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An Enriched Environment Ameliorates Oxidative Stress and Olfactory Dysfunction in Parkinson's Disease with α-Synucleinopathy

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Abstract

Parkinson's disease (PD) features nonmotor symptoms such as olfactory dysfunction referred to as hyposmia, an initial sign of disease progression. Metabolic dysfunction can contribute to neurodegenerative diseases, and various xenobiotics and endogenous compounds are also involved in the pathogenesis of PD. Although aerobic exercise was found to induce preservation or improvement in olfactory function in PD patients in a recent study, the exact underlying mechanism for this effect is not clear. We aimed to investigate the influence of an enriched environment (EE) on olfactory dysfunction especially via metabolic pathways related to detoxification enzymes. Eight-month-old transgenic (Tg) PD mice that overexpress human A53T α-synuclein (α-syn) were randomly allocated to an EE or standard conditions for 2 mo. The buried food test showed that EE group had significantly improved olfactory function compared to the control group. Reverse transcription polymerase chain reaction (PCR) and real-time quantitative PCR showed that expression of the detoxification enzymes—*cytochrome P450 family 1 subfamily A member 2*, *paraoxonase 1*, *alcohol dehydrogenase 1*, *UDP glucuronosyltransferase family 2 member A1 complex locus*, *aldehyde oxidase homolog 2*, and *aldehyde glutathione peroxidase 6*—was significantly increased in the olfactory bulb (OB) of the PD control group, but these enzymes were normalized in the EE group. Immunohistochemical staining of the OB showed that oxidative stress and nitrated α-syn were significantly increased in the control group but decreased in the EE group. In conclusion, we suggest that exposure to an EE decreases both oxidative stress and nitrated α-syn, resulting in normalized detoxification enzymes and amelioration of olfactory dysfunction.

Keywords

enriched environment, olfactory dysfunction, Parkinson's disease, detoxification enzymes, oxidative stress

Introduction

An enriched environment (EE) has been used in various research studies of rearing animals for therapeutic effects through the complex combination of physical, cognitive, and social stimulation[1,2]. When animals are exposed to an EE, even the adult brain can undergo biochemical and histologic changes, consequently promoting brain function[3]. Biochemical changes including neurogenesis, axonal sprouting, and dendritic arborization are stimulated by an EE[4,5]. As a result, performance in various behaviors is improved by an EE, and previous studies have reported that an EE is a potential therapeutic strategy for recovery from brain damage[6–9].

Parkinson's disease (PD) is one of the most common adult neurodegenerative diseases. Hyposmia is an initial sign and one of the earliest symptoms of PD. As an olfactory dysfunction, hyposmia can be detected before motor symptoms are displayed in PD[10–16]. The loss of dopaminergic neurons in the substantia nigra is known as an essential discovery in PD, and Lewy bodies, eosinophilic cytoplasmic

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inclusions composed of abnormal aggregates of proteins such as α-synuclein (α-syn), have an important role in several neurodegenerative diseases because they can induce inflammatory reactions, mitochondrial dysfunction, and subsequent oxidative stress[17]. It is thought that numerous etiologies including genetic susceptibility and environmental exposure play an important role in the pathophysiology of PD[18]. Additionally, previous research has shown that metabolic dysfunction can contribute to neurodegenerative diseases[17], and various xenobiotics and endogenous compounds are involved in the pathogenesis of PD[19–23].

In previous studies using a mouse model of PD pathology by the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydrophyridine (MPTP), the therapeutic effect of an EE in behavioral recovery of motor function has been reported[24,25]. On the other hand, there is no research on the effect of an EE on olfactory dysfunction, which is a non-motor symptom, in PD mice. Additionally, in a recent study, aerobic exercise induced the preservation or improvement of olfactory function in PD patients[26]. However, the exact mechanism underlying olfactory dysfunction in PD is not yet known, and it is not clear why exercise can lead to an improvement in olfactory function. Thus, this study aims to confirm the recovery of olfactory dysfunction by an EE in a PD transgenic (Tg) mouse model in which human A53T α-syn is overexpressed. Through this Tg mouse model of PD, we investigated the influence of EE on olfactory dysfunction, especially via the metabolic pathways related to detoxification enzymes.

Materials and Methods

Tg Mouse Model

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

Genotyping

Genotyping of mice was performed based on a protocol from Jackson Laboratories. 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, Hamilton, New Zealand). The tissue from the mouse tail was incubated with 1 μL of *prep*GEM, 10 μL of Buffer Gold (ZyGEM, Hamilton, New Zealand), and 89 μL of autoclaved 3′ distilled water at 75 °C for 15 min and 95 °C for 5 min. The following primers were used for the polymerase chain reaction (PCR): transgene forward, 5′-TCATGAAAGGACTTTCAAAGGC-3′ transgene reverse, 5′-CCTCCCCC AGCCTAGACC-3′ (transgene = ~500 bp); internal positive control forward, 5'-CTAGGCCACAGAATTGAAAGATCT-3′; internal positive control reverse, 5′-GTAGGTGGAAA TTCTAGCA TCC-3′ (internal positive control = 324 bp). Electrophoresis was performed by loading 10 μL of each PCR product on a 1.5% agarose gel (Medicago, Quebec, Canada).

EE

For the EE, mice were housed for 2 mo in a large cage (86 × 76 × 31 cm^3) containing novel objects such as tunnels, shelters, toys, and running wheels for voluntary exercise and allowing for social interaction (12 to 15 mice/cage), whereas the control group mice were housed in standard cages (27 × 22.5 × 14 cm^3) without social interaction (3 to 5 mice/cage). A schematic time line of this experiment from 8 to 10 mo of age is provided in Fig. 1A. An image of EE is provided in Fig. 1B.

Buried Food Test

The buried food test measures how quickly an overnight-fasted animal can find a small piece of familiar palatable food, such as cookies, cereal, chocolate chips, or food pellets, that is hidden underneath a layer of bedding[27]. The test was performed twice on mice at 8 and 10 mo of age. Before starting the test, the mice were fasted for 14 to 18 h to ensure motivation. The mice were placed individually in clean holding cages for 5 min, transferred to test cages for 2 min, and then returned to the holding cage, while a pellet was buried approximately 0.5 cm below the bedding in a random location to eliminate a learning component. After food placement, each mouse was placed in the center of the test cage and given 5 min to find the pellet, while the latency to sniff, dig up, and begin eating food was recorded using a stop watch[28].

RNA Preparation

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

Reverse Transcription PCR (RT-PCR)

We performed RT-PCR for genes that were related to detoxification enzymes. For RT-PCR, the following reaction-specific primers were used: *cytochrome P450 family 1 subfamily A member 2* (*CYP1A2*), forward 5′-GCTTCTCCATAGCCTCGGAC-3′ and reverse 5′-TTAGCCACCGATTCCACCAC-3′;

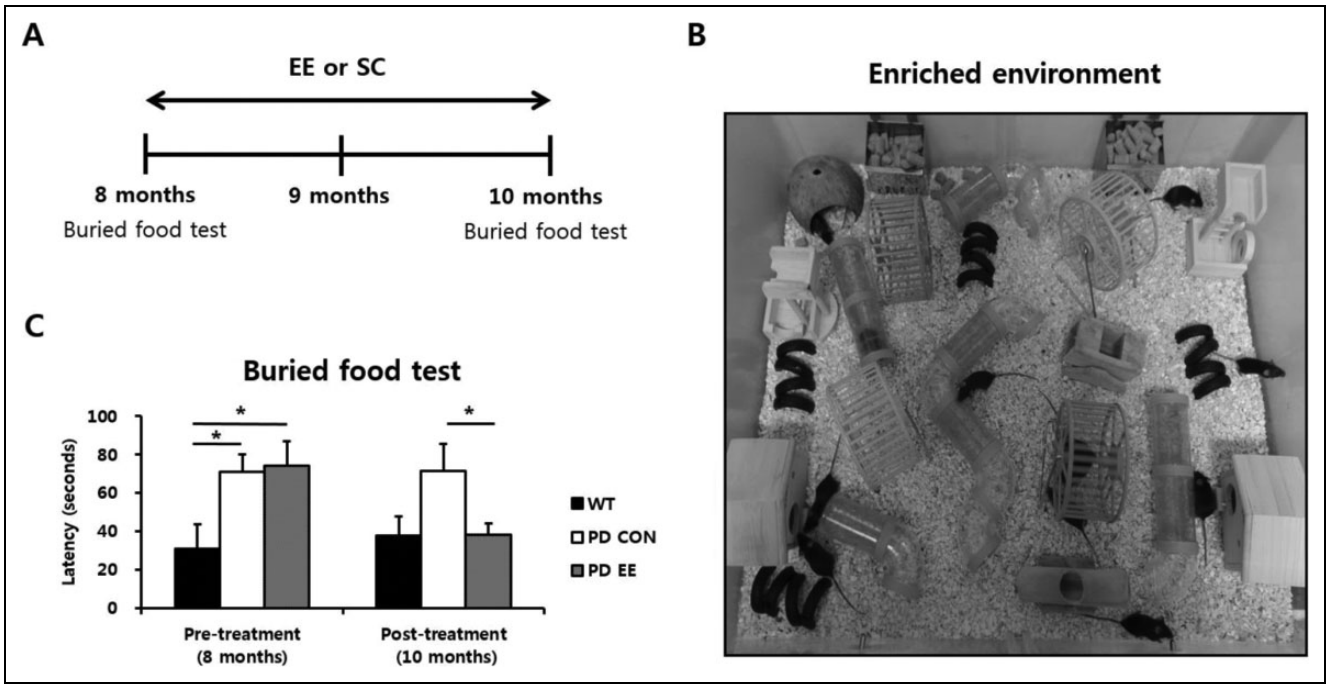

Fig. 1. Experimental design and effect of an enriched environment on olfactory dysfunction in Parkinson's disease. (A) Schematic timeline of the experiment in a mouse model of PD. (B) An image of EE. (C) Buried food test result. The latency time of finding food in PD control group ($N = 14$, 71.0 $\pm$ 9.2 sec, $P < 0.05$) and PD EE group ($N = 11$, 74.2 $\pm$ 12.8 sec, $P < 0.05$) significantly increased compared to WT group ($N = 8$, 30.7 $\pm$ 4.6 sec) at 8 mo of age. The result of latency time to find food at 10 mo of age showed that the PD EE group (37.9 $\pm$ 6.1 sec, $P < 0.05$) significantly decreased compared to PD control group (71.3 $\pm$ 14.2 sec). Abbreviations: PD = Parkinson's disease; EE = enriched environment; WT = wild type. *$P < 0.05$ is based on a one-way analysis of variance followed by a post hoc test.

paraoxonase 1 (*PON1*), forward 5′-ATGACGCAGAGAATC CTCCC-3′ and reverse 5′-TTTGTACACAGAGGCGAC CG-3′; *alcohol dehydrogenase 1* (*ADH1*), forward 5′-GACA TAGAAGTCGCACCCCC-3′ and reverse 5′-CCAACGCTCT CAACAATGCC-3′; *aldehyde oxidase homolog 2* (*AOH2*), forward 5′-CTCGGGGAGTCTGGGATGTT-3′ and reverse 5′-GTTTTTGGGTCATCTCTCGGG-3′; *UDP glucuronosyltransferase family 2 member A1 complex locus* (*UGT2A1*), forward 5′-CTAGGAATGAGTCTTGGTGGGA-3′ and reverse 5′-GGCCACAAGGACAGTCACATTA-3′; *glutathione peroxidase 6* (*GPX6*), forward 5′-CAGAAGTTGTGGGG TTCCTGT-3′ and reverse 5′-TGCCAGTCACCCCTTTG TTG-3′; and *mouse glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*), forward 5′-CATCACTGCCACCCAGAA GACTG-3′ and reverse 5′-ATGCCAGTGAGCTTCCCGT TCAG-3′.

Real-time Quantitative PCR (RT-qPCR)

Complementary DNAs (cDNAs) were synthesized from sample RNAs with the ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO, Osaka, Japan). Then, 1 μL of cDNA in a total volume of 20 μL was used in the following reaction. RT-qPCR 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-min template preincubation step at 95 °C, followed by 40 cycles at 95 °C for 20 sec, 62 °C for 20 sec, and 72 °C for 15 sec. The melting curve analysis began at 95 °C for 5 sec, followed by 1 min at 60 °C. The specificity of the produced amplification product was confirmed by the melting curve analysis and showed a distinct single sharp peak with the expected melting temperature *(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.

Immunohistochemistry

Animals were euthanized and perfused with 4% paraformaldehyde. Harvested brain tissues were cryosectioned at a slice thickness of 16 μm along the sagittal plane, and immunohistochemistry staining was performed on 4 sections over a range of over 128 μm. The sections were stained with primary antibodies against induced nitric oxide (iNOS, 1:400, Abcam, Cambridge, UK) and α-syn (Nitrate Tyr 125, Nitrate Tyr 133, Fluorescein isothiocynate (FITC); 1:400, Novus, Littleton, CO, USA). Stained sections were then mounted on glass slides with fluorescent mounting medium containing 4,6-diamidino-2-phenylindole (Vectashield, Vector, Burlingame, CA, USA). Stained sections were analyzed using a fluorescent

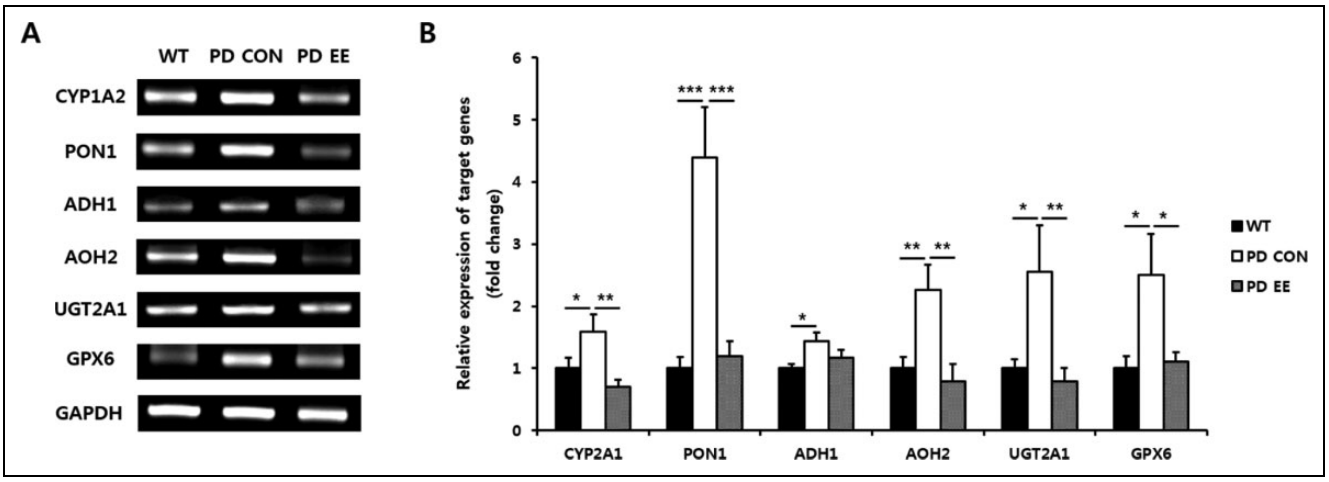

Fig. 2. Expression of genes related with detoxification enzymes in the olfactory bulb. (A) RT-PCR analysis of 6 genes related to detoxification enzymes in the OB of PD mice at 10 mo of age: Cytochrome P450 family 1 subfamily A member 2 (CYP1A2), paraoxonase 1 (PON1), alcohol dehydrogenase 1 (ADH1), aldehyde oxidase homolog 2 (AOH2), UDP glucuronosyltransferase family 2 member A1 complex locus (UGT2A1), and aldehyde glutathione peroxidase 6 (GPX6). (B) Quantitative comparison of gene expression in the PD control ($N = 5$) and PD EE group ($N = 5$) relative to WT mice ($N = 5$) at 10 mo of age determined by RT-qPCR. The expression of CYP1A2 (1.59-fold, $P < 0.05$), PON1 (4.39-fold, $P < 0.001$), ADH1 (1.43-fold, $P < 0.05$), AOH2 (2.26-fold, $P < 0.01$), UGT2A1 (2.55-fold, $P < 0.05$), and GPX6 (2.50-fold, $P < 0.05$) was significantly increased in the PD control group compared to the WT group. On the other hand, the expression of CYP1A2 (0.70-fold, $P < 0.01$), PON1 (1.20-fold, $P < 0.001$), ADH1 (1.17-fold), AOH2 (0.79-fold, $P < 0.01$), UGT2A1 (0.79-fold, $P < 0.01$), and GPX6 (1.10-fold, $P < 0.05$) was decreased in the PD EE group compared to the PD control group. Abbreviations: RT-PCR = reverse transcription polymerase chain reaction; OB = olfactory bulb; PD = Parkinson's disease; EE = enriched environment; WT = wild type; RT-qPCR = real-time quantitative polymerase chain reaction. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ are based on a one-way analysis of variance followed by a post hoc test.

microscope (Axio Imager M2, Zeiss, Stockholm, Sweden). Images of nitrated α-syn and iNOS in glomerular cell layer of OB were evaluated using ZEN Imaging Software version 2.1 (Blue edition, Zeiss). Images of the area (mm^2) of the glomerular cell layer of OB were obtained and layers were merged and converted to volume (mm^3). Area of nitrate α-syn or iNOS was also obtained using ZEN Imaging Software (Blue edition, Zeiss), and layers were merged and converted to volume. The volume of nitrate α-syn or iNOS was calculated via the formula: percent density (%) = [α-syn or iNOS (mm^3)/total volume of glomerular cell layer of OB (mm^3)] × 100.

Statistical Analysis

Statistical analyses were performed using Statistical Package for Social Sciences software version 23.0 (IBM Corporation, Armonk, NY, USA). Data are expressed as the mean ± standard error of the mean. The interaction (group × time) of results in the buried food test was analyzed using a two-way analysis of variance (ANOVA). Then, 3 groups (WT, PD control, and PD EE) were compared using a one-way ANOVA in each period pretreatment (8 mo) and posttreatment (10 mo), respectively. Both results of RT-qPCR and immunohistochemistry were analyzed using a one-way ANOVA followed by a post hoc Bonferroni test to compare 3 groups. The results of RT-qPCR in different ages (10 and 13 mo) of PD were analyzed using an independent t test. Statistical significance was accepted when $P < 0.05$.

Results

EE Ameliorates Olfactory Dysfunction in PD

We first determined whether an EE could restore olfactory dysfunction using the buried food test at 8 and 10 mo of age in a mouse model of PD (Fig. 1C). A significant interaction (group × time) of buried food test was not detected in a two-way ANOVA ($P = 0.132$), revealing there was no time effect. To identify the main effect of treatment, a one-way ANOVA was used at 8 and 10 mo of age, separately. The result based on a one-way ANOVA showed that latency time of finding food in PD control group ($N = 14$, 71.0 ± 9.2 sec, $P < 0.05$) and PD EE group ($N = 11$, 74.2 ± 12.8 sec, $P < 0.05$) were significantly increased compared to that of WT group ($N = 8$, 30.7 ± 4.6 sec) at 8 mo of age. At 10 mo of age, the latency time of finding food in PD EE group (37.9 ± 6.1 sec, $P < 0.05$) was significantly decreased compared to PD control group (71.3 ± 14.2 sec). These results suggest that EE ameliorates olfactory dysfunction in PD.

Expression of Genes Related to Detoxification Enzymes in the OB According to RT-qPCR

We performed RT-qPCR for quantitative analysis and confirmed expression patterns similar to the RT-PCR results

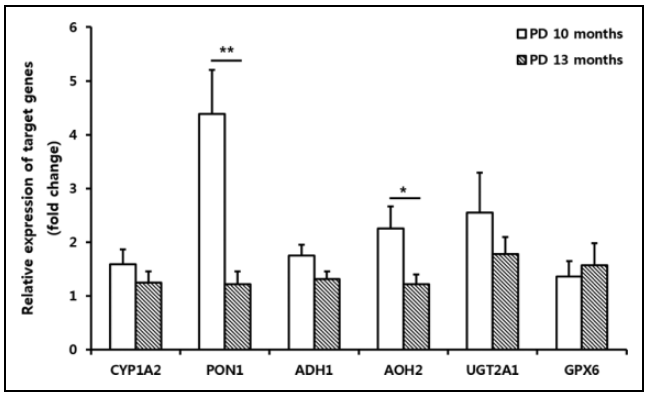

Fig. 3. Expression of genes related with detoxification enzymes in early and late stage of PD. Quantitative comparison of gene expression of detoxification enzymes in the OB of PD mice relative to the WT mice was determined by RT-qPCR. Gene expressions of early stage mice, 10 mo of age, of PD mice ($N = 5$) relative to the same age of WT mice ($N = 5$) and that of PD mice ($N = 7$) in late stage, 13 mo of age, relative to the same age of WT mice ($N = 5$) were compared. PD mice in late stage showed the decrease in CYP1A2 (0.78-fold), PON1 (0.28 fold, $P < 0.01$), ADH1 (0.75-fold), AOH2 (0.54-fold, $P < 0.05$), UGT2A1 (0.70-fold), and GPX6 (1.16-fold) compared to the PD mice in early stage. Abbreviations: PD = Parkinson's disease; OB = olfactory bulb; WT = wild type; RT-qPCR = real-time quantitative polymerase chain reaction; CYP1A2 = cytochrome P450 family 1 subfamily A member 2; PON1 = paraoxonase 1; ADH1 = alcohol dehydrogenase 1; AOH2 = aldehyde oxidase homolog 2; UGT2A1 = UDP glucuronosyltransferase family 2 member A1 complex locus; GPX6 = aldehyde glutathione peroxidase 6. *$P < 0.05$ and **$P < 0.01$ are based on an independent t test.

(Fig. 2). The expression levels of genes in the OB of the PD control ($N = 5$) and PD EE group ($N = 5$) relative to the WT mice ($N = 5$) were compared at 10 mo of age. The expression of *CYP1A2* (1.59-fold, $P < 0.05$), *PON1* (4.39-fold, $P < 0.001$), *ADH1* (1.43-fold, $P < 0.05$), *AOH2* (2.26-fold, $P < 0.01$), *UGT2A1* (2.55-fold, $P < 0.05$), and *GPX6* (2.50-fold, $P < 0.05$) was significantly increased in the PD control group compared to the WT group. On the other hand, the expression of *CYP1A2* (0.70-fold, $P < 0.01$), *PON1* (1.20-fold, $P < 0.001$), *ADH1* (1.17-fold), *AOH2* (0.79-fold, $P < 0.01$), *UGT2A1* (0.79-fold, $P < 0.01$), and *GPX6* (1.10-fold, $P < 0.05$) was decreased in the PD EE group compared to the PD control group.

Expression of Genes Related to Detoxification Enzymes in Early and Late Stage of PD

We performed RT-qPCR to compare expression of genes related to detoxification enzymes at 10 and 13 mo in the OB of PD (Fig. 3). Gene expressions of early stage mice, 10 mo of age, of PD mice ($N = 5$) relative to the same age of WT mice ($N = 5$) and that of PD mice ($N = 7$) in late stage, 13 mo of age, relative to the same age of WT mice ($N = 5$) were compared. PD mice in late stage showed the decrease in *CYP1A2* (0.78-fold), *PON1* (0.28-fold, $P < 0.01$), *ADH1* (0.75-fold), *AOH2* (0.54-fold, $P < 0.05$), *UGT2A1* (0.70-fold), and *GPX6* (1.16-fold) compared to the PD mice in early stage.

EE Ameliorates Oxidative Stress in PD

To evaluate the level of oxidative stress in the glomerular layer of the OB, we performed immunohistochemical staining of iNOS in 10-mo-old WT, PD control, and PD EE mice ($N = 5$ per group; Fig. 4A). The density of iNOS staining was significantly higher in the PD control group ($2.5 \pm 0.53\%$; $P < 0.001$) than in the WT group ($0.2 \pm 0.06\%$). However, the density of iNOS was significantly lower in the PD EE group ($0.9 \pm 0.13\%$; $P < 0.01$) than in the PD control group (Fig. 4B). These results suggest that EE ameliorates oxidative stress in PD.

EE Ameliorates Nitrated α-Syn in PD

To evaluate the density of nitrated α-syn in the glomerular layer of the OB, we also performed immunohistochemical staining of nitrated α-syn in 10-mo-old WT, PD control, and PD EE mice ($N = 5$ per group; Fig. 4C). The density of nitrated α-syn staining was significantly higher in the PD control group ($2.8 \pm 0.33\%$; $P < 0.001$) than in the WT group ($0.3 \pm 0.04\%$). However, the density of nitrated α-syn staining was significantly lower in the PD EE group ($1.1 \pm 0.21\%$; $P < 0.001$, $P < 0.05$, respectively) than both in the PD control group and WT group (Fig. 4D). These results suggest that EE ameliorates nitrated α-syn level in PD.

Discussion

Although motor symptoms of PD are caused by degeneration of dopaminergic neurons in the substantia nigra in association with Lewy bodies, it has recently been shown that degeneration starts in other sites such as the glossopharyngeal and vagal nerves and olfactory structures[16]. Since Ansari and Jonson reported olfactory dysfunction in PD in 1975[10], many reports have described impairments of odor detection and discrimination in PD, and hyposmia that precedes motor symptoms by several years[12,13,15] which can be an initial sign of PD[14]. In our study, a neurobehavioral test of olfactory function showed that EE ameliorated olfactory dysfunction in PD mice compared to the PD control group without exposure to an EE. Although a similar A53T α-syn mouse model, *B6;C3-Tg(Prnp-SNCA*A53T)83Vle/J*, of PD was previously reported to have olfactory dysfunction[27], we report on the recovery of olfactory function in PD through an EE.

Previous studies have shown that *CYP1A2* is related to detoxification of MPTP in PD[29]. MPTP is a neurotoxic compound that can lead to Parkinsonian features in humans and mice[19,20,30] and is known as one of the best molecules to establish a PD animal model[31]. Moreover, PON1, which has a role in the hydrolysis of the toxic metabolites of pesticides,

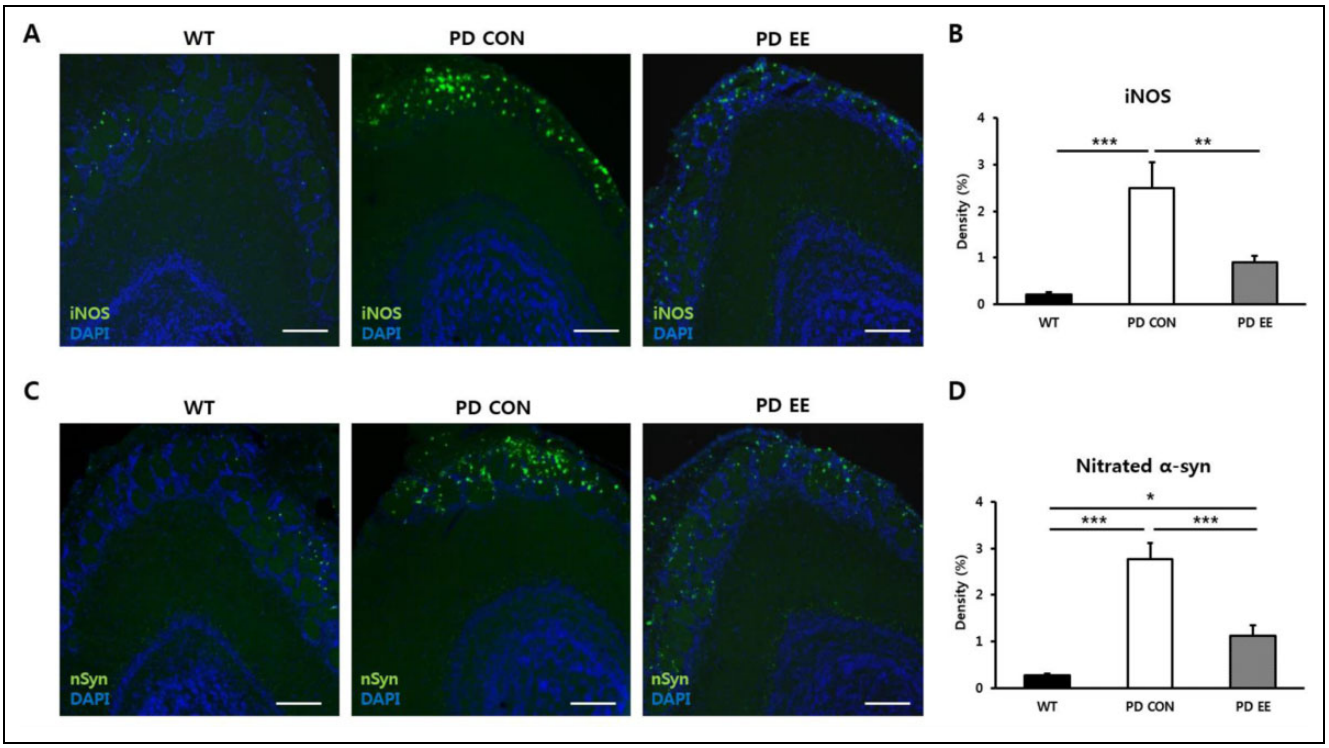

Fig. 4. An enriched environment ameliorates oxidative stress and nitrated α-syn in PD. (A) Images of the iNOS immunohistochemistry staining in the glomerular layer of the olfactory bulb. (B) The density of iNOS was significantly higher in PD control group (2.5 ± 0.53%, $P < 0.001$) than in WT group (0.2 ± 0.06%). However, the density of nitrated α-syn was significantly lower in PD EE group than in both PD control group and WT group based on a one-way ANOVA (1.1 ± 0.21%, $P < 0.001$, $P < 0.05$, respectively). Scale bars = 200 μm. Abbreviations: α-syn = human A53T α-synuclein; PD = Parkinson's disease; iNOS = induced nitric oxide; WT = wild type; EE = enriched environment; ANOVA = analysis of variance. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ are based on a one-way ANOVA followed by a post hoc test.

and has various roles in antioxidative function, anti-inflammatory ability, protection against cardiovascular disease via an inverse correlation with atherosclerosis, and metabolism of organophosphates[32–34]. Its effects on the hydrolyzation of organophosphates and antioxidant capacity suggest the putative relation of PON1 in PD pathogenesis[33]. However, its exact role should be elucidated, because many studies have shown inconsistent results between *PON1* polymorphism and the risk of PD[35–38].

As a representative enzyme for alcohol metabolism, *ADH* participates in the metabolism of aldehyde, retinol, steroid, and lipid peroxidation products as well as alcohols[39,40] and also has a defensive function against alcohols and aldehydes without production of toxic radicals[41]. It is well known that chronic consumption of ethanol can induce neurologic disorders such as neurodegenerative and neurocognitive deficits through oxidative stress and mitochondrial dysfunction[42]. There have been several studies showing the relationship between *ADH* and PD risk[43]. Furthermore, AOH, one of the molybdoflavoenzymes, is another non-P450 phase I enzyme. It has broad specificity for various substrates, endogenous molecules, and xenobiotics[44], as well as aldehydes, which have some toxicities related with neurodegenerative disease, cardiovascular diseases, stroke, and cancer[45].

UGT, as key enzymes of phase II metabolism, has an important role in biotransformation and detoxification via a glucuronidation reaction and is the most important pathway of detoxification. These enzymes have numerous substrates such as xenobiotics, including chemical carcinogens and environmental substances, as well as endogenous metabolites[46]. *UTG2A1* is expressed in the rat and mouse OB[47], indicating that *UTG2A1* as a "metabolic barrier" might have a protective role against exogenous toxins invading via the nasal cavity, which can be a direct route of entry for xenobiotics[48]. Likewise, *GPX* is an antioxidant enzyme that detoxifies hydroxyl radicals. Because oxidative stress is believed to be an important cause of nigral neuronal cell death, many human-based studies have been performed to elucidate the mechanism of PD.

In our study, these detoxification enzymes such as *CYP1A2*, *PON1*, *ADH1*, *AOH2*, *UGT2A1*, and *GPX6* significantly increased in early stage of PD as a compensatory mechanism. This result showed that detoxification enzymes were increased as a compensatory mechanism for the increment of various toxic factors after the onset of disease. From 8 to 10 months of age, EE normalized these enzymes. However, most enzymes decreased at 13 mo of age compared to 10 mo of age in PD, suggesting that detoxification enzymes attempt to detoxify the toxic factors in early stage, but in the

late stage of the disease, these detoxification enzymes finally decreased their activities, causing increased oxidative stress and exacerbating the disease at last in PD.

Histological analysis in the glomerular layer of the OB showed that oxidative stress and nitrated α-syn were significantly increased in the PD control group compared to the WT group, but those were significantly decreased in the PD EE group compared to the PD control group at 10 mo of age in PD. Oxidative stress contributes to pathogenic modification of α-syn such as nitration of tyrosine residues that have been observed in the brains of patients afflicted with PD[49]. The result of immunohistochemistry with an iNOS antibody was similar to that of nitrated α-syn. Oxidative stress in PD upregulated nitrated α-syn; however, since the mice were exposed to an EE, oxidative stress decreased and nitrated α-syn was reduced. Additionally, previous research reported that PD might occur by selective α-syn nitration[50]. Due to its nitration, α-syn becomes more difficult to degrade through proteolysis, which changes its properties as a synaptic protein. Therefore, nitration of α-syn regulated the mechanism, onset, and progression of synucleinopathy, which also depended on the formation of the nitrated α-syn form[50]. Thus, an EE alleviated olfactory dysfunction in a PD mouse model through decreased oxidative stress and nitrated α-syn in the OB.

Conclusion

In a Tg mouse model of PD that overexpressed human A53T α-syn, exposure to an EE reduced oxidative stress and nitrated α-syn, resulting in normalized detoxification enzymes and amelioration of olfactory dysfunction.

Authors' Note

Soohyun Wi and Jang Woo Lee equally contributed to this study.

Ethical Approval

The experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC 2017-0039).

Statement of Human and Animal Rights

All animals were housed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care and provided food and water *ad libitum* with alternating 12-h light/dark cycles according to animal protection regulations.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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