | Forward | 5′-CATCACTGCCACCCAGAAGACTG-3′ |
| | Reverse | 5′-ATGCCAGTGAGCTTCCCGTTCAG -3′ |

**8. Western Blot Analysis**

Striatum and nucleus accumbens were lysed in 500 μl of cold RIPA buffer (50 mM Tris-HCl, pH 7.5, 1% Triton X-100, 150 mM NaCl, 0.1% sodium dodecyl sulfate (SDS), 1% sodium deoxycholate) with a protease inhibitor cocktail (Sigma). Tissue lysates were centrifuged at 13000 rpm at 4°C for 20 minutes, the supernatant was extracted, and protein concentrations were measured using the Bradford method. Extracted protein (50 μg) was dissolved in sample buffer (60 mM Tris–HCl, pH 6.8, 14.4 mM b-mercaptoethanol, 25% glycerol, 2% SDS, and 0.1% bromophenol blue; Invitrogen), incubated for 10 minutes at 80°C, and separated on a 4-12% SDS reducing polyacrylamide gel (Invitrogen). Separated proteins were equally loaded and transferred onto polyvinylidene difluoride membranes (Invitrogen) using a trans-blot system (NovexR Mini-Cell; Invitrogen). Blots were blocked for one hour in Tris-buffered saline (TBS) (10 mM Tris–HCl, pH 7.5, 150 mM NaCl) containing 5% nonfat dry milk (Bio-Rad) at room temperature and washed three times with TBS. Blots were then incubated at 4°C overnight with a SNAP-25 (1:1,000, Abcam) antibody, a Syntaxin1 (1:1,000, Santa cruz) antibody, a VAMP-2 (1:1,000, Synaptic systems), a DAT (1:1,000, Chemicon), a DRD1 (1:1,000, Proteintech, Manchester, UK), a DRD2 (1:1,000, Abcam), and an aSyn (1:1,000, Abcam), an aSyn pSer129 (1:1,000, Abcam) in TBST (10 mM Tris pH 7.5, 150 mM NaCl, and 0.02% Tween 20) containing 3% nonfat dry milk. After incubation, the blots were washed three times with TBST and incubated for one hour with horseradish

peroxidase-conjugated secondary antibodies (1:3,000, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at room temperature. A housekeeping gene was evaluated with Actin-antibody (1:1,000, Santa Cruz Biotechnology). After washing three times with TBST, blots were visualized with an ECL detection system (Amersham Pharmacia Biotech, Little Chalfont, UK). Results of Western Blot were analyzed using Multi Gauge (Fuji photo firm, version 3.0, Tokyo, Japan).

**9. Immunohistochemistry**

Animals were euthanized and perfused transcardially with 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer, pH 7.4. Brains were removed and post-fixed 1 hour, followed by cryoprotection in 30% sucrose in Tris-buffered saline containing 0.02% sodium azide. Harvested brain tissue was cryosectioned with a slice thickness of 16-μm along the sagittal or coronal plane and immunohistochemistry staining was performed on four sections. For immunofluorescence double labeling, sections were stained with primary antibodies against rabbit anti-SNAP-25 (1:400, Abcam, Cambridge, UK), mouse anti-Syntaxin1 (1:400, Santa Cruz Biotechnology, CA, USA), mouse anti-VAMP-2 (1:400, Synaptic Systems, Gottingen, Germany) rabbit anti-TH (1:400, Abcam), rabbit anti-aSyn (1:400, Abcam), rabbit anti-phosphoserine 129 aSyn (1:100, Abcam), rabbit anti-iNOS (1:400, Abcam), anti-aSyn nitrate Tyrosine 133 (1:400, Novus, Littleton, Colorado, USA), rat anti-DAT (1:400, Chemicon, Pemecula, CA, USA), and secondary antibodies such as Alexa Fluor® 488 goat anti-Rabbit (1:400, Invitrogen, Carlsbad, CA, USA), Alexa Fluor® 594 anti-Mouse (1:400, Invitrogen), and Alexa Fluor® 594 anti-Rat (1:400, Invitrogen). Stained sections were mounted on glass slides with fluorescent mounting medium containing 4',6-diamidino-2-phenylindole (DAPI; Vectorshield, Vector, Burlingame, CA, USA). Stained sections were analyzed using a fluorescent microscope (Axio Imager M2, Zeiss, Gottingen, Germany) and confocal microscopy (LSM700, Zeiss, Gottingen, Germany).

For 3,3'-diaminobenzidine (DAB) staining of mouse brain, sagittal or coronal sectioned tissue was washed in phosphate-buffered saline (PBS) for one hour and blocked with universal blocking solution (Triton ™ X-100 (Sigma Aldrich, St Louis, MO, USA), 1% bovine serum albumin (BSA) in PBS) for one hour at room temperature. Primary antibodies of rabbit anti-TH (1:2,000, Abcam) and rabbit anti-phosphoserine129 aSyn (1:2,000, Abcam) were diluted in 0.5% BSA and incubated overnight at 4°C according to manufacturer instructions. The following day, brain tissue was treated with rabbit biotin secondary antibody (1:350), followed by avidin-biotin complex (Vector Laboratories, Burlingame, CA, USA) and visualized with 3,3'-diaminobenzidine (DAB) peroxidase substrate solution (0.05% DAB, 0.012% $H_2O_2$ in 0.1M PB). After dehydration, coverslips were mounted on glass slide using Permount ™ mounting medium (Fisher Scientific, Hampton, NH, USA ). Stained samples were analyzed under a bright-field microscope (Olympus, Tokyo, Japan).

**10. Statistical analysis**

Statistical analysis was performed using Statistical Package for Social Sciences (SPSS) software IBM Corp. Released 2014. IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp). Data are expressed as the mean ± standard error of the mean (SEM). The results of behavioral tests, RT-qPCR, Western blot, and immunohistochemistry were analyzed by the one-way ANOVA followed by *a post-hoc* Bonferroni test to adjust the variance for multiple testing effects (wild-type, A53T SC and A53T EE). A P-value $< 0.05$ was considered statistically significant.

## III. RESULTS

### 1. Environmental enrichment had no effect on human A53T aSyn mRNA and protein expression

Eight-month old A53T mice were randomly allocated to either EE or SC group (n = 15~25 per each group) for two months. The RT-qPCR result (Figure 5A) shows that relative expression levels of human A53T aSyn mRNA did not significantly differ between A53T SC (n = 6, 1.0 ± 0.04) and A53T EE (n = 6, 1.0 ± 0.04). The Western Blot result (Figure 5B) shows that relative expression levels of human A53T aSyn protein did not significantly differ between A53T SC (n = 6, 1.0 ± 0.04) and A53T EE (n = 6, 1.0 ± 0.03).

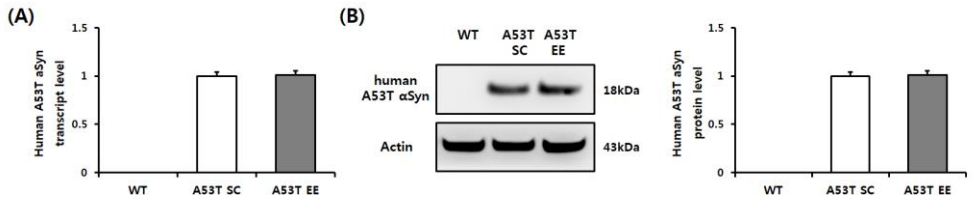


**Figure 5. Expression of human A53T aSyn in striatum and nucleus accumbens.**

(A) mRNA levels of human A53T aSyn in RT-qPCR did not significantly differ between A53T SC and A53T EE. (B) Protein levels of human A53T aSyn in Western Blot also did not significantly differ between A53T SC and A53T EE.

**2. Dopaminergic neurons of SNpc and VTA of A53T mice were not degenerated at 10 months of age**

To investigate whether dopaminergic neurons in substantia nigra pas compata (SNpc) and ventral tegmental area (VTA) were degenerated at 10 months of age, immunostaining was performed for analysis of tyrosine hydrosylase (TH) positive cells. The immunostaining results (Figure 6) showed that TH positive cells (% of wild-type) in SNpc did not differ significantly in three groups; WT (n = 3, 100% ± 8.60), A53T SC (n = 3, 95.2% ± 6.92), and A53T EE (n = 3, 95.1% ± 4.56). Also, TH positive cells (% of wild-type) in VTA did not differ significantly between the three groups; WT (n = 3, 100% ± 14.83), A53T SC (n = 3, 104.7% ± 6.21), and A53T EE (n =3, 96.5% ± 6.21).

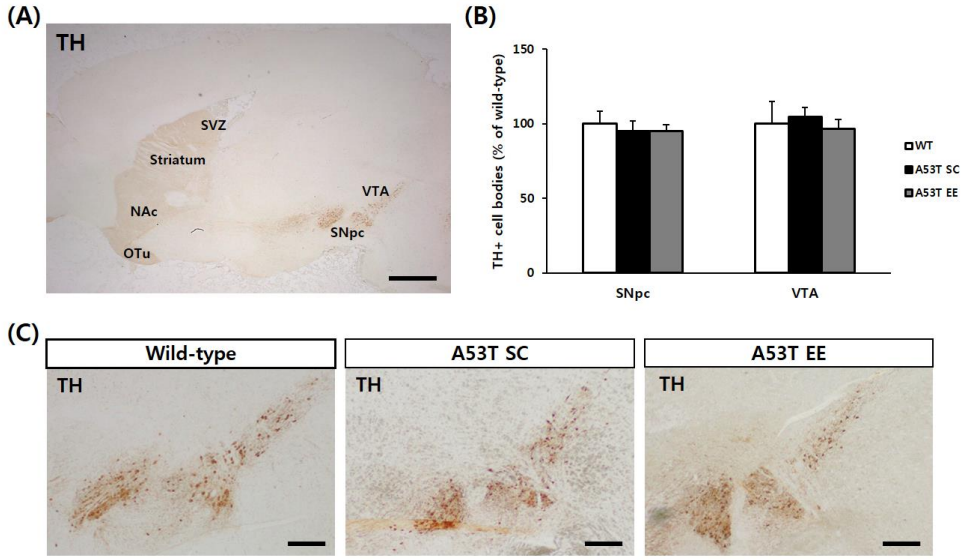


**Figure 6. Tyrosine hydroxylase positive neurons in substantia nigra pars compata and ventral tegmental area.** (A) Sagittal view of dopaminergic neurons and dopaminergic nerve terminals. (B) Results of immunostaining TH positive cells in SNpc did not differ significantly among groups. TH positive cells in VTA also did not differ significantly among groups. (C) Represenatative images of TH positive cells in SNpc and VTA in three groups. Scale = 1 mm, TH; tyrosine hydroxylase, SC; standard cage, EE; environmental enrichment, WT; wild-type, SVZ; subventricular zone, NAc; nucleus accumbens, OTu; olfactory tubercle, SNpc; substantia nigra pars compata, VTA; ventral tegmental area

**3. Dopaminergic nerve terminals of striatum and nucleus accumbens of A53T mice were slightly degenerated at 10 months of age**

Immunostaining was performed for analysis of TH density to investigate whether dopaminergic nerve terminals in striatum and nucleus accumbens were degenerated at 10 months of age and the effects of EE exposure on dopaminergic nerve terminals in striatum and nucleus accumbens. Immunostaining results (Figure 7) showed that dopaminergic nerve terminals in striatum decreased significantly in A53T SC (n = 3, 78.9% ± 1.36, $p < 0.01$) and A53T EE (n = 3, 83.2% ± 4.89, $p < 0.05$) compared to WT (n = 3, 100% ± 3.16). Those in the nucleus accumbens decreased significantly in A53T SC (n = 3, 77.9% ± 7.04, $p < 0.01$) compared to WT (n = 3, 100% ± 6.69) and tended to increase in A53T EE (n = 3, 88.7% ± 20.91) compared to A53T SC. Western Blot analysis of TH protein levels in the striatum was performed. Western Blot results (Figure 8) showed significantly decreased TH protein levels in A53T SC (0.84 ± 0.03, $p < 0.05$) and A53T EE (0.86 ± 0.05, $p < 0.05$) compared to WT.

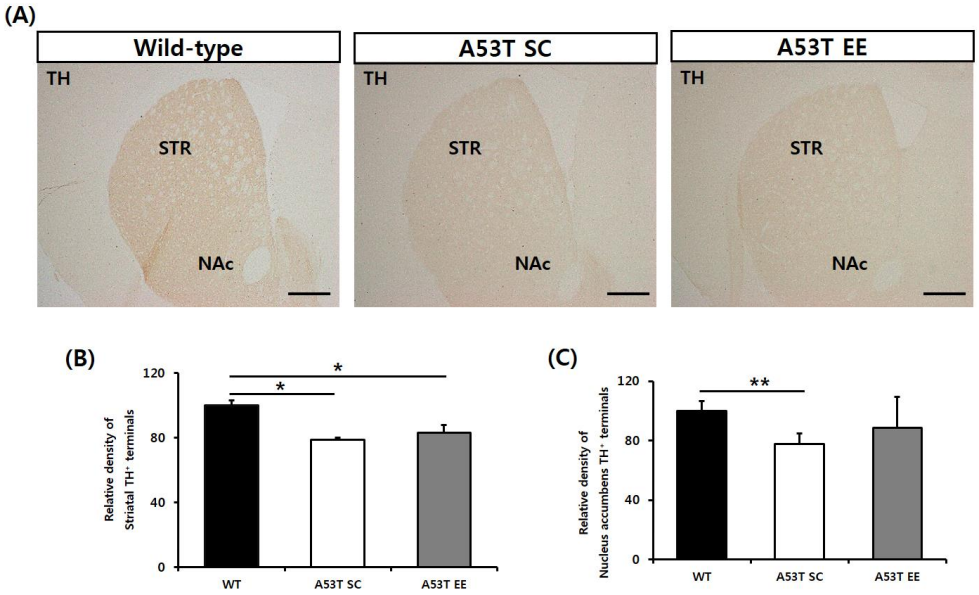


**Figure 7. Optical density of dopaminergic nerve terminals in striatum and nucleus accumbens.** (A) Representative imge of immunostaining of striatum (STR) and nucleus accumbens (NAc) in the three groups. (B) Immunostaining results showed that relative density of striatum of dopaminergic nerve terminals significantly decreased in both A53T SC and A53T EE groups compared to WT. (C) Immunostaining results showed that relative density of ventromedial striatum of dopaminergic nerve terminals significatly decrease in A53T SC compared to WT. * $p < 0.05$, $p < 0.01$, scale bars = 500 μm, TH; tyrosine hydroxylase, SC; standard cage, EE; environmental enrichment, WT; wild-type, STR; striatum, NAc; nucleus accumbens

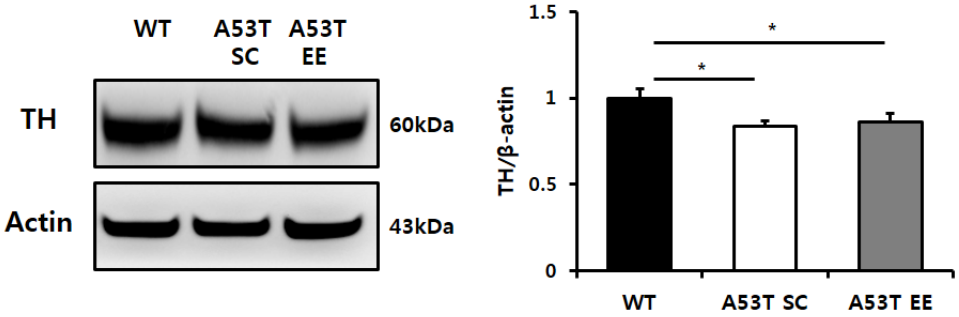


**Figure 8. Expression of tyrosine hydroxylase protein in striatum and nucleus accumbens.** The Western Blot results showed that the protein level of TH in stiratum and nucleus accumbens significantly decrease in both A53T SC and A53T EE groups compared to WT. * $p < 0.05$, TH; tyrosine hydroxylase, SC; standard cage, EE; environmental enrichment, WT; wild-type

**4. Motor symptoms of A53T mice did not appear at 10 months of age**

Tests of motor function were performed to determine whether there were motor symptoms of A53T mice at 10 months of age. Grip strength test results (Figure 9A) showed that the grip power of both forepaws did not differ significantly between WT (n = 6, 119.7 ± 2.9 gram × force), A53T SC (n = 16, 117.5 ± 3.8 gram × force) and A53T EE (n = 14, 120.0 ± 3.1 gram × force). The hanging wire test endurance time (Figure 9B) did not differ significantly between WT (n = 7, 11.6 ± 3.9 seconds), A53T SC (n = 11, 11.2 ± 2.6 seconds) and A53T EE (n = 18, 13.4 ± 2.4 seconds). The cylinder rearing test rearing count (Figure 9C) did not significantly between WT (n = 10, 15.6 ± 2.1), A53T SC (n = 14, 21.1 ± 2.3) and A53T EE (n = 14,17.9 ± 3.4).

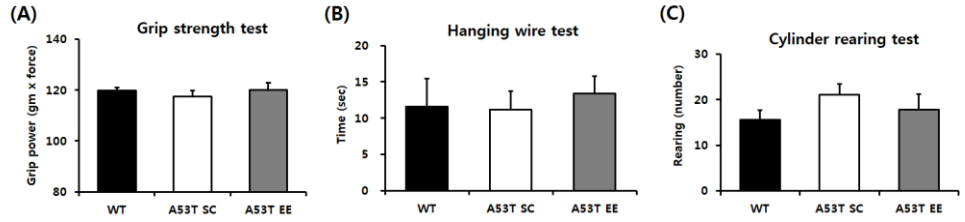


**Figure 9. The results of behavioral tests for assessment of motor function.** (A) The grip strength test results showed that grip power of both forepaws did not differ significantly among the three groups. (B) The hanging wire test endurance time did not differ significantly among groups. (C) The cylinder rearing test rearing count did not differ significantly among three groups. SC; standard cage, EE; environmental enrichment, WT; wild-type

**5. The open field test showed that EE ameliorated hyperactivity of A53T mice**

An open field test was performed for 25 minutes at 10 months of age to determine whether EE exposure influences locomotor activity (Figure 10). During the 25 minutes (Figure 11A), total distance significantly increased in A53T SC (n = 15, 38,313 ± 1,614 cm, $p < 0.001$) compared to WT (n = 12, 14,377 ± 512 cm). However, the total distance was significantly decreased in A53T EE (n = 12, 21,910 ± 2,274 cm, $p < 0.001$) compared to A53T SC and increased in A53T EE (n = 12, 21,910 ± 2,274 cm, $p < 0.01$) compared to WT. Open field test data were collected every five minutes for 25 minutes (Figure 11B). The distance of the first interval—from 0 to 5 minutes—significantly increased in A53T SC (5,536 ± 489 cm, $p < 0.05$) compared to WT (4,206 ± 184 cm) and significantly decreased in A53T EE (3,960 ± 403 cm, $p < 0.01$) compared to A53T SC. The distance of the second interval significantly increased in A53T SC (7,161 ± 352 cm, $p < 0.001$) compared to WT (2,998 ± 170 cm) and significantly decreased in A53T EE (4,292 ± 629 cm, $p < 0.001$) compared to A53T SC. The distance of the third interval significantly increased in A53T SC (8,337 ± 395 cm, $p < 0.001$) compared to WT (2,256 ± 153 cm) and significantly decreased in A53T EE (4,584 ± 582 cm, $p < 0.001$) compared to A53T SC. The distance of the fourth interval significantly increased in A53T SC (8,725 ± 363 cm, $p < 0.001$) compared to WT (2,632 ± 183 cm) and significantly decreased in A53T EE (4,365 ± 683 cm, $p < 0.001$) compared to A53T SC. The distance of the last interval significantly increased in A53T SC (8,554 ± 684 cm, $p <$

0.001) compared to WT (2,285 ± 139 cm) and significantly decreased in A53T EE (4,708 ± 683 cm, $p < 0.001$) compared to A53T SC.

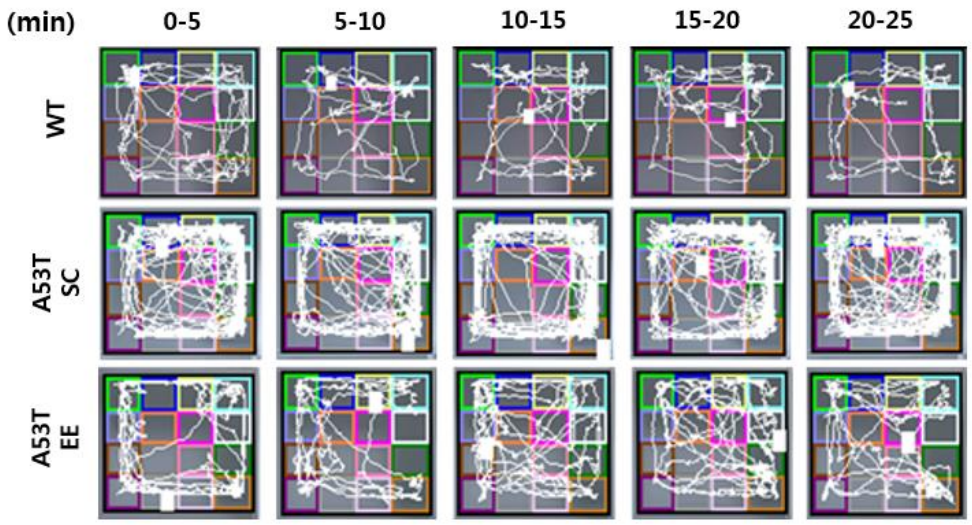


**Figure 10. Representative images of open field test in each WT, A53T SC and A53T EE.** The A53T SC group showed more hyperactivity and higher anxiety than A53T EE group. SC; standard cage, EE; environmental enrichment, WT; wild-type

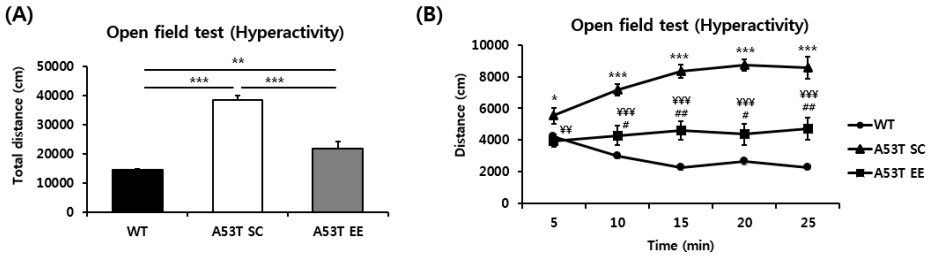


**Figure 11. Results of open field test related to hyperactivity.** (A) Over 25 minutes, total distance significantly increased in A53T SC compared to WT. However, total distance significantly decreased in A53T EE compared to A53T. (B) To evaluate the data, the results were divided into five intervals over successive five-minutes time intervals. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ¥¥ $p < 0.01$, ¥¥¥ $p < 0.001$, # $p < 0.05$, ## $p < 0.01$, SC; standard cage, EE; environmental enrichment, WT; wild-type. * ; WT compared to A53T SC, ¥ ; A53T SC compared to A53T EE, # ; A53T EE compated to WT

**6. The open field test showed that EE ameliorated anxiety of A53T mice**

The open field test was performed for 25 minutes at 10 months of age to determine whether EE exposure affects anxiety (Figure 10). Over 25 minutes, the percentage of time spent in the inner zone compared to the outer zone (inner zone /outer zone remain time × 100) significantly decreased in A53T SC (n = 12, 7.3 ± 0.8%, $p < 0.001$) compared to WT (n = 10, 20.2 ± 2.7%). However, the percentage significantly increased in A53T EE (n = 11, 13.1 ± 2.4%, $p < 0.05$) compared to A53T SC and decreased in A53T EE (n = 11, 13.1 ± 2.4%, $p < 0.05$) compared to WT (Figure 12A). The open field test results were divided into five intervals over successive five-minute time intervals (Figure 12B) decreased significantly in the percentage of the inner zone/outer zone of the last interval—from 20 to 25 minutes—in A53T SC (8.3 ± 2.0%, $p < 0.01$) compared to WT (19.7 ± 2.4%) and significantly increased in A53T EE (15.9 ± 2.9%, $p < 0.05$) compared to A53T SC.

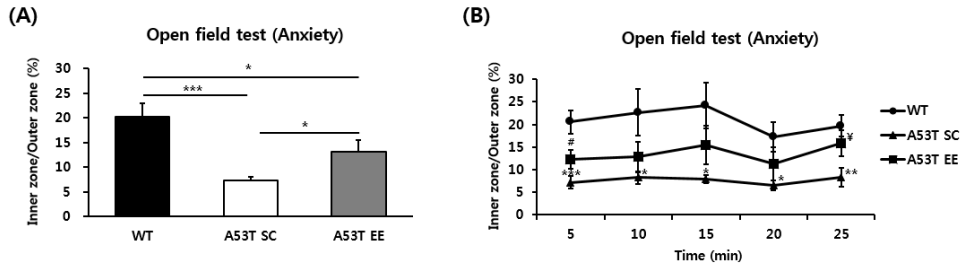


**Figure 12. The open field test results related to anxiety.** (A) Over 25 minutes, the percentage of time of spent in the inner zone compared to the outer zone (inner zone /outer zone remain time × 100) significantly decreased in A53T SC compared to WT. However, the percentage significantly increased in A53T EE compared to A53T. (B) To evaluate the data, the results were divided into five intervals over successive five-minutes time intervals. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, $^{¥}p < 0.05$, $^{\#}p < 0.05$, SC; standard cage, EE; environmental enrichment, WT; wild-type. * ; WT compared to A53T SC, ¥; A53T SC compared to A53T EE, #; A53T EE compated to WT

**7. Expressions of DAT and DRD1 were changed by EE exposure with no changes of DRD2 on striatum and nucleus accumbens in A53T mice**

Reverse transcription polymerase chain reaction (RT-PCR) (Figure 13A) and quantitative real time polymerase chain reaction (RT-qPCR) (Figure 13B) was conducted to investigate effects of EE on expression of dopamine receptors and dopamine transporter such as DRD1, DRD2 and DAT in striatum and nucleus accumbens of A53T mice at 10 months of age. The RT-qPCR results showed the relative mRNA expression of DRD1 significantly decreased in A53T EE (n = 4, 1.3 ± 0.1, $p < 0.05$) compared to A53T SC (n = 4, 1.5 ± 0.1). The expression of DAT significantly increased in A53T EE (0.6 ± 0.1, $p < 0.05$) compared to A53T SC (0.4 ± 0.05). The expression of DRD2 in A53T SC (1.1 ± 0.1) and A53T EE (1.1 ± 0.1) did not differ significantly.

Western Blot results (Figure 14) showed that the relative protein expression of DRD1 significantly decreased in A53T EE (n = 4, 0.8 ± 0.05, $p < 0.001$) compared to A53T SC (n = 4, 1.2 ± 0.1). The relative protein expression of DAT significantly increased in A53T EE (0.9 ± 0.1, $p < 0.05$) compared to A53T SC (0.7 ± 0.1). Expression of DRD2 in A53T SC (1.1 ± 0.1) and A53T EE (1.1 ± 0.1) did not differ significantly.

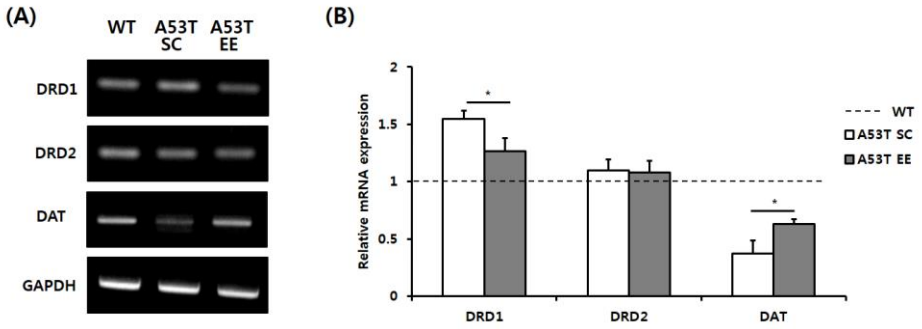


**Figure 13. RNA expression of dopamine receptors and dopamine transporter in striatum and nucleus accumbens.** (A) Image of RT-PCR. (B) The RT-qPCR results showed that relative mRNA expression of DRD1 significantly decreased in A53T EE compared to A53T SC. DAT expression significantly increased in A53T EE compared to A53T SC. DRD2 expression in A53T SC and A53T EE did not differ significantly. * $p < 0.05$, SC; standard cage, EE; environmental enrichment, WT; wild-type, DRD1; dopamine receptor 1, DRD2; dopamine receptor 2, DAT; dopamine transporter

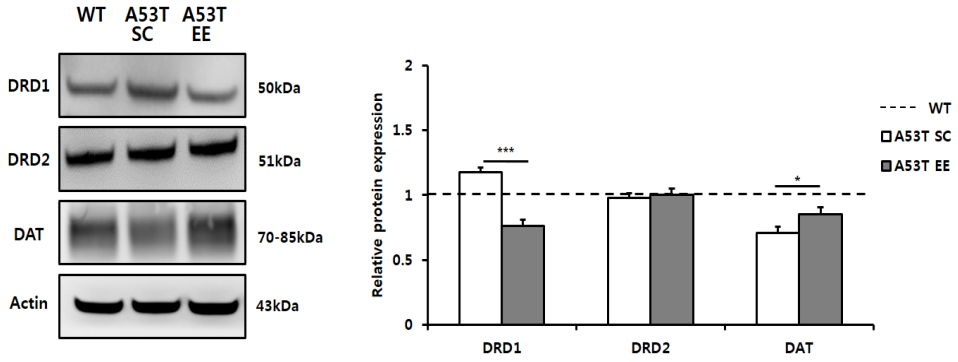


**Figure 14. Protein expression of dopamine receptors and dopamine transporter in striatum and nucleus accumbens.** Western Blot results showed DRD1 relative protein expression significantly decreased in A53T EE compared to A53T SC. DAT relative protein expression significantly increased in A53T EE compared to A53T SC. DRD2 expression in A53T SC and A53T EE did not differ. * $p < 0.05$, *** $p < 0.001$, SC; standard cage, EE; environmental enrichment, WT; wild-type, DRD1; dopamine receptor 1, DRD2; dopamine receptor 2, DAT; dopamine transporter

**8. Expression of SNARE genes—SNAP-25, Syntaxin1 and VAMP-2—was changed by EE exposure on striatum and nucleus accumbens in A53T mice**

Quantitative real time polymerase chain reaction (RT-qPCR) and Western Blot were conducted to investigate effects of EE on expression of SNARE genes and proteins SNAP-25, Syntaxin1 and VAMP-2 in striatum and nucleus accumbens of A53T mice 10 months of age. The RT-qPCR results (Figure 15) showed that the relative mRNA expression of SNAP-25 tended to increase in A53T EE (n = 4, 0.9 ± 0.04) compared to A53T SC (n = 4, 0.8 ± 0.03). Syntaxin1 expression increased significantly in A53T EE (0.5 ± 0.03, $p < 0.05$) compared to A53T SC (0.4 ± 0.03). The expression of VAMP-2 increased significantly in A53T EE (0.9 ± 0.04, $p < 0.01$) compared to A53T SC (0.7 ± 0.1).

Western Blot results (Figure 16) showed that relative protein expressions such as SNAP-25 increased significantly in A53T EE (n = 4, 0.9 ± 0.04, $p < 0.05$) compared to A53T SC (n = 4, 0.8 ± 0.02). Sytaxin1 expression increased significantly in A53T EE (0.9 ± 0.1, $p < 0.05$) compared to A53T SC (0.7 ± 0.04). The expression of VAMP-2 tended to increase in A53T EE (0.95 ± 0.1) compared to A53T SC (0.7 ± 0.1).

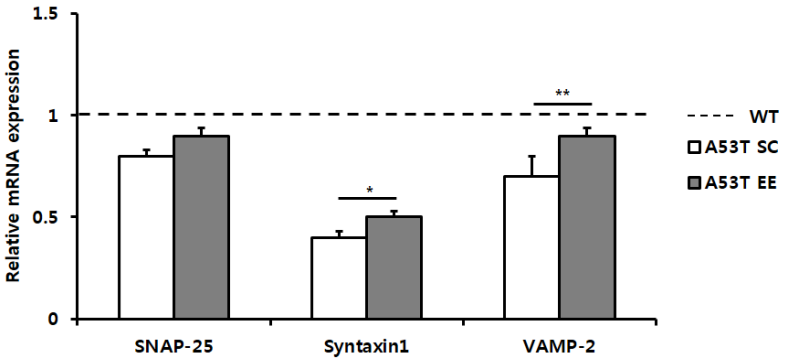


**Figure 15. RNA expression of SNARE genes SNAP-25, Syntaxin1, VAMP-2 in striatum and nucleus accumbens.** RT-qPCR results showed that relative mRNA expression of SNAP-25 tended to increase in A53T EE compared to A53T SC. Syntaxin1 and VAMP-2 expression increased significantly in A53T EE compared to A53T SC. * $p < 0.05$, ** $p < 0.01$, SC; standard cage, EE; environmental enrichment, WT; wild-type, SNARE; Soluble N-ethylmaleimide-sensitive factor attachment protein receptor, SNAP-25; Synaptosomal-associated protein 25, VAMP-2; vesicle-associated membrane protein 2

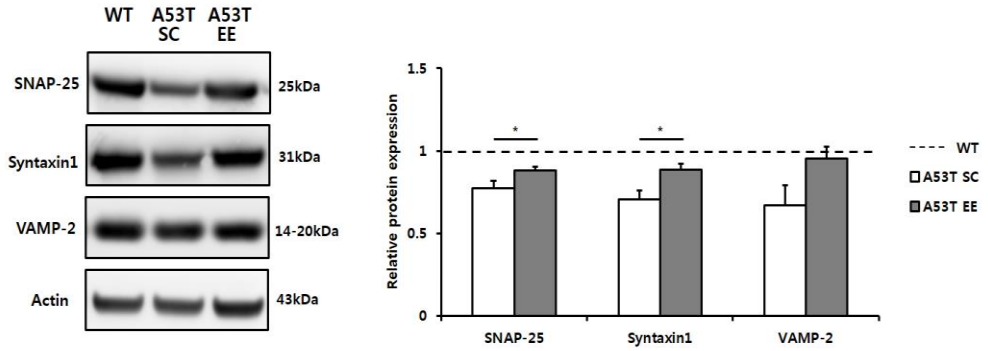


**Figure 16. Protein expression of SNARE genes SNAP-25, Syntaxin1, VAMP-2 in striatum and nucleus accumbens.** Western Blot results showed that the relative protein expressions of both SNAP-25 and Syntaxin1 increased significantly in A53T EE compared to A53T SC. VAMP-2 expression tended to increase in A53T EE compared to A53T SC. * $p < 0.05$, SC; standard cage, EE; environmental enrichment, WT; wild-type, SNARE; Soluble N-ethylmaleimide-sensitive factor attachment protein receptor, SNAP-25; Synaptosomal-associated protein 25, VAMP-2; vesicle-associated membrane protein 2

**9. Environmental enrichment ameliorates aggregation of α-synuclein in cortex, striatum and nucleus accumbens in A53T mice**

Immunohistochemistry of phosphoserine 129 (pSer129) α-synuclein were conducted to investigate effects of EE on aggregation of α-synuclein in striatum and nucleus accumbens at 10 months of age. The representative images of DAB staining (Figure 17) showed that the number of pSer129 tended to decrease in A53T EE compared to A53T SC in striatum and cortex. Immunohistochemistry results (Figure 18) showed that pSer129 counts tended to decrease in A53T EE (n = 3, 42.8 ± 7.5 pSer129 count / $mm^3$, $p$ = 0.05) compared to A53T SC (n = 3, 78.5 ± 16.0 pSer129 count / $mm^3$).

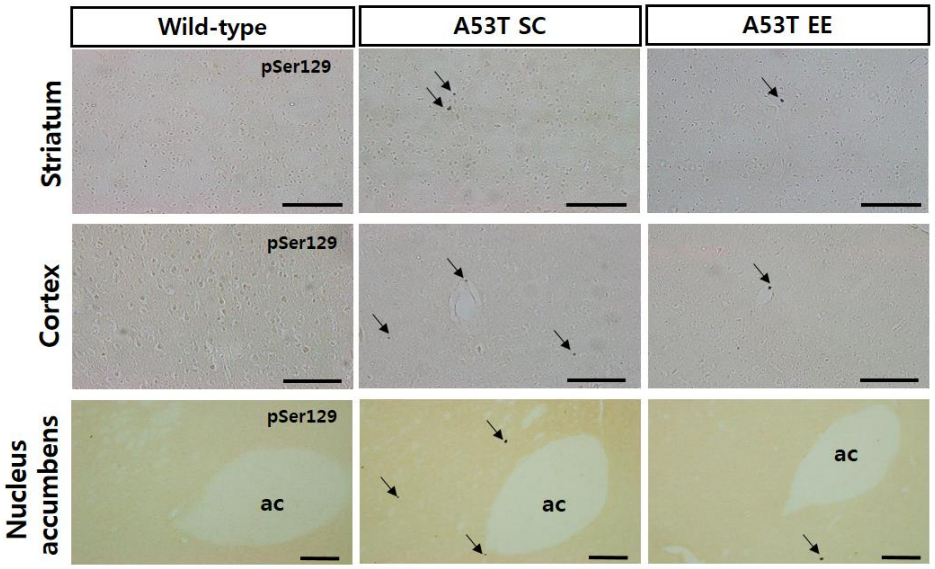


**Figure 17. Representative images of immunostaining of phosphoserine α-synuclein in striatum, cortex and nucleus accumbens in each WT, A53T SC and A53T EE.** The pSer129 counts in the striatum, cortex, and nucleus accumbens decreased in A53T EE compared to A53T SC, shown by the arrow. SC; standard cage, EE; environmental enrichment, WT; wild-type, DAB; 3,3'-diaminobenzidine, pSer129; phosphoserine 129 α-synuclein, Scale bar = 50 μm

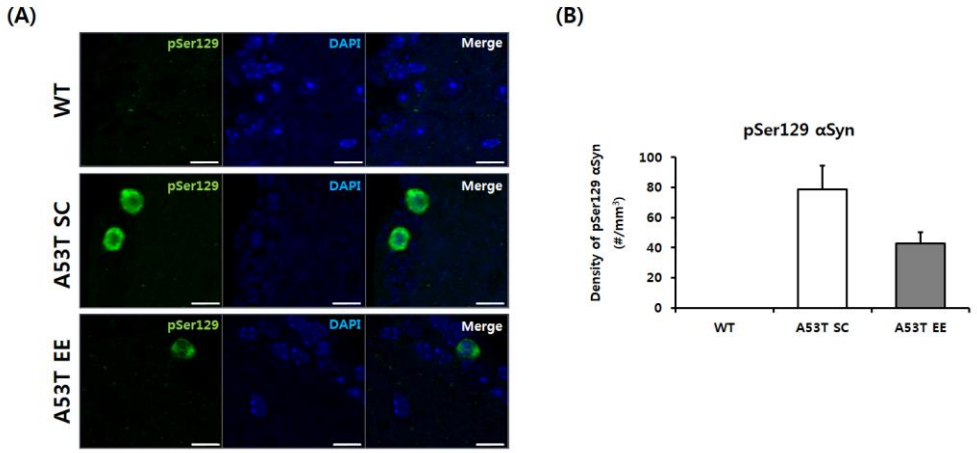


**Figure 18. Immunohistochemistry of phosphoserine 129 α-synuclein in subventricular zone.** (A) Representative images of pSer 129 immunohistochemistry in three groups (WT, A53T SC, and A53T EE). (B) Density of pSer129 αSyn tended to decrease in A53T EE compared to A53T SC. SC; standard cage, EE; environmental enrichment, WT; wild-type, pSer129; phosphoserine 129 α-synuclein, Scale bar = 10 μm

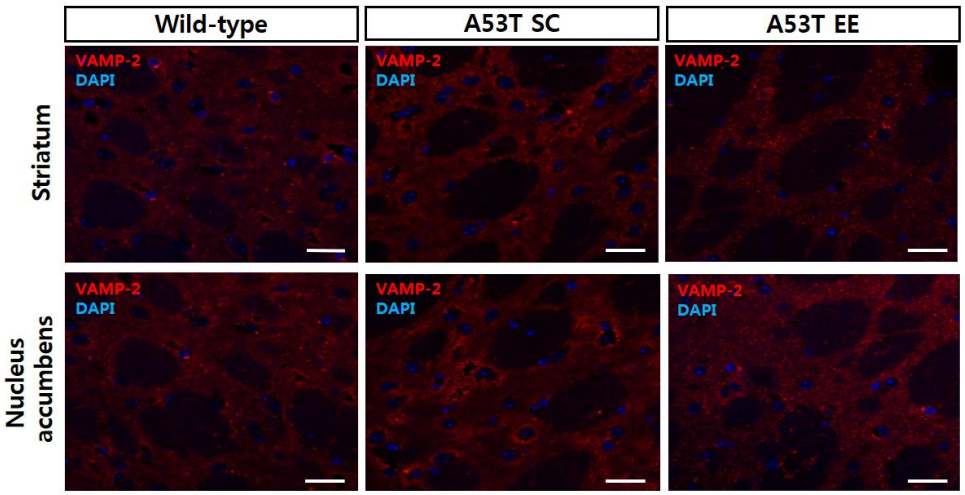


**Figure 19. Representative images of immunohistochemistry of VAMP-2 in striatum and nucleus accumbens.** In the striatum, representative images of immunohistochemistry of VAMP-2 showed no change in any three group. However, a representative image of immunohistochemistry of VAMP-2 in nucleus accumbens showed redistribution of VAMP-2 and less aggregation in A53T SC group compared to WT and A53T EE. SC; standard cage, EE; environmental enrichment, WT; wild-type, Scale bar = 20 μm, VAMP-2; vesicle-associated membrane protein 2

## IV. DISCUSSION

Motor impairments of PD are primarily due to a 50 to 70% loss of dopamine neurons in the substantia nigra pars compacta.[25] Dopaminergic terminals are thought to degenerate before cell bodies.[26] The mouse model used in our study expressing human A53T aSyn by mouse Prp promoter does not show degeneration in the substantia nigra at the age of 13- to 14-months old.[27] In our study, dopaminergic neuron in striatum at 10 months of age in A53T mice revealed only mild degeneration about 20% compared to that in WT. The mice did not show motor deficit in a grip strength test, hanging wire test or cylinder test, but non-motor symptoms, olfactory dysfunction, hyperactivity and anxiety, were revealed by the olfactory bulb test and open field test results. Before motor onset, olfactory impairment and anxiety are common as initial sign of PD.[28,29] Olfactory dysfunction, anxiety, and hyperactivity in A53T mice were ameliorated by EE exposure. Thus, EE could be one of the therapeutic factors that improves motor functions as well as non-motor symptoms in neurodegenerative diseases including PD.

Kurz et al. reported that increased striatal dopamine levels were observed in young (8 months) and old (18 months) A53T mice and revealed elevated striatal DRD1 and DRD2 levels in the absence of neurodegeneration.[30] The aforementioned study explained progressively increased striatal dopamine levels as an early consequence of A53T overexpression prior to overt neurodegeneration. Upregulation of

dopamine receptors and postsynaptic hypersensitivity are compensatory efforts associated with deficient dopamine signaling.[30,31] More recently, increasing dopamine levels in A53T mice induce aSyn oligomers resulting in progressive nigrostriatal degeneration.[32] In this study, by alteration of DAT and DRD1, EE exposure may normalize dopamine levels in striatal dopaminergic neuron and decrease phosphorylated aSyn in A53T mice.

aSyn is abundantly localized in the presynaptic nerve terminals.[33,34] While the physiological functions of aSyn have yet to be defined, several lines of evidence implicated this protein in the modulation of neurotransmitter release through the regulation of the formation of the soluble N-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex and the size of the synaptic vesicle pool.[35-37] Vesicle-associated membrane protein-2 (VAMP-2) present in the synaptic vesicles, and syntaxin and synaptosomal-associated protein of 25 kDa (SNAP-25) in the presynaptic plasma membrane form the core SNARE complex, regulate the docking and fusion of synaptic vesicles to the presynaptic membrane.[38] A recent study showed the physical interaction of aSyn with VAMP-2 to promote SNARE assembly.[36] Importantly, abnormal redistribution of SNARE proteins has been observed in human PD patients and mice overexpressing a truncated form of human aSyn.[39] Therefore, presynaptic SNARE dysfunction is considered an initial pathogenic event in α-synucleinopathies. In this study, EE exposure improved the expression of SNARE complex downregulated in striatum of A53T overexpressing

mice at 10 months of age. In conclusion, this study suggests that EE induces of synaptic plasticity in dopaminergic nerve terminals of striatum and nucleus accumbens and ameliorates even non-motor symptoms in PD.

## V. CONCLUSION

Summarizing my results (Figure 20), orginally functions of v and t-snare proteins helps synaptic vesicle to docking and fusion to exocytosis. These functions were interrupted by aggregated aSyn proteins; therefore, dopamine release was decreased in the A53T SC group. Additionally, cytosol dopamine level in striatum may be higher because the function of synaptic vesicles that recollect dopamine to vesicles and loading was interrupted by aggregated aSyn. Dopamine transporters that reuptake dopamine to pre-synapse were decreased by compensatory effect of increasing level of cytosol dopamine with lacking dopamine in synaptic cleft. Furthermore, as a compensatory effect DRD 1 increased to receive more synaptic signals. On the other hand, EE reduced aggregated aSyn, so snare proteins may recover their functions. Therefore, synaptic vesicles re-circulated and normalized, so cytosolic dopamine level may normalize in striatum and nucleus accumbens. Since dopamine transporters and/or dopamine receptors were normalized, pre-motor symptoms were ameliorated and the progress of disease was delayed.

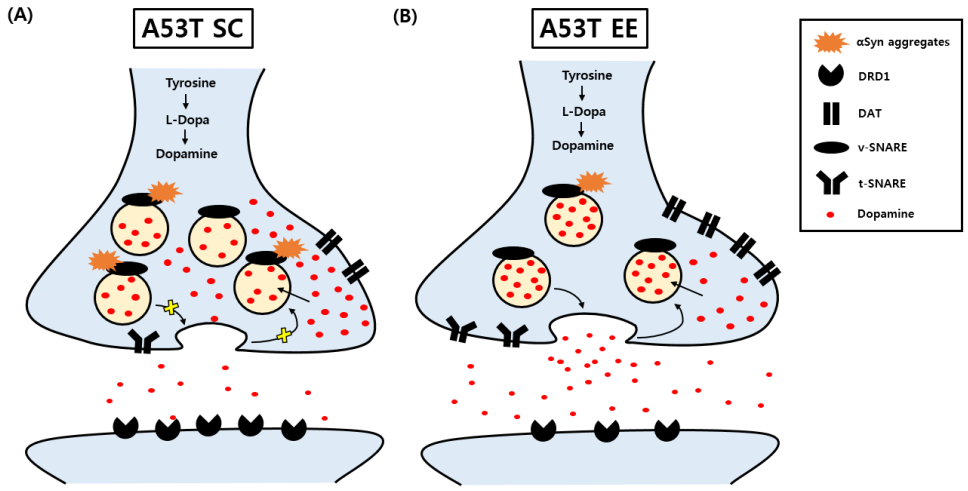


**Figure 20. Synopsis of abnormal synaptic signaling in A53T mice recovered by EE.** This schematic illustration summerizes progressive pathology of dopaminergic nerve terminal in striatum and nucleus accumbens. (A) Orginally functions of v and t-snare proteins helps synaptic vesicle to docking and fusion to exocytosis. These functions were interrupted by aggregated aSyn proteins; therefore, dopamine release was decreased in the A53T SC group. Additionally, cytosol dopamine level in striatum may be higher because the function of synaptic vesicles that recollect dopamine to vesicles and loading was interrupted by aggregated aSyn. Dopamine transporters that reuptake dopamine to pre-synapse were decreased by compensatory effect of increasing level of cytosol dopamine with lacking dopamine in synaptic cleft. Furthermore, as a compensatory effect DRD 1 increased to receive more synaptic signals. (B) EE reduced aggregated aSyn, so snare proteins may recover their functions. Therefore, synaptic vesicles re-circulated and normalized,

so cytosolic dopamine level may normalize in striatum and nucleus accumbens. Since dopamine transporters and/or dopamine receptors were normalized, pre-motor symptoms were ameliorated and the progress of disease was delayed.

# REFERNCE

1. Spillantini MG, Schmidt ML, Lee VM, Trojanowski JQ, Jakes R, Goedert M. Alpha-synuclein in Lewy bodies. Nature 1997;388:839-40.
2. Spillantini MG, Crowther RA, Jakes R, Hasegawa M, Goedert M. alpha-Synuclein in filamentous inclusions of Lewy bodies from Parkinson's disease and dementia with lewy bodies. Proc Natl Acad Sci U S A 1998;95:6469-73.
3. Spillantini MG, Crowther RA, Jakes R, Cairns NJ, Lantos PL, Goedert M. Filamentous alpha-synuclein inclusions link multiple system atrophy with Parkinson's disease and dementia with Lewy bodies. Neurosci Lett 1998;251:205-8.
4. Polymeropoulos MH, Lavedan C, Leroy E, Ide SE, Dehejia A, Dutra A, et al. Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. Science 1997;276:2045-7.
5. Anderson JP, Walker DE, Goldstein JM, de Laat R, Banducci K, Caccavello RJ, et al. Phosphorylation of Ser-129 is the dominant pathological modification of alpha-synuclein in familial and sporadic Lewy body disease. J Biol Chem 2006;281:29739-52.
6. Jankovic J. Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 2008;79:368-76.
7. Chaudhuri KR, Schapira AH. Non-motor symptoms of Parkinson's disease: dopaminergic pathophysiology and treatment. Lancet Neurol 2009;8:464-74.
8. Rosenzweig MR, Bennett EL, Hebert M, Morimoto H. Social grouping cannot account for cerebral effects of enriched environments. Brain Res 1978;153:563-76.
9. Janssen H, Ada L, Bernhardt J, McElduff P, Pollack M, Nilsson M, et al. An enriched environment increases activity in stroke patients undergoing rehabilitation in a mixed rehabilitation unit: a pilot non-randomized controlled trial. Disabil Rehabil 2014;36:255-62.
10. White JH, Alborough K, Janssen H, Spratt N, Jordan L, Pollack M. Exploring staff experience of an "enriched environment" within stroke rehabilitation: a qualitative sub-study. Disabil Rehabil 2014;36:1783-9.

11. Chen H, Zhang SM, Schwarzschild MA, Hernan MA, Ascherio A. Physical activity and the risk of Parkinson disease. Neurology 2005;64:664-9.
12. Xu Q, Park Y, Huang X, 2010 #129} X., Hollenbeck A, Blair A, Schatzkin A, et al. Physical activities and future risk of Parkinson disease. Neurology 2010;75:341-8.
13. Cotman CW, Berchtold NC. Physical activity and the maintenance of cognition: learning from animal models. Alzheimers Dement 2007;3:S30-7.
14. Berchtold NC, Castello N, Cotman CW. Exercise and time-dependent benefits to learning and memory. Neuroscience 2010;167:588-97.
15. Petzinger GM, Fisher BE, Van Leeuwen JE, Vukovic M, Akopian G, Meshul CK, et al. Enhancing neuroplasticity in the basal ganglia: the role of exercise in Parkinson's disease. Mov Disord 2010;25 Suppl 1:S141-5.
16. Faherty CJ, Raviie Shepherd K, Herasimtschuk A, Smeyne RJ. Environmental enrichment in adulthood eliminates neuronal death in experimental Parkinsonism. Brain Res Mol Brain Res 2005;134:170-9.
17. Goldberg NR, Haack AK, Meshul CK. Enriched environment promotes similar neuronal and behavioral recovery in a young and aged mouse model of Parkinson's disease. Neuroscience 2011;172:443-52.
18. Im SH, Yu JH, Park ES, Lee JE, Kim HO, Park KI, et al. Induction of striatal neurogenesis enhances functional recovery in an adult animal model of neonatal hypoxic-ischemic brain injury. Neuroscience 2010;169:259-68.
19. Cummins RA, Walsh RN, Budtz-Olsen OE, Konstantinos T, Horsfall CR. Environmentally-induced changes in the brains of elderly rats. Nature 1973;243:516-8.
20. Fan LW, Lin S, Pang Y, Lei M, Zhang F, Rhodes PG, et al. Hypoxia-ischemia induced neurological dysfunction and brain injury in the neonatal rat. Behav Brain Res 2005;165:80-90.
21. Denenberg VH. Open-field bheavior in the rat: what does it mean? Ann N Y Acad Sci 1969;159:852-9.
22. Walsh RN, Cummins RA. The Open-Field Test: a critical review. Psychol Bull 1976;83:482-504.
23. Carola V, D'Olimpio F, Brunamonti E, Mangia F, Renzi P. Evaluation of the elevated plus-maze and open-field tests for the assessment of

anxiety-related behaviour in inbred mice. Behav Brain Res 2002;134:49-57.
24. Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J Pharmacol 2003;463:3-33.
25. Fearnley JM, Lees AJ. Ageing and Parkinson's disease: substantia nigra regional selectivity. Brain 1991;114 ( Pt 5):2283-301.
26. Cheng HC, Ulane CM, Burke RE. Clinical progression in Parkinson disease and the neurobiology of axons. Ann Neurol 2010;67:715-25.
27. Daher JP, Pletnikova O, Biskup S, Musso A, Gellhaar S, Galter D, et al. Neurodegenerative phenotypes in an A53T alpha-synuclein transgenic mouse model are independent of LRRK2. Hum Mol Genet 2012;21:2420-31.
28. Shiba M, Bower JH, Maraganore DM, McDonnell SK, Peterson BJ, Ahlskog JE, et al. Anxiety disorders and depressive disorders preceding Parkinson's disease: a case-control study. Mov Disord 2000;15:669-77.
29. Pellicano C, Benincasa D, Pisani V, Buttarelli FR, Giovannelli M, Pontieri FE. Prodromal non-motor symptoms of Parkinson's disease. Neuropsychiatr Dis Treat 2007;3:145-52.
30. Kurz A, Double KL, Lastres-Becker I, Tozzi A, Tantucci M, Bockhart V, et al. A53T-alpha-synuclein overexpression impairs dopamine signaling and striatal synaptic plasticity in old mice. PLoS One 2010;5:e11464.
31. Seeman P, Niznik HB. Dopamine receptors and transporters in Parkinson's disease and schizophrenia. FASEB J 1990;4:2737-44.
32. Mor DE, Tsika E, Mazzulli JR, Gould NS, Kim H, Daniels MJ, et al. Dopamine induces soluble alpha-synuclein oligomers and nigrostriatal degeneration. Nat Neurosci 2017;20:1560-8.
33. Maroteaux L, Campanelli JT, Scheller RH. Synuclein: a neuron-specific protein localized to the nucleus and presynaptic nerve terminal. J Neurosci 1988;8:2804-15.
34. Iwai A, Masliah E, Yoshimoto M, Ge N, Flanagan L, de Silva HA, et al. The precursor protein of non-A beta component of Alzheimer's disease amyloid is a presynaptic protein of the central nervous system. Neuron 1995;14:467-75.
35. Chandra S, Gallardo G, Fernandez-Chacon R, Schluter OM, Sudhof TC. Alpha-synuclein cooperates with CSPalpha in preventing neurodegeneration. Cell 2005;123:383-96.

36. Burre J, Sharma M, Tsetsenis T, Buchman V, Etherton MR, Sudhof TC. Alpha-synuclein promotes SNARE-complex assembly in vivo and in vitro. Science 2010;329:1663-7.
37. Darios F, Ruiperez V, Lopez I, Villanueva J, Gutierrez LM, Davletov B. Alpha-synuclein sequesters arachidonic acid to modulate SNARE-mediated exocytosis. EMBO Rep 2010;11:528-33.
38. Sudhof TC. The synaptic vesicle cycle. Annu Rev Neurosci 2004;27:509-47.
39. Garcia-Reitbock P, Anichtchik O, Bellucci A, Iovino M, Ballini C, Fineberg E, et al. SNARE protein redistribution and synaptic failure in a transgenic mouse model of Parkinson's disease. Brain 2010;133:2032-44.

**ABSTRACT (IN KOREAN)**

# 알파 시누클레인 병변을 가진 파킨슨 질병에서

# 재활 환경에 의한 시냅스 가소성 유도

<지도교수 조성래>

연세대학교 대학원 의과학과

위수현

파킨슨 질병은 후각 기능 소실, 인지 기능 저하, 우울증 및 불안과 같은 비 운동 증상을 동반하는 신경 퇴행성 질환으로 조기 또는 운동 증상이 나타나기 전 단계에 나타난다. 신체적, 인지적, 사회적 자극의

복잡한 조합을 통한 재활 환경은 시냅스 가소성 및 행동 기능을 향상시킨다. 그러나, 알파 시뉴클레인 병변을 나타내는 유전자 변형 마우스의 운동증상이 나타나기 전 단계의 비 운동 증상에 대한 재활 환경의 효과에 대한 연구는 밝혀지지 않는 상태이다. 본 연구는 파킨슨 질병 초기 단계에서 재활 환경에 의한 선조체와 중격핵의 시냅스 가소성을 밝히고자 하였다. 이를 위해 8 개월 된 인간 A53T 알파시누클레인 과발현 마우스를 재활 환경 (EE) 또는 표준 환경 (SC)에 2 개월 동안 무작위로 사육하였다. 오픈 필드 검사 결과, 재활 환경은 A53T 마우스의 과다 활동과 불안을 상당히 개선 시켰다. 10 개월째에 A53T 마우스는 흑질과 배쪽피개부의 도파민성 뉴런은 퇴행하지 않았다. 선조체의 도파민성 신경 말단은 야생형에 비해 A53T SC 와 A53T EE 에서 약 20% 감소를 나타내었으나, 중격핵의 도파민성 신경 말단은 야생형에 비해 A53T SC 에서 유의하게 감소하였고 A53T EE 에서는 A53T SC 보다 증가하는 경향을 보였다. 재활 환경에 의한 도파민 운반체 (DAT)와 도파민 수용체 1 (DRD1)의 발현 변화에 의해 선조체의 도파민 성 신경 말단의 도파민 수치를 정상화시키고 인산화 된 알파 시누클레인을 감소시켰다. 또한 재활 환경은 A53T 마우스의 선조체에서 SNARE-25, Syntaxin 1 및 VAMP-2 와 같은 SNARE 복합체 형성 유전자의 발현감소를 정상화시켰다. 결론적으로, 본 연구는 재활 환경이 선조체와 중격핵에서 도파민 성 신경 말단의 시냅스 소성의 유도함으로써 파킨슨 질병의 운동증상이 나타나기 전 단계에서 비 운동 증상을 개선한다고 제안하였다.

---

핵심되는 말: 파킨슨 질병, 재활 환경, 시냅스 가소성, A53T 유전자 변형 마우스, 도파민, 인산화 된 알파시누클레인, SNARE 단백질

# PUBLICATION LIST

1. **Wi S**, Yu JH, Kim M, Cho SR. In Vivo Expression of Reprogramming Factors Increases Hippocampal Neurogenesis and Synaptic Plasticity in Chronic Hypoxic-Ischemic Brain Injury. Neural Plast 2016;2016:2580837.

2. Kim M, Yu JH, Seo JH, Shin YK, **Wi S**, Baek A, et al. Neurobehavioral Assessments in a Mouse Model of Neonatal Hypoxic-ischemic Brain Injury. J Vis Exp 2017; doi:10.3791/55838.

3. **Wi S**, Lee JW, Kim M, Park CH, Cho SR. An Enriched Environment Ameliorates Oxidative Stress and Olfactory Dysfunction in Parkinson's Disease with alpha-Synucleinopathy. Cell Transplant 2018; doi:10.1177/0963689717742662.963689717742662.

4. Jin Y, Lee JS, Kim J, Min S, **Wi S**, Yu JH, et al. Three-dimensional brain-like microenvironments facilitate the direct reprogramming of fibroblasts into therapeutic neurons. Nat Biomed Eng 2018. Accepted on 21 June 2018