Endurance Exercise Mediates Neuroprotection Against MPTP-mediated Parkinson’s Disease via Enhanced Neurogenesis, Antioxidant Capacity, and Autophagy

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Abstract—Parkinson’s disease (PD) is a neurodegenerative disorder caused by loss of dopaminergic neurons in the substantia nigra, leading to motor dysfunction. Growing evidence has demonstrated that endurance exercise (EE) confers neuroprotection against PD. However, the exact molecular mechanisms responsible for exercise-induced protection of dopaminergic neurons in PD remain unclear. Since oxidative stress plays a key role in the degenerative process of PD. We investigated whether EE-induced neuroprotection is associated with enhanced antioxidative capacity and autophagy, using a mouse model of PD induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration. C57BL/6 male mice were randomly assigned to four groups: control (CON, n = 12), exercise (EXE, n = 12), MPTP (MPTP, n = 12) and MPTP + exercise (MPTP + EXE, n = 12).

Our data demonstrated that while MPTP treatment impaired motor function, EE restored MPTP-induced motor deficits. Our biochemical data showed that EE-induced neuroprotection occurs in combination with multiple synergic neuroprotective pathways: (1) increased neurogenesis shown by an increase in BrdU-positive neurons; (2) diminished loss of dopaminergic neurons evidenced by upregulated tyrosine hydroxylase (TH) and dopamine transporter (DAT) levels; (3) increased antioxidant capacity (e.g., CuZnSOD, CATALASE, GPX1/2, HO-1, DJ1 and PRXIII); and (4) enhanced autophagy (LC3 II, p62, BECLIN1, BNI3, LAMP2, CATHEPSIN L and TFEB). Our study suggests that EE-induced multiple synergic protective pathways including enhanced neurogenesis, antioxidative capacity, and concordant autophagy promotion contribute to restoration of impaired dopaminergic neuronal function caused by PD. Thus, PD patients should be encouraged to actively participate in regular EE as a potent nonpharmacological therapeutic strategy against PD.

Key words: Parkinson’s disease, neurogenesis, oxidative stress, autophagy, endurance exercise, antioxidant.

INTRODUCTION

Parkinson’s disease (PD) is a progressive neurodegenerative disorder caused by the selective degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc) in mostly elderly population, resulting in motor dysfunctions such as bradykinesia, tremor, rigidity and postural instability (Yang et al., 2016). These motor deficits critically contribute to death. Currently, the exact pathogenic mechanism of PD remains largely unknown. Nevertheless, recent studies suggest that reduced neurogenesis in hip-pocampus is associated with motor impairment and cognitive deficit (Zhu et al., 2011, 2015; Winner et al., 2012; Klein et al., 2016).

Moreover, oxidative stress has been linked to possible etiology of PD in numerous studies. In this regard, mitochondria are major sources of reactive oxygen species (ROS), leading to neuronal cell death (Pankratz and Foroud, 2007; Dias et al., 2013; Blesa et al., 2015). While endogenous antioxidants including copper zinc superoxide dismutase (CuZnSOD), CATALASE, glutathione peroxidase (GPX), heme oxygenase I (HO-1) in cytosol and manganese superoxide dismutase (MnSOD), peroxiredoxin III (PRX III) in mitochondria are known to antagonize deleterious oxidative stress (Day, 2009; Buettner, 2011), excess ROS disrupts redox balance leading toward oxidative stress and thus results in cellular damages.

Autophagy, a lysosome-dependent catabolic process by which dysfunctional organelles or damaged proteins are removed, has been on the rise in the etiology of PD since dysregulated autophagy has been reported to
contribute to loss of dopaminergic cells in SNpc (Lynch-Day et al., 2012; Takalo et al., 2013; Rivero-Rios et al., 2016). By contrast, autophagy promotion mitigates cell death of dopaminergic neuronal cells (Pan et al., 2009; Rodriguez-Navarro et al., 2010; Giordano et al., 2014).

A mitochondrial dopaminergic neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has been used in PD research because it targets and impairs dopaminergic neurons including SNpc (Koo and Cho, 2017) and hippocampus (Zhu et al., 2011; Klein et al., 2016). Since MPTP has been known to trigger mitochondria dysfunction and impair autophagy, resembling clinical PD, it has been widely used to study pathophysiology of PD.

Although pharmacological therapeutic approaches have been made to mitigate poor progression of PD, long-term administration generates serious side effects. On the contrary, a nonpharmacological therapeutic strategy, regular endurance exercise (EE), has been reported to confer neuroprotective effects against PD, exhibiting improved motor function along with dopaminergic neuron survival and neurogenesis with relatively no side effects (Gerecke et al., 2010; Paillard et al., 2015; Klein et al., 2016; Shin et al., 2016). Despite this therapeutic potential, clear molecular mechanisms of EE-induced neuroprotection remain still poorly understood. Given that reduced neurogenesis in hippocampus and increased antioxidative stress, and dysregulated autophagy are involved in pathogenesis of PD, examination of whether EE improves endogenous antioxidant capacity and promotes autophagy may help reveal a novel mechanism responsible for exercise-induced neuroprotection against PD.

In the present study, we tested our hypothesis that EE-induced neurogenesis, improvement in antioxidative capacity, and autophagy would be associated with amelioration of impaired motor function caused by PD.

EXPERIMENTAL PROCEDURES

Animals
Male C57BL/6 mice (7-week-old) were purchased from Envigo (USA) and were housed in controlled environment (12:12-h dark–light cycle at 22 ± 2 °C with 50% relative humidity) with ad libitum access to a standard chow diet. Upon arrival, the animals were randomly divided into four groups: control (CON, n = 12), EE (EXE, n = 12), MPTP (MPTP, n = 12) and MPTP + endurance exercise (MPTP + EXE, n = 12). All procedures were approved by the Institutional Animal Care and Use Committees at the University of West Florida.

MPTP-induced mouse model of PD
MPTP is a common neuro toxin used to induce a PD like symptom in an animal model of PD (Meredith and Rademacher, 2011). Amphiphilic structure of MPTP permits rapid penetration of blood–brain barrier into brain cells, remaining nontoxic until it is converted into MPP⁺ by astrocytes, after which MPP⁺ is transported into dopaminergic neurons (e.g., SNpc) via dopamine transporters (DATs) (Tieu, 2011). MPP⁺ particularly accumulates in mitochondria of the neurons, then disturbs the normal function of the complex I of the electron transport chain, and induces oxidative stress, leading to cell death of dopaminergic neurons and subsequently motor impairment (Meredith and Rademacher, 2011).

One week after acclimation to the animal housing facility, mice assigned to MPTP and MPTP + EXE groups were intraperitoneally injected with MPTP (25 mg/kg, Sigma–Aldrich) daily for one week to induce PD, whereas mice assigned to CON and EXE groups were injected with saline.

EE protocol
Our animal exercise protocol has been used in our previous study and studies from others (Lee et al., 2016; Koo and Cho, 2017; Koo et al., 2017). Briefly, four weeks after the last MPTP or saline administration, mice assigned to EXE and MPTP + EXE groups were acclimated to running exercise (8 m/min, 30 min/day) for 5 days on a motorized treadmill. Two days after the acclimation, the mice performed regular EE at 12 m/min for 60 min per day, 5 days/week for 6 weeks. It should be noted that animals assigned to exercise training intervention ran at slower running speed (12 m/min, ~60% VO₂max) rather than 15–16 m/min, ~75% VO₂max (Schefer and Talan, 1996) because we concerned about possibility that MPTP-treated animals might not be able to run full speed possibly due to a significant motor deficit. Also, despite the same absolute running study between groups, it is possible that that relative exercise intensity for MPTP-treated animals might be higher than untreated animals because of significant motor deficit. All animals successfully completed exercise intervention.

Pole test
The pole test is a useful method to measure motor coordination and balance in several mouse models of PD (Ju et al., 2010). Briefly, mice were placed head upward on top of a rough-surfaced wooden pole (50 cm in length and 1 cm in diameter). The time the mice take to turn their heads completely downward (T-turn) and to reach the bottom (T-total) was recorded respectively with a cut-off limit of 30 s. Each mouse performed three trials, and average value of the trials was used to assess their motor function.

5-Bromo-2-Deoxyuridine (BrdU) injection
In order to assess neurogenesis, BrdU, a thymidine analog which is incorporated into newly synthesized DNA was used. Mice (n = 3 per group) had been intraperitoneally injected with BrdU (50 mg/kg/day) for 5 days before they were sacrificed.

Tissue preparation
After motor function assessment, mice (n = 9/group) were euthanized by cervical dislocation for biochemical and immunohistochemical assays. Briefly, once mice...
reached the surgical plane, the brain was rapidly excised, and the substantia nigra was dissected and stored at −80 °C until needed. For immunohistochemistry, the dissected whole brain (n = 3 mice/group) were covered with OCT freezing compound, submerged into pre-cooled isopentane under liquid nitrogen, and allowed to be gradually frozen for 30 s. The frozen brain tissues were transferred to a −80 °C freezer and stored until needed.

Western blotting

The brain tissues (substantia nigra) were homogenized in iced-cold T-PER lysis buffer (ThermoFisher, USA), and protein concentration was determined by the Bradford assay. Proteins (30 μg) were separated by 10–12% SDS–PAGE and transferred to nitrocellulose or polyvinylidene fluoride membrane (PVDF). For verification of protein transfer and equal loading, proteins on the membranes were stained with Ponceau S solution (0.1% in 5% acetic acid, Sigma–Aldrich, USA), and images were captured for quantitative analysis using a ChemiDoc XRS device (Bio-Rad, USA). Then, the membranes were blocked with 5% skim milk for 1 h at room temperature. The membranes were incubated overnight at 4 °C with the following primary antibodies: TH (Millipore, dilution, 1:1000); CATHEPSIN L and TFEF (Abcam, dilution, 1: 1000); LC3 A/B, P62, BECLIN1 and BNIP3 (Cell Signaling, Boston, MA, U.S.A, 1:1000); HEME OXIGENASE 1, CATALASE, GPX1/2 and PRX III (Santa Cruz, dilution, 1:1000); DAT, CuZn SOD and NRF2 (NOVUS, dilution, 1:1000); and LAMP2 and DJ1 (Thermo Fisher, dilution, 1:1000). After overnight incubation, the membranes were washed with TBS containing 0.05% Tween 20 (TBS-T) and incubated with secondary antibodies (HRP-conjugated goat anti-rabbit or rabbit anti-goat, or goat anti-mouse (dilution, 1:5000) for one hour at room temperature. Target proteins were identified using the ECL Western blotting detection substrates (GE Healthcare, USA) and a ChemiDoc XRS software (Bio-Rad, USA). Band intensity obtained from the target protein was normalized against intensity of total protein of each corresponding lane acquired from Ponceau S stain on the membrane. We chose this normalization method because conventional housekeeping proteins for normalization such as GAPDH, β-tubulin have been reported to change across different experimental conditions (Perez-Perez et al., 2012) and Ponceau S staining method has been used in recent studies.

Detection of oxidized proteins

Amounts of oxidized proteins containing carbonyl groups were detected with the OxyBlot kit (Millipore, Germany). Briefly, 5 μL of tissue lysates containing the equal amount proteins was incubated with 2,4-dinitrophenyl hydrazine (DNPH) for 15 min, and the chemical reaction was terminated by the addition of 7.5 μL of neutralization solution. The prepared protein samples were then separated by 10% SDS–PAGE, transferred to nitrocellulose, and blocked with PBS-T containing 5% skim milk for 1 h. The membranes were incubated for 1 h at room temperature with DNP antibodies (1:150), after which they washed with PBS-T and incubated with goat anti-rabbit IgG conjugated to HRP (1:300) for 1 h at room temperature. Oxidized proteins were identified using the ECL Western blotting detection system (GE Healthcare, USA), and the density of the protein was assessed using a ChemiDoc XRS software (Bio-Rad, USA).

Immunohistochemistry

Frozen brain tissue containing SNpc (anatomical location of −2.9 to −4.0 mm of bregma) was cryo-sectioned into 10-μm thickness using a sliding cryostat (Leica, Germany), according to the mouse brain stereotaxic atlas (Paxinos and Franklin, 2013). Six tissue sections from each sample were collected (n = 3/group). The tissue sections were fixed with 4% paraformaldehyde solution prepared in PBS (pH 7.4) for 15 min on ice, after which they were rinsed three times with PBS solution for 10 min, permeabilized with 0.2% Triton X-100 at room temperature for 10 min, and blocked with 10% normal goat serum for 1 h at room temperature. After blocking, the tissue sections were incubated overnight with TH antibodies (Millipore, 1:200) at 4 °C, washed with PBS, and incubated with Horseradish peroxidase (HRP)-conjugated goat anti-rabbit for 1 h at room temperature, respectively. Then, avidin–biotin peroxidase complex (ABC kit, Vector Laboratories) was applied and incubated for 30 min at room temperature. After washing, the sections were incubated with diaminobenzidine (DAB) substrate kit (Vector Laboratories) for 2 min at room temperature. The tissue sections were dehydrated in 80%, 90%, 100% ethanol in order, cleared using xylene, and mounted using a Permount solution (Fisher Scientific, USA). The sections were examined using a digital light microscope (EVOS, Thermo Fisher, USA). The numbers of TH-positive cells from both brain hemispheres on the images (×100 magnification) were manually counted, and average numbers were reported as results. Six tissue sections from each sample were chosen, and average number of TH-positive cells from the sections was used as results.

Immunofluorescence microscopy

Tissue sections containing hippocampus (anatomical location of −1.6 to −2.7 mm of bregma) were washed in 0.01 M PBS for 10 min, permeabilized with 0.2% Triton X-100, and blocked with 10% normal goat serum for 1 h at room temperature. The sections were incubated with BrdU (Cell Signaling, 1:200) and NeuN (Invitrogen, Grand Island, NY, U.S.A, 1:200) or LC3 A/B (Cell Signaling, 1:200) antibodies overnight at 4 °C. The tissue sections were washed with 0.01 M PBS and incubated with secondary antibodies Alexa 488-conjugated goat anti-rabbit and Cy3-conjugated goat anti-mouse (Jackson Immunoresearch, USA) for 1 h. After being washed with PBS, the sections were mounted with VectaShield (Vector Laboratories, Burlingame, CA, USA) and examined using an immunofluorescence microscope (EVOS, ThermoFisher, USA).
Statistical analysis

All statistical analysis was performed with SPSS version 18.0 (SPSS, Chicago, IL, USA). All data was analyzed using a two-way ANOVA, followed by Tukey’s post hoc test when statistical significance was found to identify group differences. Data were represented as mean ± S EM, and statistical significance was set at p < .05.

RESULTS

EE restores a motor deficit caused by MPTP treatment

Impairment in motor function is a common observation in PD patients. To confirm if MPTP treatment developed a PD-like symptom, we assessed animals’ motor function via a pole test. In this test, time taken by mice to turn their heads from upside to downside (T-turn) and to reach the floor (T-total) was measured. MPTP treatment significantly delayed T-turn time (7.69 ± 0.9 vs. 3.84 ± 1.2 s) as well as T-total time (10.18 ± 1.13 vs. 7.01 ± 1.29) compared with the CON group (Fig. 1A, B). After the confirmation of a MPTP-induced mouse model of PD, MPTP-treated mice underwent 8 weeks of EE intervention. Strikingly, MPTP + EXE treatment exhibited a recovery of impaired motor function, evidenced by reduction in T-turn (1.21 ± 0.14 vs. 3.04 ± 0.16 s) and T-total time (4.41 ± 0.8 vs. 6.96 ± 0.79 s), compared with MPTP group (Fig. 1C, D).

EE restores dopaminergic neurons via enhanced neurogenesis

Since loss of dopaminergic neurons is a primary cause of motor impairment, we examined if EE-induced restoration of motor function against MPTP-mediated motor deficits is due to compensatory induction of neurogenesis. Using an immunohistochemical technique, we assessed the numbers of TH-positive neurons and found that MPTP destroyed many cells, whereas MPTP with EE intervention regenerated many cells up to a level similar to the CON group (Fig. 2A, B). Furthermore, Western blot data confirmed that MPTP + EXE reestablished TH and DAT protein up to levels similar to CON and EXE groups (Fig. 2C–E). To further verify EE-induced neurogenesis, we sought to detect the newly generated neuronal cells in the hippocampal dentate gyrus (DG) of mice treated with BrdU and observed that both EXE group and MPTP + EXE group significantly increased the numbers of BrdU-positive cells, compared with the CON group and MPTP group (Fig. 2F, G).

EE-induced improvement in antioxidant capacity reduces oxidative damages in SNpc

Oxidative stress is one of the main causes of loss of dopaminergic neurons, and EE has been suggested to improve antioxidant capacity and thus help overcome adverse oxidative stress. Given this premise, we examine whether EE enhances antioxidant capacity and contribute to mitigating MPTP-induced oxidative stress. Our results showed that EXE and MPTP + EXE groups significantly elevated cytosolic antioxidant enzymes CuZnSOD, CATALASE, GPX1/2 and heme oxygenase-1 (HO-1), compared with CON and MPTP group (Fig. 3A–E). Since mitochondrion is the origin of free radicals as well as their common target and, we also assessed mitochondria-specific antioxidant enzyme levels. While no changes in MnSOD levels were observed (data not shown), MPTP treatment significantly diminished DJ1 and PRX III levels; conversely, MPTP with EE intervention (MPTP + EXE) prevented a MPTP-induced decline in DJ1 and PRX II levels (Fig. 3A–E). Consistent with this, while MPTP treatment raised the levels of protein oxidation, the enhanced proactive antioxidative capacity in the MPTP + EE group prohibited MPTP-induced protein oxidation (Fig. 3H, I).

We next sought to examine a candidate transcription factor responsible for EE-induced upregulation of antioxidant enzymes. Since the nuclear factor erythroid 2–related factor 2 (NRF2) has been known as a master transcription regulator of various antioxidant enzymes,
we measured NRF2; interestingly, its expression was not significantly altered in any groups, although EXE and MPTP + EXE groups showed a trend of an increase compared with CON and MPTP groups (Fig. 3 J, K).

**EE promotes autophagy in SNpc of MPTP-treated mice**

Autophagy is an important catabolic process involved in maintaining cellular homeostasis and survival. We examined if EE induces autophagy and its induction is associated with EE-induced neuroprotection. Data from fluorescence microscopy showed that although EE per se did not modulate LC3-II levels, EE intervention after MPTP treatment (MPTP + EE group) significantly increased LC3 (green) puncta, compared with CON, EXE and MPTP groups (Fig. 4A, B). Consistent with the results, Western blot data also confirmed that only MPTP + EXE group increased LC3-II levels (Fig. 4A,

**Fig. 2.** Endurance exercise reestablishes a network of dopaminergic neurons eliminated by MPTP administration. (A) Representative immunohistochemical staining of TH in the substantia nigra par compacta (SNpc), n = 3/group. A scale bar = 400 μm. Endurance exercise restores dead dopaminergic neurons. (B) The number of TH-positive cells in the SNpc. (C) Representative Western blot data in the SNpc (n = 9/group). (D, E) Quantification of TH and DAT levels. Ponceau staining was used as a loading control. (F) Representative double-labeled Immunofluorescence staining of NeuN (red) and BrdU (green) in the dentate gyrus (n = 3/group). A scale bar = 200 μm. (G) Computation of BrdU positive cells in the dentate gyrus. Values are mean ± SEM. *Denotes a statistically significant difference compared to CON, EXE and MPTP + EXE groups at p < 0.05. # Denotes a statistically significant difference compared to CON and MPTP groups at p < 0.05. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)
Since an autophagy adapter protein p62 has been used as a marker of autophagy flux, we measured p62 levels. Interestingly, p62 was also elevated in only MPTP + EXE group, compared to other groups (Fig. 4A). To examine if autophagy induced by the combinatorial effect between MPTP and EE undergoes the same autophagy paradigm, we measured BECLIN1 and BNIP3 levels to evaluate an inductive process of autophagy. Both EXE and MPTP + EXE group exhibited significant increases in BECLIN1 compared to CON and MPTP groups, whereas only MPTP + EXE group showed BNIP3 upregulation (Fig. 4B, C, and D). We next assessed levels of lysosomal proteins such as LAMP2, CATHESIN L and TFEB that are associated with regulation of autophagy flux. In proportion to upregulated LC3-II, these proteins were significantly elevated in only MPTP + EXE group (Fig. 4E, F, I and J) compared with other groups.

**DISCUSSION**

EE has been widely recommended as a preventive as well as therapeutic strategy against neurodegenerative diseases including PD, with side effects relatively negligible. However, its protective mechanisms at molecular and cellular levels have not been clearly elucidated. In the present study, we show that six weeks of EE restores motor function of mice undergoing MPTP-induced PD. Importantly, its amelioration is linked to enhanced antioxidant capacity and autophagy in parallel with neurogenesis.

We used a pole test to examine motor function, whose validity was also confirmed by other research groups (Okuda et al., 2006; Zhang et al., 2016) and observed that EE restores MPTP-induced motor deficit, evidenced by significant improvement in time for T-turn and total test completion time. Since PD-mediated motor dysfunction...
is mainly caused by loss of dopaminergic neurons (DN) in SNpc (Drui et al., 2014), it is plausible to expect that improvement in motor function correlates with amounts of live neurons. In many studies, assessment of tyrosine hydroxylase (TH), a rate-limiting enzyme for dopamine biosynthesis and DAT have been used as an alternative to direct dopamine measurement (Hallett et al., 2014; Churchill et al., 2017). We found that TH and DAT levels diminishes upon MPTP treatment, but EE intervention restores those levels comparable to CON and EXE groups. This result suggests that EE-induced regeneration of dopaminergic neurons may be a key element that reestablishes improved motor function that was impaired by PD.

Generally, two areas of the brain are known for neurogenesis: DG of the hippocampus and subventricular zone (SVZ) (Urban and Guillemot, 2014). However, while role of neurogenesis of SVZ in PD remains inconsistent, inverse relationship between neurogenesis in DG of hippocampus and PD has been reported.
In line with these studies, our data and others show reduced neurogenesis levels in hippocampus in response to MPTP treatment; however, EE intervention reverses MPTP-induced neurogenesis deprivation in the hippocampus concurrent with improved motor function (Kim et al., 2014, 2016; Koo and Cho, 2017). This suggests that EE-induced neurogenesis in the hippocampus may be associated with regeneration of dopaminergic neurons in SNpc. Moreover, death of neurons in SNpc reduces neuroplasticity of hippocampus (Zhu et al., 2015; Klein et al., 2016; Bonato et al., 2018); thus, increased neuron numbers of SNpc via EE may be a positive loop of neurogenesis executed by hippocampus and SNpc.

Preservation of intact neurons in SNpc upon MPTP treatment is another way to attenuate or prevent poor progression of PD. Since damaged mitochondria facilitate excessive oxidative stress and thus initiate cell death, several studies have focused on antioxidant capacity as a means to prevent cell death of dopaminergic neurons caused by PD (Pankratz and Foroud, 2007; Jin et al., 2014; Romuk et al., 2017). Supporting this notion, our study for the first time provides strong evidence that EE-induced upregulation of endogenous antioxidant enzymes (e.g., CuZnSOD, CATALASE, and GPX1/2) and resultant reduction in oxidized proteins in SNpc are associated with neuroprotection against a MPTP model of PD.

Numerous studies have reported NRF2 as a master transcription regulator of various antioxidant enzymes (Nguyen et al., 2009; Vomhof-Dekrey and Picklo, 2012); however, it has remained unknown whether NRF2 is associated with EE-induced upregulation of antioxidant enzymes in SNpc. Therefore, we attempted to measure NRF2 levels, but interestingly found no significant changes in response to any interventions (e.g., EE, MPTP, and EE + MPTP). Our study infers that other transcription-regulating proteins rather than NRF2 may be involved in upregulation of EE-mediated antioxidant enzymes.

Another potential mechanism to explain protective effects of EE against PD is autophagy because recent studies have implicated autophagy dysfunction in pathogenesis of PD (Wang et al., 2016) and autophagy promotion improves PD (Lynch-Day et al., 2012). Now that EE has been known to be a potent inducer of autophagy (He et al., 2012; McMillan et al., 2015; Lee et al., 2016) and its promotion is associated with multiple health benefits (Rocchi and He, 2017) in various tissues, it seems plausible that EE-induced autophagy in SNpc may be linked to neuroprotection against PD. Our results support this notion by providing evidence that EE enhances autophagy (e.g., an increase in LC3-II in conjunction with upregulation of BECLIN-1 and BNIP3) in the SNpc of MPTP-treated mice (MPTP + EE group) and that this elevation coincides with improved motor function. This exercise effect on autophagy in SNpc has also been observed in a recent study (Koo and Cho, 2017).

Upregulation of LC3-II concurrent with decline in p62 exemplifies bona fide autophagy flux in various tissues (Winner et al., 2012; Zhu et al., 2015; Klein et al., 2016). Interestingly, we failed to observe the same findings; by contrast, we rather observed a significant rise in p62 levels. In line with our results, a recent study has shown the similar findings that 9 weeks of EE raises both LC3-II and p62 levels in the white adipose tissue, but the authors interpreted their observation as interrupted autophagy in that no concomitant increase in lysosomal autophagy proteins such as LAMP2 were found (Tanaka et al., 2015). However, in our study, EE-induced p62 elevation concurs with upregulation of several key proteins involved in lysosomal activities including a lysosomal membrane fusion protein LAMP2, a protease CATHEPSIN L and a master transcription regulator of lysosomal biogenesis TFEB. Multiple studies have shown that BECLIN1 and BNIP3 play a crucial role in inducing autophagy in various tissues including skeletal and cardiac muscles and brain (Kim et al., 2012; Lee et al., 2016; Koo and Cho, 2017). In line with these studies, our study also shows remarkable increases in those proteins in SNpc. Therefore, we infer that elevation of EE-induced LC3-II and autophagy inducer proteins with lysosomal biosynthesis is an indication of improved autophagy flux.

CONCLUSION

Our study demonstrates that 6 weeks of EE improves motor dysfunction observed in the MPTP neurotoxin model of PD by enhancing hippocampal neurogenesis and preventing dopaminergic neuronal loss. Our results provide clear evidence that EE-induced multiple beneficial effects (e.g., neurogenesis, increased antioxidative capacity, and enhanced autophagy) in the SNpc are associated with dopaminergic neuronal protection against MPTP-mediated PD. Taken together, our results provide a key insight into EE as a potent nonpharmacological therapeutic strategy for prevention and management of PD.

AUTHOR CONTRIBUTIONS

Y.J. and Y.L. developed the concept and design of research; Y.J. and I.K. collected and analyzed data; Y.J., I.K., W.S. and Y.L. interpreted results; Y.J. prepared table and figures; Y.J. and Y.L. drafted manuscript; Y.J., I.K., W.S., L.M.K. and Y.L. edited and revised the manuscript; Y.J., I.K., W.S., L.M.K. and Y.L. approved the final version of manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interests.

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